Thesis reference number: ……


**University of Science and Technology of Hanoi**


# CHEMICAL CONSTITUENTS AND HYPOGLYCEMIC EFFECTS OF *COMMELINA DIFFUSA* BURM.F.


**Nguyen Xuan Tung**

**Department of Life Sciences**

**Specialty: Pharmacological, Medical and Agronomical Biotechnology**

**Assoc. Prof. Vu Duc Loi, Supervisor**

Vietnam University of Traditional Medicine

**Assoc. Prof. Nguyen Thi Van Anh, Co-supervisor**

University of Science and Technology of Hanoi, Vietnam Academy of Science and Technology


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The thesis has been successfully defended on July 14th, 2026, in front of a jury composed of:

Assoc. Prof. Nguyen Hai Dang, USTH, Chairman

Prof. Byung Sun Min, Daegu Catholic University, South Korea, Reviewer

Assoc. Prof. Pham Thi Nguyet Hang, National Institute of Medicinal Materials, Reviewer

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Dr. Le Thanh Huong, USTH, Secretary

**Hanoi – 2026**

# Acceptance of Supervisors

**1. Assoc. Prof. Vu Duc Loi,** Vietnam University of Traditional Medicine

I approve all the modifications and corrections made in the revised version of the thesis.

**Assoc. Prof. Vu Duc Loi**

**2. Assoc. Prof. Nguyen Thi Van Anh,** University of Science and Technology of Hanoi, Vietnam Academy of Science and Technology

I approve all the modifications and corrections made in the revised version of the thesis.

**Assoc. Prof. Nguyen Thi Van Anh**

# Acceptance of the Chairman of the Examination Jury

**Assoc. Prof. Nguyen Hai Dang,** University of Science and Technology of Hanoi, Vietnam Academy of Science and Technology

I approve all the modifications and corrections made in the revised version of the thesis.

**Assoc. Prof. Nguyen Hai Dang**

# Declaration

I hereby certify that the thesis entitled “Chemical constituents and hypoglycemic effects of *Commelina diffusa* Burm.f.” is the result of my own research conducted under the scientific guidance of the group of supervisors. All information and data obtained from other sources have been properly cited and acknowledged. The research findings presented in this thesis have been published jointly with co-authors, with their full consent for inclusion in this work. All data and results reported herein are truthful and have not been previously published in any other form, except in the author’s own published works. This thesis was completed during my time as a PhD student at the University of Science and Technology of Hanoi, Vietnam Academy of Science and Technology.

**PhD student**

**Nguyen Xuan Tung**

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**Nguyen Xuan Tung**

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# List of Abbreviations

| | |
|---|---|
| **AA** | α-amylase |
| **ADMET** | Absorption, distribution, metabolism, excretion, and toxicity |
| **AG** | α-glucosidase |
| **ALT** | Alanine aminotransferase |
| **AMP** | Adenosine monophosphate |
| **AST** | Aspartate transaminase |
| **ATP** | Adenosine triphosphate |
| **CC** | Column chromatography |
| **CD.EAF** | Ethyl acetate fraction of *Commelina diffusa* Burm.f. |
| **CNS** | Central nervous system |
| **DEPT** | Detorsionless enhancement by polarization transfer |
| **ESI-MS** | Electrospray ionization – Mass spectrometry |
| **EtOAc** | Ethyl acetate |
| **GC-MS** | Gas chromatography – Mass spectrometry |
| **GIP** | Glucose-dependent insulinotropic polypeptide |
| **GLP-1** | Glucagon-like peptide-1 |
| **HbA1c** | Haemoglobin A1c |
| **HE** | Hematoxylin & Eosin |
| **HFD** | High-fat diet |
| **HDL-C** | High-density lipoprotein cholesterol |
| **HMBC** | Heteronuclear multiple bond correlation spectroscopy |
| **HSQC** | Heteronuclear single quantum coherence spectroscopy |

| | |
|---|---|
| **HPLC** | High-performance liquid chromatography |
| **$IC_{50}$** | Half-maximal inhibitory concentration |
| **IDF** | International Diabetes Federation |
| **IRS** | Insulin receptor substrate |
| **$LD_{50}$** | The median lethal dose |
| **LDL-C** | Low-density lipoprotein cholesterol |
| **MeOH** | Methanol |
| **MIC** | Minimum inhibitory concentration |
| **NFD** | Normal-fat diet |
| **NMR** | Nuclear magnetic resonance |
| **NSAIDs** | Non-steroidal anti-inflammatory drugs |
| **OECD** | Organization for Economic Cooperation and Development |
| **PCs** | Pure compounds |
| **PDB** | Protein Data Bank |
| **PPAR** | Peroxisome proliferator-activated receptors |
| **Rf** | Retention factor |
| **RMSD** | Root mean square deviation |
| **SGLT2** | Sodium-glucose co-transporter-2 |
| **STZ** | Streptozotocin |
| **TC** | Total cholesterol |
| **TG** | Triglyceride |
| **TLC** | Thin-layer chromatography |
| **UV** | Ultraviolet |
| **W** | Water |

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# Abstract

Diabetes mellitus has increased rapidly in recent decades and has become one of the most pressing public health problems globally, with type 2 diabetes comprising nearly all diagnosed cases. It has been proven that α-amylase and α-glucosidase are two enzymes involved in carbohydrate metabolism that contribute to postprandial hyperglycemia in patients with type 2 diabetes. Therefore, inhibition of these enzymes is an established therapeutic strategy for reducing glucose absorption and controlling blood glucose levels.

*Commelina diffusa* (*C. diffusa*) Burm.f. is a species belonging to the *Commelina* genus, which contains several medicinal plants that have been proven to exhibit antihyperglycemic activity, such as *C. communis* and *C. benghalensis*. Traditionally, *C. diffusa* has been used in Vietnamese medicine to treat respiratory disorders, hypertension, dysentery, and urinary infections. Modern pharmacology studies have confirmed its anti-inflammatory, antioxidant, antimicrobial, nephroprotective, hepatoprotective, diuretic, and CNS-depressant activities. Furthermore, phytochemical research has revealed that this plant contains phenolics, flavonoids, alkaloids, tannins, steroids, and vitamins. However, the scientific evidence supporting its hypoglycemic potential and the identification of its bioactive constituents remains scarce. On that basis, this thesis aimed to (1) extract, isolate, and determine the chemical structures of compounds from the extract fractions of *C. diffusa*, (2) evaluate *in vitro* the α-glucosidase and α-amylase inhibitory activities of the extract fractions and isolated compounds, (3) assess the acute toxicity and *in vivo* hypoglycemic effect of the most active fraction, and (4) study the α-glucosidase and α-amylase inhibitory effects of the isolated compounds using the molecular docking method.

In this study, the powdered plant material was extracted with methanol and subsequently fractionated using *n*-hexane, ethyl acetate, and distilled water. The pure compounds were isolated by thin-layer chromatography and column chromatography methods. Their structures were elucidated using spectroscopic techniques and compared

to the literature. The *in vitro* hypoglycemic effect of the fractions and their compounds was evaluated by their capacity to inhibit the α-glucosidase and α-amylase enzymes. The acute toxicity experiment was determined through the median lethal dose ($LD_{50}$) values, while the *in vivo* assay was conducted on the high-fat diet and streptozotocin-induced mouse model. Molecular docking studies were conducted using MOE software. The drug-likeness properties of the isolated compounds were assessed based on Lipinski's Rules of Five, while their pharmacokinetic parameters were predicted via the pkCSM online tool.

The obtained results showed that 14 compounds were isolated from the fractions of *C. diffusa*, including corosolic acid (**1**), 3*α*-acetyl-20*S*,24*S*-epoxydammarane-25-ol (**2**), 11-oxo-α-amyrin acetate (**3**), stigmasterol (**4**), lyratol F (**5**), 4-hydroxybenzoic acid (**6**), methyl gallate (**7**), *N-trans*-feruloyltyramine (**8**), *N-trans-p*-coumaroyl-3',4'-dihydroxyphenylethylamine (**9**), 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**), daucosterol (**11**), quercitrin (**12**), benzyl-O-β-D-glucopyranoside (**13**), and isoschaftoside (**14**). To the best of our knowledge, compounds **1–3**, **5**, **8–10**, and **13** were isolated for the first time from *C. diffusa* and had not previously been reported from the genus *Commelina*. Compounds **7**, **11**, **12**, and **14** were also reported for the first time from *C. diffusa*. These findings expand the known chemical profile of the species and provide additional chemical constituents for investigating its potential antihyperglycemic activity.

Among the fractions, the ethyl acetate fraction (CD.EAF) exhibited the strongest inhibitory effects against both α-glucosidase and α-amylase enzymes. Consequently, it was subjected to further investigation to assess its acute toxicity and antihyperglycemic efficacy in an *in vivo* model. In addition, the isolated compounds performed concentration-dependent inhibitory effects on both tested enzymes, with compounds **9**, **10**, and **14** showing the most potent effects.

Regarding the acute toxicity test, the CD.EAF was relatively safe, with an $LD_{50}$ value greater than 2000 mg/kg. In the *in vivo* experimental assay, after 14 days of treatment, the repeated daily administration of the CD.EAF at both doses of 100 and

300 mg/kg/day showed significant antihyperglycemic activities as compared to the diabetic control group ($p < 0.05$). Although no significant changes were observed in lipid parameters, histopathological examination revealed notable protective effects of this fraction on liver and pancreatic tissues.

The docking analysis presented that compounds **10** and **14** exhibited the strongest binding affinities toward both target enzymes, as reflected by their lower docking scores compared with the positive control, acarbose. These compounds were predicted to form stable interactions with key amino acid residues within the active sites of the investigated enzymes. Furthermore, the isolated compounds from *C. diffusa* were evaluated for their physicochemical characteristics and ADMET properties using *in silico* approaches.

In conclusion, our findings have contributed to providing information and enriching knowledge about the chemical composition of *C. diffusa* and its antihyperglycemic effects. From there, it opens up further research directions to find the main active components that can be applied in drug development, orienting the application in the prevention and treatment of type diabetes.

# Tóm tắt

Đái tháo đường đã gia tăng nhanh chóng trong những thập kỷ gần đây và trở thành một trong những vấn đề sức khỏe cộng đồng cấp bách nhất trên toàn cầu; trong đó, đái tháo đường týp 2 chiếm gần như tất cả các trường hợp được chẩn đoán. Nhiều nghiên cứu đã chứng minh rằng α-amylase và α-glucosidase là hai enzym tham gia vào quá trình chuyển hóa cacbohydrat, góp phần gây tăng đường huyết sau ăn ở bệnh nhân đái tháo đường týp 2. Do đó, việc ức chế các enzym này là một chiến lược điều trị đã được chứng minh để giảm hấp thu glucose và kiểm soát lượng đường trong máu.

Thài lài trắng (*Commelina diffusa* (*C. diffusa*) Burm.f.) là một loài thuộc chi *Commelina*, bao gồm một số dược liệu đã được chứng minh là có hoạt tính chống đái tháo đường, chẳng hạn như *C. communis* và *C. benghalensis*. Theo y học cổ truyền Việt Nam, thài lài trắng đã được sử dụng để điều trị nhiều bệnh khác nhau, chẳng hạn như rối loạn hô hấp, tăng huyết áp, kiết lỵ và nhiễm trùng tiết niệu. Các nghiên cứu dược lý hiện đại đã xác nhận các tác dụng chống viêm, chống oxy hóa, kháng khuẩn, bảo vệ thận, bảo vệ gan, lợi tiểu và ức chế thần kinh trung ương của *C. diffusa*. Bên cạnh đó, nghiên cứu hóa thực vật đã tiết lộ rằng dược liệu này chứa các hợp chất phenolic, flavonoid, alkaloid, tannin, steroid và vitamin. Tuy nhiên, bằng chứng khoa học hỗ trợ tác dụng hạ đường huyết và xác định các thành phần hoạt tính sinh học của loài cây này vẫn còn khan hiếm. Trên cơ sở đó, luận án này được tiến hành nhằm mục đích (1) chiết xuất, phân lập và xác định cấu trúc hóa học của các hợp chất từ các phân đoạn dịch chiết của thài lài trắng, (2) đánh giá hoạt tính ức chế α-glucosidase và α-amylase *in vitro* của các phân đoạn dịch chiết và các hợp chất phân lập được, (3) đánh giá độc tính cấp và tác dụng hạ đường huyết của phân đoạn có hoạt tính ức chế enzym tốt nhất trên mô hình *in vivo* và (4) nghiên cứu tác dụng ức chế α-glucosidase và α-amylase của các hợp chất phân lập được bằng phương pháp docking phân tử.

Trong nghiên cứu này, bột cây thài lài trắng được chiết xuất bằng methanol và sau đó được chiết phân đoạn với *n*-hexan, etyl axetat và nước cất. Các hợp chất tinh khiết

được phân lập bằng phương pháp sắc ký lớp mỏng và sắc ký cột. Cấu trúc của chúng được làm sáng tỏ bằng các kỹ thuật quang phổ và được so sánh với tài liệu tham khảo. Tác dụng chống đái tháo đường *in vitro* của các phân đoạn và hợp chất của chúng được đánh giá bằng khả năng ức chế các enzym α-glucosidase và α-amylase. Độc tính cấp được xác định thông qua giá trị liều gây chết trung bình ($LD_{50}$), trong khi thử nghiệm *in vivo* được tiến hành trên mô hình chuột mắc đái tháo đường gây ra bởi streptozotocin và chế độ ăn nhiều chất béo. Các nghiên cứu docking phân tử được thực hiện bằng phần mềm MOE. Tính chất giống thuốc của các hợp chất được phân lập được đánh giá dựa trên Quy tắc Năm của Lipinski, trong khi các thông số dược động học của chúng được dự đoán thông qua công cụ trực tuyến pkCSM.

Kết quả thu được cho thấy đã phân lập được 14 hợp chất từ các phân đoạn dịch chiết của *C. diffusa*, bao gồm axit corosolic (**1**), 3α-acetyl-20S,24S-epoxydammaran-25-ol (**2**), 11-oxo-α-amyrin axetat (**3**), stigmasterol (**4**), lyratol F (**5**), axit 4-hydroxybenzoic (**6**), methyl gallat (**7**), *N*-trans-feruloyltyramin (**8**), *N*-trans-*p*-coumaroyl-3',4'-dihydroxyphenylethylamin (**9**), 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-N1,N2-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalen dicarboxamid (**10**), daucosterol (**11**), quercitrin (**12**), benzyl-O-β-D-glucopyranosid (**13**) và isoschaftosid (**14**). Trong số các chất này, các hợp chất **1-3**, **5**, **8-10** và **13** lần đầu tiên được phân lập từ *C.diffusa* và chưa từng được báo cáo từ chi *Commelina*. Đồng thời, đây là nghiên cứu đầu tiên tách chiết và phân lập được các hợp chất **7**, **11**, **12** và **14** từ *C. diffusa*. Những kết quả này đã mở rộng cơ sở dữ liệu về thành phần hóa học của loài cây này và cung cấp thêm các thông tin để nghiên cứu về hoạt tính hạ đường huyết tiềm năng của nó.

Trong số các phân đoạn thử nghiệm, phân đoạn ethyl acetate (CD.EAF) thể hiện tác dụng ức chế mạnh nhất đối với cả hai enzyme α-glucosidase và α-amylase. Do đó, phân đoạn này đã được lựa chọn để nghiên cứu sâu hơn về độc tính cấp và hiệu quả hạ đường huyết trên mô hình *in vivo*. Ngoài ra, các hợp chất được phân lập cho thấy tác dụng ức chế phụ thuộc vào nồng độ đối với cả hai enzyme được thử nghiệm, trong đó các hợp chất **9**, **10** và **14** thể hiện tác dụng mạnh nhất.

Về thử nghiệm độc tính cấp tính, CD.EAF tương đối an toàn với giá trị $LD_{50}$ thu được lớn hơn 2000 mg/kg. Trong nghiên cứu *in vivo*, sau 14 ngày điều trị, việc sử dụng lặp lại CD.EAF hàng ngày ở cả hai liều 100 và 300 mg/kg/ngày đều cho thấy hoạt tính chống tăng đường huyết đáng kể so với nhóm đối chứng mắc bệnh tiểu đường ($p < 0,05$). Mặc dù không quan sát thấy sự thay đổi đáng kể nào về các thông số lipid, mô bệnh học của gan và tụy ở chuột được điều trị bằng CD.EAF có sự cải thiện đáng kể.

Phân tích docking cho thấy hợp chất **10** và **14** thể hiện ái lực liên kết mạnh nhất với cả hai enzym đích, thể hiện qua điểm ghép nối thấp hơn của chúng so với chứng dương acarbose. Các hợp chất này được dự đoán sẽ tạo thành tương tác ổn định với các gốc axit amin quan trọng trong các vị trí hoạt động của các enzym được nghiên cứu. Hơn nữa, các hợp chất phân lập từ *C. diffusa* cũng đã được đánh giá về các đặc điểm lý hóa và tính chất ADMET bằng phương pháp *in silico*.

Tóm lại, những phát hiện của chúng tôi trong luận án này đã góp phần cung cấp thông tin và làm phong phú thêm kiến thức về thành phần hóa học của cây thài lài trắng và tác dụng chống đái tháo đường của nó. Từ đó, mở ra những hướng nghiên cứu tiếp theo nhằm tìm ra các thành phần hoạt chất chính có thể được ứng dụng để phát triển thuốc, định hướng phòng ngừa và điều trị bệnh đái tháo đường týp 2.

# Chapter I – Introduction

## 1.1. Definition, classification, and prevalence of diabetes mellitus

### 1.1.1. Definition and classification of diabetes mellitus

According to the International Diabetes Federation (IDF), diabetes mellitus, simply called diabetes, is defined as a serious, chronic condition that occurs when blood glucose levels increase because the hormone insulin cannot be produced enough in the body or cannot be used effectively [1]. Diabetes can be diagnosed based on one or more following criteria, including (a) a fasting plasma glucose level equal or greater than 7.0 mmol/L (≥ 126 mg/dL), or (b) a two-hour plasma glucose value during oral glucose tolerance test (using a 75 g oral glucose load) equal or greater than 11.1 mmol/L (≥ 200 mg/dL), or (c) an HbA1C level equal or greater than 6.5% (48 mmol/mol), or (d) a random plasma glucose value in patients having classic symptoms of hyperglycemia or hyperglycemia crisis equal or greater than 11.1 mmol/L (≥ 200 mg/dL) [1,2].

Diabetes can be classified into four major categories:

- *Type 1 diabetes:*

Type 1 diabetes can appear at any age; however, this type of diabetes is mainly found in children and young adults. The pathogenesis mechanism of type 1 diabetes is identified by an autoimmune process leading to the destruction of β cells in the pancreas, which prevents the body from producing insulin. The combination of genetic sensitivity and environmental factors is considered the main cause of this damage process. Patients with type 1 diabetes require daily insulin treatment to maintain the serum glucose concentration in an appropriate range. The classic symptoms of type 1 diabetes include polydipsia, polyuria, fatigue, sudden weight loss, constant hunger, blurred vision, and diabetic ketoacidosis [1,2].

- *Type 2 diabetes:*

Type 2 diabetes, which was previously known as non-insulin-dependent diabetes or adult-onset diabetes, is responsible for 90 – 95% of all diabetes cases. Along with the increase in inappropriate food consumption and little or no physical activity in children, type 2 diabetes is increasing in children, becoming a serious public health problem [1]. The pathophysiology of type 2 diabetes mellitus is primarily characterized by a progressive decline in insulin-secreting β-cell function of the pancreas and insulin resistance [3]. Due to insulin resistance, in the early stages, β-cells compensate and increase insulin secretion into the blood. If insulin resistance persists or worsens, β-cells will not secrete enough insulin, and clinical type 2 diabetes will appear. Thus, the early symptoms of β-cell dysfunction frequently go unnoticed, and hyperglycemia develops gradually for many years before a diagnosis of this diabetes form is made [2].

- *Gestational diabetes:*

Gestational diabetes accounts for 75 – 90% of all cases of hyperglycemia in pregnancy. This syndrome may appear at any time during the prenatal period without occurrence in the postpartum period. Because of the similarity between typical symptoms of gestational diabetes and normal pregnancy symptoms, it is recommended to examine the oral glucose tolerance test for screening gestational diabetes between 24 and 28 weeks of pregnancy or earlier in high-risk women. Older age, obesity, diabetes anamnesis, overweight during pregnancy, smoking, polycystic ovary syndrome, and a history of miscarriage or giving birth to a congenitally abnormal infant are risk factors of gestational diabetes. Pregnant women with gestational diabetes can sustain their blood glucose values by controlling their weight, doing moderate exercise, eating a healthy diet, and monitoring serum glucose frequently [1,2].

- *Specific types of diabetes:*

According to the World Health Organization (WHO) report, other specific types of diabetes mellitus can be classified as shown in Table 1.1.

**Table 1.1.** Specific types of diabetes mellitus [4]

| Types of Diabetes | Causes |
|---|---|
| Monogenic diabetes | Monogenic causes β-cell dysfunction with clinical features including transient neonatal diabetes, permanent neonatal diabetes, maturity-onset diabetes of the young, and genetic syndromes associated with insulin-deficient diabetes. Besides, monogenic causes defects in insulin resistance with typical manifestations, such as polycystic ovarian disease, hyperinsulinemia, virilization, and acanthosis nigricans. |
| Pancreatic diabetes | Diseases of the pancreas, including infection, trauma, pancreatectomy, pancreatitis, and pancreatic cancer. |
| Endocrine disorders | Disorders associated with excess secretion of hormones (e.g., cortisol, epinephrine, glucagon, and growth hormone) that antagonize insulin action (e.g., Cushing's syndrome, phaeochromocytoma, acromegaly, and glucagonoma). |
| Drug- and chemical-induced diabetes | Drugs or chemicals damage insulin action or pancreatic β-cells (e.g., Glucocorticoids, thyroid hormone, thiazides, alpha-adrenergic agonists, beta-adrenergic agonists, dilantin, pentamidine, nicotinic acid, pyrinuron, interferon-alpha, etc). |
| Infection-related diabetes | Viral infections associated with β-cell destruction, such as congenital rubella, coxsackie B, cytomegalovirus, adenovirus, and mumps. |

| | |
|---|---|
| Uncommon specific forms of immune-mediated diabetes | Insulin receptor antibodies, stiff person syndrome, and immunological diseases other than those that lead to type 1 diabetes. |
| Other genetic syndromes sometimes associated with diabetes | Alstrom syndrome, Prader-Willi syndrome, Bardet-Biedl syndrome, Turner's syndrome, Down's syndrome, Klinefelter's syndrome, Huntington's chorea, Friedreich's ataxia, and myotonic dystrophy. |

### 1.1.2. Prevalence of diabetes mellitus

Diabetes mellitus has increased significantly over the last few decades, making it one of the most imperative public health issues worldwide. The IDF reported that in 2021, approximately 537 million adults (20 – 79 years old) were living with diabetes, a number predicted to rise to 783 million by 2045. This sharp increase is largely attributed to the rise in type 2 diabetes, which is closely associated with lifestyle factors such as obesity and physical inactivity. Type 1 diabetes, although less common, has also seen an increase in incidence, particularly in younger populations [1].

The epidemiology of diabetes shows significant variation across nations and regions, caused by differences in genetics, lifestyle, socioeconomic factors, and healthcare infrastructure. Globally, the Middle East and North Africa regions, which had the highest prevalence (~16.2%), were heavily affected owing to genetic predisposition, high obesity rates, and dietary patterns. Southeast Asia (11.3%) and the Western Pacific (11.9%) also reported substantial burdens, with India (~77 million cases) and China (~140 million cases) leading due to rapid urbanization, aging populations, and lifestyle changes. North America and the Caribbean (14.5%) reported high prevalence, especially in the United States, where over 37 million adults were affected, largely influenced by obesity and sedentary behavior. In Europe (9.2%), aging populations in countries like Germany and Italy contributed to a stable yet high burden. In comparison, Africa (6.7%) had the lowest prevalence but was experiencing the fastest growth due to urbanization

and limited healthcare access. Additionally, undiagnosed diabetes remained a global challenge, with Africa (~55%) and Asia (~50%) having the highest proportions of unrecognized cases. Urban areas showed a higher prevalence than rural areas because of lifestyle differences, and certain ethnic groups, including South Asians, Pacific Islanders, and African Americans, were at higher risk owing to genetic susceptibility [5,6].

Diabetes is not only a significant cause of morbidity and mortality globally, but it is also a major economic burden because of its complications, which include cardiovascular diseases, kidney failure, blindness, and amputations. The global cost of diabetes, including direct medical expenses and indirect costs such as lost productivity, exceeded $966 billion in 2021, representing a 316% increase over the past 15 years. Direct medical costs include hospitalizations, medications, diagnostic tests, and diabetes-related complications such as cardiovascular disease and kidney failure. Indirect costs arise from reduced workforce participation, absence, early mortality, and caregiving responsibilities. The economic impact is particularly severe in low- and middle-income countries, where healthcare systems are less equipped to manage the disease, leading to higher out-of-pocket expenses and catastrophic financial consequences for families. In high-income countries, diabetes accounts for a substantial share of healthcare spending, often surpassing 10% of total expenditures. Complications of diabetes significantly increase the costs, with patients experiencing complications requiring up to three times the medical resources of those without complications. Beyond financial costs, diabetes affects economic development by reducing the quality of life and lifespan of working-age populations [1,2,7].

In Vietnam, diabetes is a growing public health concern, reflecting the rising trend of this disease globally. According to the IDF, the number of adults aged 20 – 79 with diabetes rose from approximately 1.7 million in 2000 to about 5 million in 2021, with projections suggesting this could reach over 6 million by 2045 [1]. In 2015, the WHO reported a 4.1% prevalence of diabetes among adults aged 18 – 69, with only 28.9% of these individuals receiving management [8]. A systematic review and meta-analysis

study reported that the prevalence rate of type 2 diabetes in Vietnam increased from 3% during 2000 – 2004 to 9% in 2016 – 2020, and the incidence of type 2 diabetes was higher in males than in females [9]. Additionally, in a study conducted in 2023, Vu Thi Hoang Lan and her colleagues found that 7.9% of men and 6.1% of women had diabetes among respondents aged 25 – 64, with a higher prevalence in urban areas than in rural regions [10]. Diabetes imposes a substantial economic burden on Vietnam's healthcare system and society. In 2017, the total direct medical costs for type 2 diabetes mellitus were estimated at USD 435 million, including hospitalizations (24%), outpatient care (20%), emergency care (7%), non-diabetes-related medications (36%), and antihyperglycemic medications (13%). Notably, approximately 40% of these costs were attributed to diabetes-related complications, reflecting the financial impact of inadequate preventative care [11]. Besides, the combined direct non-medical and indirect costs of diabetes were estimated at USD 239 million, of which USD 78 million were spent on direct non-medical costs and USD 161 million on indirect costs, with presenteeism, absenteeism, and premature mortality accounting for 73%, 17%, and 10% of these costs, respectively [12]. These findings highlighted the growing burden of diabetes in Vietnam, underscoring the need for enhanced public health strategies to resolve this escalating health concern.

## 1.2. Pathogenesis mechanism and pharmacological treatment of type 2 diabetes

### 1.2.1. Pathogenesis mechanism of type 2 diabetes

Type 2 diabetes is associated with relative insulin deficiency due to insulin secretion disorders and insulin resistance [1,2]. It is proven that insulin secretory dysfunction might lead to the development of insulin resistance, and both occur before the appearance of clinical manifestations of diabetes (pre-diabetes stage) [3]. Patients with type 2 diabetes who are not overweight mainly show signs of decreased insulin. On the contrary, in patients with obesity, insulin resistance is the main problem [13].

***Insulin secretion disorders***

In patients with insulin resistance, in the early stages, the pancreatic islets increase insulin secretion, or the weight of the pancreas increases to compensate for insulin resistance. In the case of patients without insulin resistance, insulin secretion or pancreatic weight may be reduced. However, in the later stages, if serum glucose concentrations continue to increase, the ability of the insulin secretory response to glucose is further reduced [14]. Type 2 diabetes only has clinical manifestations when β-cells of the pancreas are damaged and are unable to secrete insulin to bring blood glucose levels to the normal range [2].

The causes of insulin secretion disorders might be neurological factors, abnormal biochemical processes in the body, genetic features, or hormones [1,2]. Besides, the impairment of pancreatic β-cell function in type 2 diabetes is also due to pancreatic β-cell mass reduction, pancreatic β-cell exhaustion, toxicity due to increased glucose, toxicity due to increased lipids, amyloid accumulation, and insulin secretion reduction due to reduced secretion of glucagon-like peptide-1 (GLP-1) or reduced insulin-stimulating action of glucose-dependent insulinotropic polypeptide (GIP) [15].

***Insulin resistance***

Insulin resistance is a condition of reduced responsiveness of target organs or tissues to normal circulating insulin levels. This syndrome is considered the primary defect or main defect of type 2 diabetes. In addition, insulin resistance is also an indirect cause leading to impaired insulin secretion function of pancreatic β-cells [16].

At the cellular level, the occurrence of insulin resistance can be due to pre-receptor, receptor, or post-receptor derangements. Pre-receptor defects in insulin action, such as insulin abnormalities or anti-insulin antibodies, appear rarely and do not generate significant clinical features. Furthermore, the binding ability of insulin to its receptors rarely changes. In type 2 diabetes, insulin resistance is mainly due to abnormalities in the intracellular insulin signaling pathway. In particular, the inactivation

of the serine/threonine protein kinase B (PKB, also known as Akt) and the activation of forkhead box 01 (FOXO1), along with the repression of insulin receptor substrate-1 (IRIS-1) and IRIS-2, are fundamental mechanisms of impairing insulin action [16,17].

The main causes leading to increased expression of factors causing insulin resistance are genetic factors and acquired factors, of which being overweight and obesity are the most important causes. Several studies have also mentioned other causes, including physical inactivity, increased glucagon, or central nervous system disorders [18].

### 1.2.2. Pharmacological agents for type 2 diabetes treatment

Type 2 diabetes can not be entirely cured. The main goal of type 2 diabetes treatment and management is good glycemic control, achieved by promoting lifestyle changes, such as eating a healthy diet, regularly exercising, maintaining a healthy body weight, and quitting smoking. Drug therapy is usually initiated if lifestyle modifications are insufficient to maintain serum glucose concentrations. In diabetic patients with long-term complications, drug therapy often requires several pharmacological agents with different mechanisms of action. Currently, antidiabetic medication can be divided into oral agents and injected agents [19-21].

#### 1.2.2.1. Oral agents

##### *Metformin*

Metformin is considered the first-line medicine for type 2 diabetes treatment [1]. In the liver, it can prevent gluconeogenesis through four mechanisms of action, including (1) Activating AMP-activated protein-kinase (AMPK) in hepatocytes and reducing energy charge; (2) Blocking adenyl cyclase to inhibit the production of glucagon-induced cAMP; (3) Decreasing ATP levels and increasing the ratio of AMP/ATP through the prevention of NADH coenzyme Q oxidoreductase in the mitochondrial electron transport chain; and (4) Inhibiting mitochondrial glycerol phosphate dehydrogenase (mG3PDH) and affecting the transportation of NADH from the cytoplasm into the

mitochondrion, which leads to the suppression of gluconeogenesis procedure from lactate. Metformin can reduce fasting serum glucose by about 20% and HbA1c index by approximately 1.5%. However, this antihyperglycemic medication has several adverse effects, such as nausea, diarrhea, anorexia, abdominal discomfort, and lactic acidosis. Besides, patients having long-term treatment with metformin might be associated with a reduction of vitamin B12 absorption in the intestine [22,23].

***Insulin secretagogues (Sulfonylureas and meglitinides)***

Although sulfonylureas and meglitinides are two different classes of oral antidiabetic agents and have different receptor binding sites, their mechanisms of action are similar [22]. They can promote insulin secretion through the closure induction of ATP-sensitive potassium channels located in the membrane of the pancreatic β-cells and cell depolarization, which leads to an increase in calcium levels in the cytoplasm [24]. Sulfonylureas and meglitinides exhibit effective impacts on reducing glucose levels when used as monotherapy or in combination with other oral hypoglycemic agents or insulin. Generally, the glucose-lowering effectiveness of sulfonylureas is higher than that of meglitinides. The most frequent side effects of these drugs are hypoglycemia, weight gain, and loss of efficacy [22,25].

***Alpha-glucosidase inhibitors***

Many studies have demonstrated that postprandial hyperglycemia is an important factor contributing to the development of type 2 diabetes. Therefore, controlling postprandial hyperglycemia is one of the main strategies for treating this disease. α-Glucosidase is an important carbohydrate metabolic enzyme involved in postprandial hyperglycemia in patients with type 2 diabetes. This enzyme is responsible for hydrolyzing α-1,4-glycoside residues at the non-reducing end of carbohydrates to release monosaccharides that can be absorbed into the blood. Therefore, inhibiting the α-glucosidase enzyme can reduce carbohydrate hydrolysis and slow down the absorption of glucose into the blood [26]. α-glucosidase inhibitors have three available agents: acarbose, miglitol, and voglibose [25]. Their inhibitory effect on the α-glucosidase

enzyme is due to their similar structures to natural oligosaccharides and their higher affinity for α-glucosidase. α-glucosidase inhibitors do not improve insulin secretion and affect body weight [27]. However, diarrhea, abdominal pain, and flatulence are the main adverse effects of these drugs. Apart from reducing blood glucose concentrations, acarbose can also decrease the risk of cardiovascular diseases and reduce the development of diabetes in patients with impaired glucose tolerance [28].

***Thiazolidinediones***

Thiazolidinediones act on the liver, adipose tissue, and muscle to enhance glucose utilization and reduce glucose production, causing an increase in insulin sensitivity. Peroxisome proliferator-activated receptor (PPAR) gamma, which is commonly found in adipose tissue, macrophages, pancreatic β-cells, vascular endothelium, and the central nervous system, has been proven to increase its concentrations in the skeletal muscle of patients with obesity and diabetes [29]. Meanwhile, PPAR alpha is commonly found in vascular walls, skeletal muscle, liver, and heart. Thiazolidinediones can bind to PPARs and express their effectiveness in improving lipid metabolism. In addition, they also provide the preservation of the function of the pancreatic β-cells. Although they have similar efficiency to metformin in monotherapy, physicians do not usually choose them because of their cost and side effects. In there, weight gain, heart failure, bone density reduction, and fracture risk development are the main problems related to the usage of these agents. As a result, thiazolidinediones are contraindicated in diabetic patients having a history of low bone mass or heart failure [22].

***Dipeptidyl peptidase-4 inhibitors***

Dipeptidyl peptidase-4 (DPP4) is a glycoprotein that can selectively cleave the N-terminal dipeptides from neuropeptides, growth factors, cytokines, and incretin hormones and inactivate these substrates [30]. The incretin hormones, including glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), are produced by intestinal L cells. Their roles are inhibiting glucagon in response to nutrient inputs and increasing insulin secretion. Therefore, DPP4 inhibitors can enhance islet

function and glycemic control through the increase of the active concentrations of incretin agents. DPP4 inhibitors are assigned as monotherapy in patients with inadequate diet and exercise management or given in combination with metformin, thiazolidinediones, and insulin. There are five commercially available DPP4 inhibitors, including Saxagliptin, Linagliptin, Vildagliptin, Alogliptin, and Sitagliptin. DPP4 inhibitors are well tolerated and considered very safe, with rare adverse effects [22]. Several studies have reported that hepatic dysfunction, acute pancreatitis, upper respiratory tract infection, headache, and nasopharyngitis are side effects of these drugs [31,32].

***Sodium-glucose co-transporter-2 inhibitors***

Sodium-glucose co-transporter-2 (SGLT2), detected only in the proximal tubule, is responsible for approximately 90% of glucose reabsorption. Meanwhile, the remaining glucose is reabsorbed by SGLT1, which is found mainly in the kidney proximal convoluted tubule and the small intestine [33]. Consequently, SGLT2 inhibitors can reduce hyperglycemia due to the inhibition of the SGLT2 transporter, leading to the reduction of glucose reabsorption and the enhancement of its excretion in the urine. Because of the increase in diuresis and glucosuria, body weight and blood pressure are decreased. SGLT2 inhibitors have an insulin-independent mode of action, so they do not produce fatigue and overstimulate the pancreatic β-cells. Besides, their mechanism depends on the function of the glomerular tubular; hence, their effectiveness in patients with renal failure is reduced [34]. Currently, clinical trials have revealed several adverse events of SGLT2 inhibitors, such as urogenital tract infections, orthostatic hypotension, volume depletion, and euglycemic ketoacidosis [22].

**1.2.2.2. Injecting agents**

***Insulin***

Human insulin is a non-glycosylated peptide hormone composed of 51 amino acids and arranged in two polypeptide chains, including the A chain (21 amino acids)

and the B chain (30 amino acids). Insulin is synthesized in β-cells of the pancreas in the form of preproinsulin, which is a precursor molecule containing 86 amino acids. The N-terminal signal peptide of this molecule is cleaved by proteolytic enzymes to obtain proinsulin. The two polypeptide chains of insulin are created due to the cleavage of proinsulin, and the disulfide bonds between chains A and B form the final structure of insulin [35].

Insulin is used to treat all types of diabetes. It can be administered by any parental route, including intravenous, intramuscular, or subcutaneous. The currently available insulins are divided into five main categories: rapid-acting, short-acting, intermediate-acting, long-acting, and insulin mixtures. Their onset of action, peak action, and duration of action are presented in Table 1.2. Hypoglycemia is considered the major side effect of insulin therapy, along with weight gain, local reactions, mitogenic properties, and cardiovascular disease [36].

**Table 1.2.** Types of insulin and their pharmacodynamics [36]

| **Category/ Name of insulin** | **Onset of action (Hours)** | **Peak effect (Hours)** | **Duration of action (Hours)** |
|---|---|---|---|
| **Rapid-acting** | | | |
| Insulin Lispro | 5 – 15 min | 0.5 – 1.5 | 3 – 5 |
| Insulin Aspart | 5 – 15 min | 0.5 – 1.5 | 3 – 5 |
| Insulin Glulisine | 5 – 15 min | 0.5 – 1.5 | 3 – 5 |
| **Short-acting** | | | |
| Regular human | 0.5 – 1 | 2 – 3 | 5 – 8 |
| Actrapid® | ~ 0.5 | 1 – 3 | ~ 8 |

| | | | |
|---|---|---|---|
| **Intermediate-acting** | | | |
| NPH human | 2 – 4 | 4 – 10 | 10 – 16 |
| Insulin Lente | 3 – 4 | 4 – 12 | 12 – 18 |
| Insulatard® HM | 1 – 1.5 | 4 – 12 | ~ 24 |
| Insulatard® FlsxPen | 1 – 1.5 | 4 – 12 | ~ 24 |
| **Long-acting** | | | |
| Insulin Ultralente | 6 – 10 | 10 – 16 | 18 – 24 |
| Insulin Glargine | 2 – 4 | Flat | 20 – 24 |
| Insulin Detemir | 2 – 4 | 6 – 14 | 16 – 20 |
| **Insulin mixtures** | | | |
| Aspart Mix 70/30 | 0.1 – 0.2 | 1 – 4 | 18 – 24 |
| Mixtard® HM 70/30 | ~ 0.5 | 2 – 8 | ~ 24 |
| Mixtard® 30 FlexPen | ~ 0.5 | 2 – 8 | ~ 24 |
| Novomix® 30 FlexPen | 10 – 20 min | 1 – 4 | ~ 24 |

***GLP-1 receptor agonists***

GLP-1 is a gut-derived incretin hormone, secreted after nutrient intake to improve glucose-dependent insulin secretion. It is clarified that GLP-1 receptor agonists can reduce plasma glucose levels due to three modes of action: (1) Stimulating insulin secretory response and reducing inappropriate glucagon secretion in a glucose-dependent manner, (2) Slowing gastric emptying, reducing body weight and lipid mass

by increasing satiety and decreasing food intake, and (3) Preventing the progression and inflammation of aortic atherosclerotic plaques. GLP-1 receptor agonists are utilized as monotherapy or in combination with other oral antidiabetic agents or insulin. They have a low risk of hypoglycemia when used alone. Besides, their effects on decreasing HbA1c levels, fasting blood glucose and postprandial blood glucose concentrations, body weight, systolic blood pressure, and improving β-cell function have been proven. Thus, these agents are recommended as the first choice in diabetic patients who are at risk of cardiovascular disease. In addition to therapeutic effects, GLP-1 receptor agonists might cause nausea, vomiting, diarrhea, acute pancreatitis, local irritation, abscess, cellulitis, and local subcutaneous nodule formation. Furthermore, diabetic patients with a personal or family history of multiple endocrine neoplasia type 2 and medullary thyroid cancer should not use GLP-1 receptor agonists [22,37,38].

### 1.2.3. Role of inhibiting α-glucosidase and α-amylase enzymes in type 2 diabetes management

Enzymes are protein molecules that facilitate complex biochemical reactions within the body by acting as catalysts. They exhibit specificity in catalyzing particular chemical reactions and ensure these reactions occur in one direction at a rhythmic rate in living organisms [39]. On the other hand, enzyme inhibitors are substances that reduce or hinder enzyme-catalyzed reactions. These inhibitors can block a substrate from binding to the active site of an enzyme, thereby disrupting the enzymatic process [40].

The α-amylase (EC 3.2.1.1) is a key digestive enzyme, primarily secreted by the pancreas (about 5–6%) and salivary glands, and is widely distributed in bacteria, fungi, plants, and higher organisms. This enzyme belongs to a family of endoamylases that initiate the hydrolysis of starch and glycogen by cleaving α-D-(1→4) glycosidic bonds, producing shorter oligosaccharides of different lengths. Although other amylolytic enzymes contribute to starch hydrolysis, the participation of α-amylase is essential to initiate this process. If excessive hydrolysis of starch into smaller sugar moieties leads to an increase in blood glucose levels, insulin will signal cells to metabolize the excess

glucose and store it as an energy source. However, if the α-amylase enzyme is overactive and there is either a deficiency or resistance to insulin, hyperglycemia might appear. To control this condition, α-amylase inhibitors, often referred to as starch blockers, are recommended. Nevertheless, the inhibition of pancreatic α-amylase must be carefully regulated. Excessive suppression of this enzyme activity can lead to undigested carbohydrates reaching the colon, where they undergo bacterial fermentation, potentially resulting in gastrointestinal side effects such as diarrhea and flatulence [41,42].

The α-glucosidase (EC 3.2.1.20) is an exoenzyme with a mechanism similar to glucoamylase, catalyzing the hydrolysis of α-1,4-glycosidic linkages at the non-reducing terminals of carbohydrate substrates through an anomer-retaining reaction mechanism to release α-D-glucose molecules. This enzyme plays an important role in the breakdown and utilization of starch and oligosaccharides across various organisms, including microbes, plants, and animals. The typical substrates of α-glucosidase are disaccharides, oligosaccharides, and other aryl- and alkyl-α-glucopyranosides. The α-glucosidase enzyme possesses duplicated glycoside hydrolase domains, which are responsible for catalyzing the hydrolysis of α-glycosidic bonds in disaccharides and oligosaccharides. Beyond carbohydrate digestion, these glycosidases are also involved in other biological processes, such as lysosomal glycoconjugate degradation and post-translational modification of glycoproteins [43-45].

Generally, in humans, digestion begins with the generation of α-amylase enzyme by the pancreatic glands and the salivary glands, which initially activate the digestive process of starch and glycogen by hydrolysing the polysaccharides into oligosaccharides. After that, the α-glucosidase enzyme, which is localized in the epithelium of the small intestine, is responsible for the hydrolysis of α-D-(1,4) glycosidic linkages in carbohydrates to generate monosaccharide glucose for intestinal absorption. Hence, inhibiting the activity of these two digestive enzymes can delay the digestion of carbohydrates and suppress the glucose uptake rate, thereby reducing blood sugar levels [42,45]. Besides, scientific studies have demonstrated the relationship between the action

of these two enzymes and postprandial hyperglycemia, emphasizing the critical role of managing postprandial glucose levels in the treatment of type 2 diabetes. Currently, the three commercial drugs used in clinical practice for limiting carbohydrate digestion and absorption by inhibiting the activities of α-amylase and α-glucosidase enzymes are acarbose, voglibose, and miglitol. However, severe stomach pain, flatulence, and diarrhea are the main undesirable clinical outcomes of these marketed agents [41,43]. On that basis, it is essential to research and develop novel drugs with fewer side effects and strong inhibitory activity derived from natural sources, especially medicinal herbs that have been widely used in traditional medicine. The capacity of natural α-amylase and α-glucosidase inhibitors derived from plants, including plant extracts and their secondary metabolites, in controlling blood glucose levels in human biological systems is represented in Figure 1.1.

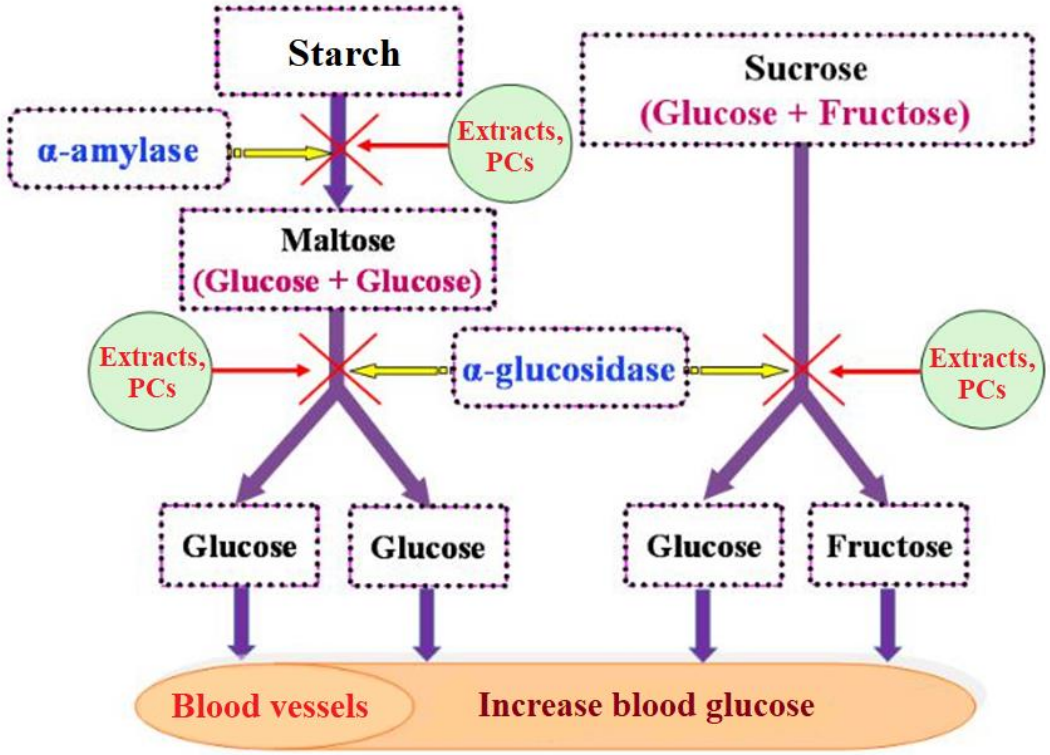



**Figure 1.1.** The schematic illustration of α-amylase and α-glucosidase inhibitory effects of plant extracts and their isolated pure compounds (PCs)

Medicinal plants and traditional remedies have been used for centuries to manage a variety of health conditions, including diabetes. In addition to their effectiveness, herbal therapies are generally associated with fewer side effects and represent a cost-effective alternative to synthetic hypoglycemic drugs. Therefore, plant-derived α-amylase and α-glucosidase inhibitors have recently gained more attention. Phytochemical studies have identified several bioactive compounds responsible for these inhibitory effects, supporting their potential for therapeutic development [41,44]. The lists of natural α-amylase and α-glucosidase inhibitors isolated from a wide range of botanical species are shown in Tables 1.3 and 1.4, respectively. However, most evidence relies on *in vitro* studies, with relatively limited validation in animal models and scarce clinical research in humans. While initial results are encouraging, further investigation is essential to advance these natural inhibitors into clinically viable antidiabetic therapies.

**Table 1.3.** Bioactive compounds isolated from various plant species as α-amylase inhibitors [41]

| No | Compound groups | Names of bioactive compounds |
|---|---|---|
| 1 | Flavonoids | 5-hydroxy-2-(4 methoxy-3-((E)-3-methylbut-1-enyl)-5-(3-methylbut-3-enyl)phenyl)chroman-4-one, luteolin, isoquercitrin, epicatechin gallate, puerarin, tricetin, tricetin 4'-O-β-glucopyranoside, rutin, quercetagetin-7-O-β-D-glucopyranoside, luteolin-7-O-α-L-rhamnoside, hypolaetin 8-O-β-D-galactopyranoside. |
| 2 | Terpenoids | Wedtriloside A, wedtriloside B, oleanolic acid, carnosol, 12-methoxycarnosic acid, glochidon, ligularoside A. |
| 3 | Alkaloids | 3,3',5,5',8-pentamethyl-3,3'-bis(4-methylpent-3-en-1-yl)-3,3',11,11'-tetrahydro-10,10'-bipyrano[3,2-a]carbazole, berberine. |
| 4 | Phenolic acid and its derivatives | Rosmarinic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, dehydrodieugenol B. |
| 5 | Tannins | Chingiitannin A, lambertianin A, sanguiin H-6, 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose. |
| 6 | Xanthones | Mangoxanthone A, garcixanthone D, garcinone E. |
| 7 | Others | Jionoside D, xyloglucan, sasastilboside A, halfordin, drevoluoside Q, gymsyloside B, gymsyloside C, gymsyloside D, fucoxanthin, proanthocyanidins, 5,7-dihydroxy-6-oxoheptadecanoic acid, β-sitosterol. |

**Table 1.4.** Bioactive compounds isolated from various plant species as α-glucosidase inhibitors [43]

| No | Compound groups | Names of bioactive compounds |
|---|---|---|
| 1 | Flavonoids | Catechin, epicatechin, naringenin, apigenin, scutellarein, hispidulin, nepetin, quercetin-4'-O-glucoside, quercetin-3-O-α-L-rhamnopyranoside-2"-gallate, myricetin-3-O-(2"-O-galloyl)-α-L-rhamnoside, myricetin-3-O-(4"-O-galloyl)-α-L-rhamnoside, vitexin, quercetagetin-7-O-β-D-glucopyranoside, (-) epigallocatechin-gallate, calodenin A, globunone A, globunone B, globunone C, globunone F, dehydrolophirone C, lophirone P. |
| 2 | Terpenoids | Scolopianate A, akebonoic acid, 3-oxolupenal, katononic acid, cypaliuruside J, betulin, betulinic acid, margoclin, andrographolide, gauleucin E, taxumariene F, ent-atisane-3-oxo-16β,17-acetonide. |
| 3 | Phenolic acid and its derivatives | Ellagic acid, 3-O-methylellagic acid, 3,3'-O-dimethylellagic acid, 3,3'-O-dimethylellagic-4-O-β-D-xylopyranoside, vanillin, tergallic acid dilactone. |
| 4 | Tannins | Procyanidin A2, 1,2,3-tri-O-galloyl-β-D-glucopyranose. |
| 5 | Stilbenes | Rhaponticin, scirpusin B. |
| 6 | Anthraquinones | Alaternin, chysalodin. |
| 7 | Others | Pelargonidin-3-O-rutinoside, cyanidin, gymnepregoside F, 3β,8β,14β,20-tetrahydroxy-(20S)-pregn-5-ene-3-O-β-D-glucopyranosyl-(1→4)-O-β-D-digitaloside-20-O- |

| | | |
|---|---|---|
| | | 3-isoval-β-D-glucopyranoside, mulberrofuran K, 2-(3',4'-dihydroxyphenyl)-2,3-dihydro-4,6-dihydroxy-2-(methoxy)-3-benzofuranone, mangoxanthone A. |

### 1.3. The genus *Commelina* L.

#### 1.3.1. Botanical classification and distribution

According to the Cronquist taxonomy (the classification system of flowering plants developed by Arthur Cronquist) [46], the genus *Commelina* belongs to:

**Kingdom:** Plantae

**Subkingdom:** Tracheobionta

**Superdivision:** Spermatophyta

**Division:** Magnoliophyta

**Class:** Liliopsida

**Subclass:** Commelinidae

**Order:** Commelinales

**Family:** Commelinaceae

**Genus:** *Commelina* L.

The genus *Commelina* L. is the largest and most complex genus in the family Commelinaceae, containing between 205 and 215 species. It is distributed mainly in tropical and subtropical regions [47,48]. The two most common species of this genus include *C. benghalensis,* found in subtropical Asia and Africa, and *C. erecta,* found in the Americas, sub-Saharan Africa, and the south of the Arabian Peninsula. Among the species of the genus *Commelina*, *C. dielsii* is the only species found in exactly one place

in the world. This plant was first discovered in 1940 and has only been found in Concepción del Uruguay, Entre Ríos province, northeastern Argentina [49,50].

### 1.3.2. Botanical characteristics

Species of the genus *Commelina* are perennials or annuals. Roots are usually in the fibrous form and rarely have tubers or rhizomes. Leaves usually grow in two rows or spirals, without a stalk or with a petiole [48,51].

Inflorescences grow at the apex and axils of the leaves on the same node as the leaves. The inflorescences contain one or two scorpion-tailed cymes, single flower axes staggered to form a scorpion-tailed cluster that grows along the main axis of the inflorescence. Flowers grow on petioles and are bilaterally symmetrical. The bracts are sometimes located below the petiole. This genus has hermaphroditic or male flowers. Three sepals are unequal and may grow freely, or two sepals may fuse. The free-growing petals are uneven. The two upper petals are larger and tucked in, meaning they narrow to a stalk at the base. Meanwhile, the lower petals are usually smaller and have a different color than the two upper petals. Flowers are usually blue, but sometimes can also be light purple, yellow, white, etc. Flowers have three fertile and three unfertilized stamens. They are free-growing and have smooth stamens. The unfertilized stamens usually grow posteriorly with anthers, having four or six lobes. Fertile stamens grow above and are longer than unfertilized stamens. The middle stamens are different in size and shape from the rest of the stamens. The ovary has two or three cells with one or two ovules in each cell [48,51].

Capsules usually have two or three cells, rarely one cell, and two or three shells. Each cell contains one or two seeds, possibly none at all. The seeds are arranged in a row, and the umbilicus is straight [51].

### 1.3.3. Phytochemicals of the *Commelina* plants

The genus *Commelina* L. is the largest in the family Commelinaceae. However, in the world, only a few species of this genus have been studied, such as *C. benghalensis*,

*C. erecta*, *C. nudiflora*, *C. appendiculata*, *C. communis*, *C. maculata, C. clavata,* and *C. diffusa*. The chemical compositions of some species of the *Commelina* genus published in previous studies are detailed in Table 1.5.

**Table 1.5.** Chemical composition of some species of the genus *Commelina* L.

| Name of species | Substances / Substance Groups | References |
|---|---|---|
| *C. benghalensis* | Polyphenols, flavonoids, tannins, alkaloids, phytosterols (stigmasterol), triterpenes (dammara-12-en-3-on; 3-(2,3,4,5,6-pentahydroxy)-cinnamoyldammara-12-en). | [52,53] |
| *C. erecta* | Luteolin, isoquercitrin, quercitrin, saccharinic lactone acid, and shikimic acid. | [54] |
| *C. nudiflora* | Phenolics, flavonoids. | [55] |
| *C. appendiculata* | Carbohydrates, flavonoids, tannins, glycosides, and alkaloids. | [56] |
| *C. communis* | Glucoluteolin, orientin, isoorientin, isoquercitrin, isorhamnetin-3-O-rutinoside, vitexin, swertisin, 1-deoxynojirimycin, (2R, 3R, 4R, 5R)-2,5-bis (hydroxymethyl)-3,4-dihydroxypyrrolidin, chrysoeriol-7-O-β-D-glucoside, methyl gallate, *p*-coumaric acid, protocatechuic acid, caffeic acid, *p*-hydroxybenzoic acid, 2-phenethyl-β-D-glycosidase, (7S, 8R)-dihydrodehydrodiconiferyl alcohol-9-O-β-D-glucoside, isovitexin, isofurcatain, isorhamnetin-3-O-β-D-glucoside, quercetin-3-O-α-L-rhamnoside, stigmasterol, | [57-59] |

| | | |
|---|---|---|
| | sitosterol, syringic acid, dodecanoic acid, coumaric acid, daucosterol, luteolin, apigenin, and 3,3',4',7-tetramethoxyflavone. | |
| *C. maculata* | Hexadecanoic acid, 2-hexadecen-1-ol, and heptadecan-3,7,11,15-tetramethyl. | [60] |
| *C. clavata* | Carbohydrates, tannins, alkaloids, and flavonoids. | [61] |
| *C. diffusa* | Steroids, alkaloids, phenolics, tannins, saponins, amino acids, and phytosterols. | [62] |

### 1.3.4. Traditional uses of the *Commelina* plants

According to traditional medicine, many species of the genus *Commelina* are used as medicine for sore throats, flu, colds, etc. *C. benghalensis* is used to relieve pain, constipation, headache, and leprosy. In many tropical regions of Asia, it is used to treat infertility in women. In India, it is used for slimming, laxatives, skin softening, anti-inflammatory, and antidepressant. Besides, *C. coelestis* is used to treat diarrhea in Mexico [50,63,64].

In Vietnam, some species of the genus *Commelina* have also been used in the treatment of various diseases for a long time. *C. benghalensis* is used as an enema, mucous membrane protector, laxative, and cooling agent. *C. communis* is used to treat edema caused by heart failure, high blood pressure, fever, eye pain, and gonorrhea [65].

### 1.3.5. Pharmacology of the *Commelina* plants

Modern pharmacological studies have demonstrated the effects of some species of the genus *Commelina*. For example, the ethanol extracts from the roots of *C. benghalensis* showed a highly potent analgesic with a mechanism of action similar to NSAIDs. In addition, this species could also relieve pain, be anti-inflammatory, anti-cancer, antioxidant, antibacterial, antiparasitic, antiviral, hepatoprotective, and

antidiabetic [50,52,53]. Another study revealed the *in vitro* antibacterial and antioxidant effects of *C. nudiflora*. In particular, the aqueous extract of this species showed the highest inhibitory activity against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhi*, while flavonoid components in the acetone, ethanol, and chloroform extracts of *C. nudiflora* expressed potent antioxidant effects [55]. Similarly, published research reported that *C. erecta* exhibits antibacterial properties against various pathogens, including *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Staphylococcus pyogenes*, *Salmonella typhii*, *Pseudomonas aeruginosa*, and *Escherichia coli*, and the leaves of *C. erecta* contain caffeic acid with significant antioxidant activity against oxidative stress induced by the free radical nitric oxide [64]. In addition, it is proven that *C. coelestis* showed good anti-diarrheal activity [63], and *C. appendiculata* had potential analgesic and cytotoxic effects due to its alkaloids, flavonoids, tannins, phenolic acids, and steroids [56]. Besides, the analgesic and anti-inflammatory activities of *C. latifolia* have been demonstrated in animal models, while the presence of 3,5,7,3′,4′-pentahydroxy flavone-7-O-β-L-ribopyranosyl-3′-O-β-D-galactopyranosyl contributes to the anti-inflammatory effect of *C. oblique* Vahl [64].

In 2004, Ji-Youn Youn et al. [66] evaluated the *in vitro* and *in vivo* activities of stabilizing blood glucose from the extracts of *C. communis* species. Notably, *C. communis* extracts showed dose-dependent α-glucosidase inhibition and amelioration of maltose- or starch-induced hyperglycemia in diabetic mice. In addition, the prolonged administration of *C. communis* tended to stabilize blood glucose in streptozocin-induced diabetic mice. The above results showed that the inhibitory effect of *C. communis* on α-glucosidase may contribute to slowing carbohydrate digestion and glucose absorption. Therefore, *C. communis* had the potential for use in the treatment of non-insulin-dependent diabetes. In 2008, Makio Shibano and colleagues isolated two potent α-glucosidase inhibitors, including 1-deoxynojirimycin (DNJ) and (2R,3R,4R,5R)-2,5-bis (hydroxymethyl)-3,4-dihydroxypyrrolidine (DMDP) from this plant. The medicinal extracts and powders of *C. communis* are promising agents for type 2 diabetes prevention and treatment [59].

## 1.4. Overview of *Commelina diffusa* Burm.f.

### 1.4.1. Botanical distribution and description

*Commelina diffusa* Burm.f. was first described in 1768 by Burman. F. This species is found throughout the tropics of the Americas, Africa, Asia, and the Pacific, as well as in the southern subtropical regions of the United States, South America, Australia, and the islands of South Asia [67]. *C. diffusa* is a tropical and subtropical weed distributed mainly in airy and moist habitats. This plant commonly grows in cultivated lands, fields, wet meadows, roadsides, and gardens. *C. diffusa* can withstand temporary floods [66]. In Vietnam, *C. diffusa* is found across the country and often grows in wet places such as fields, hillsides, roadsides, ditches, and riverbanks [68].

*C. diffusa* is an annual plant in temperate regions but is a perennial when grown in tropical and subtropical areas. The plant spreads on the ground, the trunk is branched, and the root grows at the knot. The stems are cylindrical, smooth or rough, hairless, green, and can be up to one meter long, divided into segments 2 – 10 cm long. The leaves are simple, alternate, and without stalks. The leaf plates are variegated, ranging from lanceolate to ovoid. The leaf surface may be smooth or hairy, with arched veins. The leaf sheath is striped red and covered with lanugos 0.8 – 1.7 cm long.

*C. diffusa* flowers from May to November, blooming in the morning and quickly fading after a few hours. Flowers are male or hermaphroditic and usually cymbal. Each flower grows on a single flower axis. The single flower axes stagger to form clusters like a scorpion tail and rise along the central axis of the inflorescence. The flower is enclosed in a “sack” – a leaf that has been altered and filled with mucus. There will usually be two flower cymbals. The lower cyme has about two to four flowers, while the upper cyme has one or more flowers. The upper cymbal has only one male flower and has a longer peduncle than the lower cymbal, which has hermaphroditic flowers. The flower stalk is long and cylindrical, and each stalk grows only one flower. Sepals are in the form of a thin film, difficult to see. The petals are blue or pale purple and 4.2 – 6 mm in size. Six stamens are arranged in two rings, with three blue, infertile outer stamens and three blue,

fertile stamens of different sizes. The part connecting the two other halves of the central stamen has a purple horizontal band. The pistil has an oblong ellipse, blue. The filamentous style is blue and 0.6 – 0.9 cm in size.

The capsule has three cells, a bivalve, and five black seeds. The seed coat is networked. The fruit is 4 – 6.3 mm long and 3 – 4 mm wide. The seeds are 2 – 2.8 mm long, rarely to 3.2 mm long, and 1.4 – 1.8 mm wide [51,65,69].

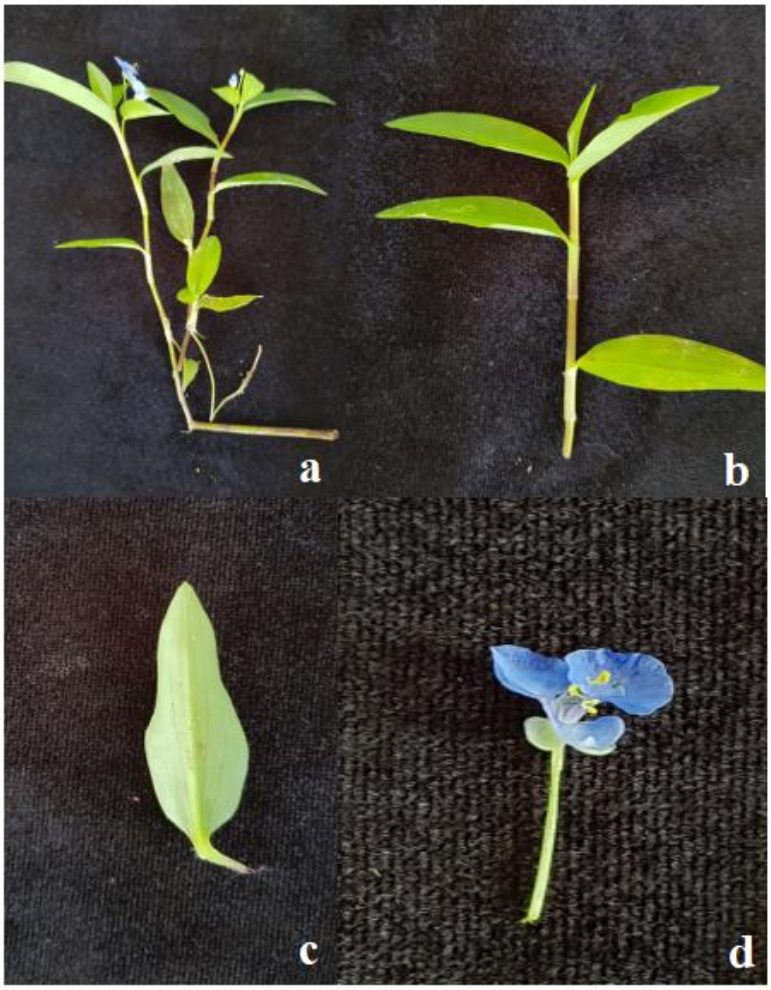



**Figure 1.2.** Plant (a), stem (b), leaf (c), and flower (d) of *C. diffusa*

### 1.4.2. Chemical constituents

By thin-layer chromatography and preliminary quantification, it was found that *C. diffusa* includes alkaloids, flavonoids, saponins, tannins, coumarins, cardiac

glycosides, phenolic acids, anthraquinones, steroids, and terpenoids. In 2019, Malarvizhi D et al. discovered, by the use of the GC-MS method, the presence of 21 phytochemical components in the methanol extract of *C. diffusa*. Among them, some important compounds have biological effects such as 2-methoxy-4-vinylphenol (antibacterial, antioxidant, anti-inflammatory); 3,7,11,15-tetramethyl-2-hexadecen-1-ol (antibacterial, anti-inflammatory); phytol (anti-inflammatory, antioxidant, diuretic); methyl stearate (anti-diarrheal, cytotoxic, anti-proliferative); 9,12-octadecadienoic acid, methyl ester (hepatoprotective, antihistamine, lowering blood cholesterol, lowering blood pressure); and pimaric acid (reduce the risk of atherosclerosis) [62]. In addition, *C. diffusa* also contains substances with high nutritional content, including protein, vitamin C, vitamin B2, vitamin B3, carotene, fat, and organic acids [70,71].

### 1.4.3. Traditional uses

According to ethnomedicinal practitioners, *C. diffusa* has been widely used as a medicinal herb for urinary tract infections, swellings, inflammation, diarrhea, hemorrhoids, enteritis, eye irritation, conjunctivitis, and ophthalmia. The use of *C. diffusa* leaves to cure edema was a common medical practice in ancient China. The stem juice treats laryngitis, sore throats, acute tonsillitis, pharyngitis, otitis media, and nose bleeding. Using this herb topically or orally can treat abscesses, boils, fever, malaria, insect, snake, and bug bites, rheumatoid arthritis, mumps, gonorrhea, common cold, cough, coughing up blood, influenza, bladder infection, and edema. The plant components are utilized in dermatitis and burns all across Latin America [72]. Hemorrhoids, an irritated uterus, laryngitis, leprosy, malaria, mumps, otitis media, painful menses, pharyngitis, rheumatoid arthritis, sore throats, snake bites, tonsillitis, and tumors are all treated with *C. diffusa* in Nepal. The plant is used as a refrigerant and tonic in Egypt to heal stomach and groin ailments. The herb is used as a tea to prevent influenza and in therapeutic baths by Caribbean Indians. It is used to treat conjunctivitis, dermatitis, and dysmenorrhea in Mexico and to treat enteritis, gonorrhea, and infertility in Paraguay. It is utilized for renal problems, leucorrhea, malaria, neurological illnesses,

postpartum pain, tumors, and venereal disorders in the Dominican Republic, Haiti, and South America. In Ecuador and Peru, the small blue blooms are cooked to form a headache-relieving tea [73].

In Vietnam, *C. diffusa* is decocted and used from 30 to 60 g per day to treat colds, upper respiratory tract infections, acute tonsillitis, pharyngitis, edema, genitourinary-urinary tract infections, enteritis, dysentery, high blood pressure, and gonorrhea. In addition, the plant is also pounded fresh to cover wounds and treat pyoderma. In the Muong ethnic group, people use a decoction of the whole plant to treat kidney stones [70,74].

### 1.4.4. Biological activities

The pharmacological studies showed that *C. diffusa* has antioxidant, anti-inflammatory, antibacterial, antifungal, hepatoprotective, nephroprotective, and central nervous system depressant activities [75].

***Antioxidant***

The leaf extract of *C. diffusa* was proved to have potent antioxidant activity with an $IC_{50}$ value of 11.35 μg/mL, compared to the standard substance, ascorbic acid ($IC_{50}$ value of 2.64 μg/mL) [76]. Moreover, the total antioxidant capacity of *C. diffusa* was 130.5 mg of ascorbic acid per gram of plant extract. The leaf extract of this herb had a high phenolic content of 193.7 mg of tannic acid per gram of extract, which might be related to the high antioxidant effect. Secondary metabolites such as reducing sugar, alkaloids, phytosterols, flavonoids, and triterpenoids were also responsible for the antioxidant and anti-inflammatory activities of this herb [77].

***Anti-inflammatory***

In 2014, AY Mensah et al. used the carrageenan-induced foot edema in a 7-day-old chick model to investigate the anti-inflammatory activity of the ethanolic extract of *C. diffusa*. The results indicated that the *C. diffusa* ethanolic leaf extract significantly reduced the total foot edema in a dose-dependent manner. The maximum percentage inhibition of inflammation given by the plant extract was 43.55% at the dose of

300 mg/kg. Similarly, at the highest doses used, diclofenac and dexamethasone, standard agents, performed dose-dependent anti-inflammatory effects with inhibitions of 49.39% and 49.78%, respectively [77].

***Antibacterial, antifungal***

In 2006, Abraham Y Mensah et al. revealed that the methanol extract of *C. diffusa* had selective antibacterial and antifungal activity against *Trichophyton* species. The minimum inhibitory concentrations (MICs) for *Bacillus subtilis*, *Trichophyton interdigitale*, and *Trichophyton tonsurans* were 500 μg/mL, 500 μg/mL, and 250 μg/mL, respectively [78]. Furthermore, another study showed that the methanol extract of *C. diffusa* species was able to inhibit seven types of bacteria, including *Aspergillus niger*, *Blastomyces dermatitidis*, *Pityrosporum ovale*, *Trichophyton spp.*, *Microsporum spp.*, and *Cryptococcus neoformans,* with MICs from 15.62 to 62.5 μg/μL. Meanwhile, the diethyl ether extract of this plant was able to inhibit *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Shigella sonnei*, *Salmonella typhi*, *Vibrio cholerae*, and *Salmonella paratyphi* with MICs from 15.62 to 125 μg/μL [79]. In 2021, A. Frankova and colleagues evaluated the *in vitro* antibacterial activity of the ethanol extract of this species. The results showed that the ethanol extract could inhibit the growth of *Staphylococcus aureus* with an MIC greater than 4 μg/mL [80].

***Hepatoprotective***

In 2019, the study of Ha Quang Thanh and colleagues investigated the hepatoprotective effect of *C. diffusa* extract on an albino rat model of liver damage caused by chronic alcohol use. The results showed that the oral administration of 96% alcohol extract from *C. diffusa* at both doses of 0.21 and 0.42 g/kg reduced AST (aspartate transaminase) and ALT (alanine aminotransferase) activities in the blood plasma, causing decreased MDA (malondialdehyde) concentration and increased GSH (glutathione) concentration in rat liver. These effects were comparable to those of the standard drug silymarin [81].

***Nephroprotective***

An experiment was conducted using the ethanolic leaf extract of *C. diffusa* at 200 and 400 mg/kg, which was given to albino rats for 20 days while they were also given doxorubicin. In this study, the rats pretreated with the extract had normal kidney stroma with Bowman's capsule, glomerulus, and renal tubules, indicating that the extract had a beneficial nephroprotective activity of the plant [82].

***Central nervous system (CNS) depressant***

The methanolic extract of *C. diffusa* was tested in mouse models using standard depression models to identify the CNS depressive activities of this herb. The mouse models were orally given 50, 100, and 200 mg/kg of plant extracts and distilled water (0.1 mL/mouse, p.o.). Diazepam (1 mg/kg) was regarded as a typical medication. Compared to the control group, the plant extract reduced locomotor activity in the open field and hole-cross tests but increased immobility time in forced swimming and tail suspension tests. In addition, it increased the amount of time spent sleeping compared to the control group [83].

## 1.5. Introduction of *in silico* research methods

Traditional drug discovery is a complex, time-consuming, and high-risk process involving biological target identification, lead compound discovery and optimization, and extensive preclinical and clinical trials. The cost of bringing a new drug to market has risen to around $1.8 billion, with failure rates reaching up to 96%. High-throughput screening (HTS) techniques were developed to speed up this process, allowing researchers to rapidly test thousands of compounds using automated systems [84].

The term "*in silico*", originating from Latin, was first introduced by Pedro Miramontes in 1989 to describe computer-based simulations of natural processes [85]. Initially, it referred specifically to the use of computer models to replicate biological or physical phenomena, rather than general computational tasks. Since then, *in silico* approaches have become important tools in drug discovery, allowing researchers to

predict pharmacological effects, side effects, and drug-likeness of a compound [86]. These computational methods can significantly reduce both time and cost in the drug development process while enabling rapid screening of potential drug candidates [87].

A key *in silico* approach is structure-based virtual screening (SBVS), which plays an increasingly important role in modern drug discovery. SBVS uses the three-dimensional structures of target proteins, often obtained via X-ray crystallography or NMR, to predict the binding affinity of candidate compounds. Compounds scoring above a certain threshold are selected for further *in vitro* and *in vivo* testing. This rational, structure-based strategy is more targeted and cost-effective than traditional methods, as it builds on a detailed understanding of disease at the molecular level [88].

### 1.5.1. Docking

Docking is a computational technique used to predict how a molecule, such as a potential drug, will bind to the active site of a target protein. It estimates the orientation, geometry, and the scoring (binding or free energy score) of the molecule. Docking algorithms typically generate numerous orientations and conformations of the molecule within the binding site of the protein and then score each one. While some software tools retain all the calculated orientations, most keep only the top-scoring configurations [89].

In computational drug discovery, docking is widely used to evaluate the inhibitory potential of compounds against specific protein targets. As a result, pharmaceutical enterprises often employ this method to screen large compound libraries, including commercially available molecules, proprietary compounds, or structures designed *in silico*. By identifying promising candidates for synthesis and experimental testing, docking helps reduce the number of compounds that need to be physically evaluated, thereby improving the efficiency of the drug discovery process [90].

Docking simulates interactions between ligands and protein binding sites, so its predictions are often comparable to biochemical assay data. However, docking does not account for factors like bioavailability, toxicity, or metabolism, which are critical in a

living organism. Therefore, its results generally do not correlate well with outcomes from cell-based assays, animal studies, or other biological evaluations [91].

The main goals of docking are to identify compounds likely to bind effectively to a target protein and to visualize their three-dimensional binding geometry. One limitation of this technique is the requirement for a known three-dimensional structure of the protein. Additionally, docking can be computationally intensive, which may limit its use when screening extremely large compound libraries. In such cases, faster methods like pharmacophore modeling or similarity searches are used to narrow down the databases of compounds before detailed docking analysis [89,91].

Widely used docking tools incorporate diverse search algorithms, including genetic algorithms, fragment-based methods, Monte Carlo simulations, and molecular dynamics approaches. Additionally, several software programs, such as DOCK, GOLD, FlexX, and ICM, are commonly utilized for high-throughput docking simulations. Depending on the specific goals of the study, docking procedures may account for either ligand and/or target flexibility or treat the interacting molecules as rigid bodies [92]. Based on molecular flexibility, docking can be classified into three types:

- Rigid docking: Both the receptor and ligand are treated as rigid bodies. This approach is fast and computationally efficient, but may miss accurate binding poses if conformational changes are essential for binding. Therefore, this method is suitable for initial screening.

- Flexible ligand docking: The ligand is allowed to explore its conformational space (e.g., rotation around rotatable bonds), while the receptor remains rigid. This is the most commonly used method in small-molecule docking. Flexible ligand docking has better accuracy than rigid docking with a moderate computational cost.

- Flexible receptor docking: The receptor is allowed to change conformation, either partially (side chains or loops) or fully (backbone movements), during docking. As a result, this approach is more realistic and accurate, but computationally demanding [93].

### 1.5.2. Molecular docking

Molecular docking is a widely used technique in structure-based drug design due to its capacity to predict how small-molecule ligands bind to specific target sites. It aims to determine the most probable three-dimensional conformations of ligands within target binding cavities and to quantitatively estimate the energy changes associated with intermolecular recognition. These energy predictions are used to rank docked compounds, assisting in the selection of ligands for experimental testing. Molecular docking involves two main steps, which are exploring the conformational space of the ligand within the binding site and evaluating the binding energy for each predicted conformation [90].

To search conformational space, molecular docking programs adjust ligand parameters such as dihedral angles, translations, and rotations. Two primary strategies, including systematic and stochastic methods, are used. Systematic methods involve slight changes to structural parameters, gradually moving toward a minimum energy conformation. While effective, these methods may generate local rather than global minimum conformations. This can be mitigated by running multiple searches from diverse starting conformations. Besides, systematic methods find all possible structural parameter combinations, leading to an exponential increase in the number of combinations with the degrees of freedom of ligands, which is known as a combinatorial explosion. Docking tools like FRED and DOCK solve this problem using incremental construction algorithms, which break the ligand into fragments and assemble them within the binding site. This reduces complexity by limiting the conformational search to individual fragments [94].

On the other hand, stochastic methods randomly adjust ligand structural parameters to explore a wider conformational space. This approach helps avoid conformations at local minima and increases the probability of finding those at global minima. Genetic algorithms (GAs) are a prominent stochastic technique, utilized in software such as AutoDock and GOLD. GAs simulate natural selection by encoding

ligand conformations as chromosomes, evolving them through selection and recombination across generations. This iterative process leads to conformations with progressively lower binding energies [94].

Apart from binding conformation prediction, molecular docking algorithms use scoring functions to estimate the binding energy of ligand–receptor complexes. Binding affinity is quantified via constants such as the binding constant (Kd) and Gibbs free energy (ΔG). Scoring functions evaluate these parameters based on three algorithm categories: force-field-based, empirical, and knowledge-based. Force-field methods compute energy by summing bonded interactions (e.g., bond stretching, angle bending) and non-bonded forces (e.g., electrostatic and van der Waals interactions) using classical mechanics. Empirical methods use regression models trained on datasets of known protein–ligand complexes, incorporating features such as hydrogen bonding, hydrophobic effects, desolvation, and entropy. Their accuracy depends on the features of the data used to create the model. Knowledge-based functions derive statistical potentials from the frequency of atom pair interactions in known complexes, assigning weights to these interactions to form a general scoring equation [90,94].

Generally, molecular docking effectively predicts small-molecule conformations within binding sites and provides binding energy estimates. Its reliability is supported by comparisons with experimental structures such as X-ray crystallography. However, the current limitation of this method is inadequate modeling of desolvation, entropy, and protein flexibility [95].

### 1.5.3. Procedure of molecular docking

The molecular docking process is carried out through three main steps: ligand preparation, protein preparation, and docking simulation.

#### *Ligand preparation*

This step involves acquiring or constructing the three-dimensional (3D) structures of small-molecule ligands. These structures can be obtained from chemical databases

such as PubChem or ZINC. If they are not available, the 3D structures of ligands can be constructed using chemical drawing tools like ChemDraw or ChemSketch. After that, the ligands must undergo pre-docking processing, which includes assigning charges, applying an appropriate force field, and performing energy minimization to ensure structural stability [96].

***Protein preparation***

The protein preparation begins by obtaining the 3D structure of the target protein, which is most commonly found in the Protein Data Bank (PDB). In cases where the experimental protein structure is unavailable, homology modeling may be employed to generate a reliable model. Subsequently, specialized software is utilized to prepare the protein for the docking simulation program, including removing irrelevant molecules (e.g., water, ligands, or ions), adding hydrogen atoms, applying force fields, and defining or analyzing the binding site. These steps are essential to ensure the protein is suitable for docking [96].

***Docking simulation***

Before conducting the docking simulation, it is necessary to define a search area or grid box, which guides the algorithm in exploring potential binding modes. The search area depends on each study. However, the grid box should be centered on the active site of the protein and appropriately sized. If the grid box is too large, it may waste computational resources and reduce precision. By contrast, if the grid box is too small, critical binding interactions may be missed. Once the search area is defined, the docking software explores different orientations and conformations of the ligand to identify those with the lowest binding energies. Post-docking, the predicted binding poses can be analyzed using visualization and interaction analysis tools such as MOE, Discovery Studio, or PyMOL to assess binding affinity and interaction types [96].

### 1.5.4. Virtual screening

Virtual screening refers to the application of rapid and cost-effective computational techniques to identify potentially active compounds from available chemical databases. It is one of the most widely adopted strategies in modern drug discovery, with several approved pharmaceutical agents originating from virtual screening attempts. When virtual screening involves docking compounds into a specific molecular target, it is referred to as structure-based virtual screening (SBVS). Apart from predicting potential binding conformations, SBVS allows for the ranking of compounds using scoring functions, facilitating the prioritization of candidates for further experimental validation. These scoring metrics can be used independently or alongside other selection criteria to identify promising hits [94,97].

A typical SBVS procedure includes four main steps: (1) selection and preparation of the molecular target, (2) selection of a compound database, (3) molecular docking, and (4) result analysis. Successful SBVS implementation depends on thorough knowledge of the structure and function of targets and known ligands. Once a suitable target structure is chosen, it undergoes a preparation process that includes the addition of hydrogen atoms, removal of non-essential water molecules, assignment of partial charges, and adjustment of protonation states for amino acid residues [98].

Next, a compound library is selected for docking, and each compound is docked into the defined binding site of the target. During docking, the software explores possible conformations and orientations of each molecule to identify low-energy binding poses. Compounds predicted to have favorable interactions are then subjected to post-docking analysis. This stage involves visual inspection of ligand–receptor complexes to assess binding modes and key intermolecular interactions. Such analysis assists in selecting top-ranked candidates for experimental testing. Additionally, comparing docking poses of known ligands with their crystallographic conformations is a validation step, ensuring that the docking protocol can reliably reproduce experimentally observed binding modes [94,98].

### 1.5.5. Prediction of physicochemical parameters, pharmacokinetic properties, and toxicity

Physicochemical properties such as molecular weight, hydrogen bond donors and acceptors, octanol–water partition coefficient (logP), and rotatable bonds are important factors in evaluating whether a compound can become a drug or not. These parameters influence essential pharmacokinetic characteristics, including water solubility and intestinal permeability, and play a key role in determining oral bioavailability. In 1997, Christopher Lipinski and colleagues proposed the Rule of Five (RO5), which outlines the optimal range for these properties in orally active drugs. According to RO5, compounds that significantly differ from these ranges are more likely to exhibit poor oral absorption. However, compliance with RO5 does not guarantee the success of a compound as a drug candidate, as the rule does not consider specific structural features that distinguish drugs from non-drugs [99].

In addition to physicochemical profiling, *in silico* prediction of pharmacokinetic properties, including absorption, distribution, metabolism, excretion, and toxicity (ADMET), has become an essential step in early drug development. According to a report, nearly 50% of drug candidates failed in clinical trials because of failure in pharmacokinetics (39%) or animal toxicity (11%) requirements [100]. Currently, ADMET screening is commonly performed at the preclinical stage, leading to the risk reduction of late-stage failures, time and resource savings, and drug safety enhancement. Nowadays, there are numerous available tools for ADMET prediction, including commercial platforms like CASE ULTRA, DEREK, META-PC, METEOR, PASS, and GUSAR, as well as freely available online tools such as ADMETlab, admetSAR, pkCSM, and SwissADME, which are often preferred due to their time and cost advantages [101].

Among online platforms, pkCSM stands out for its efficiency and predictive power. This online tool enables rapid assessment of the pharmacokinetic and toxicological properties of a compound using a simple SMILES input. pkCSM has a

large dataset of over 10,000 toxicity profiles and 18,000 metabolic compounds. Furthermore, this tool applies 14 quantitative regression and 16 binary classification models of ADMET parameters to provide accurate predictions. Its capacity to handle large datasets makes it particularly valuable for high-throughput screening and early-stage drug discovery efforts [102].

### 1.6. Objectives of the thesis

Diabetes mellitus has increased rapidly in recent decades and has become one of the most pressing public health problems globally. Type 2 diabetes, which was previously known as non-insulin-dependent diabetes, represents the predominant form of diabetes, comprising nearly all diagnosed cases [1]. It has been proven that α-amylase and α-glucosidase are two enzymes involved in carbohydrate metabolism that contribute to postprandial hyperglycemia in patients with type 2 diabetes. Therefore, inhibition of these enzymes can reduce carbohydrate hydrolysis and, consequently, slow glucose absorption into the blood [26].

*Commelina diffusa* is a species of the *Commelina* genus, which belongs to the *Commelinaceae* family. According to several pharmacological investigations, some extracts of plants from the *Commelina* genus demonstrate insulin-mimetic and antihyperglycemic activities. In 2004, Ji-Youn Youn et al. [66] evaluated the *in vitro* and *in vivo* blood glucose stabilizing effects of the extract of *C. communis* species and found that *C. communis* extract showed dose-dependent α-glucosidase inhibition and amelioration of maltose or starch-induced hyperglycemia in diabetic mice. Furthermore, prolonged administration of *C. communis* tended to stabilize blood glucose in streptozocin-induced diabetic mice. Besides, 1-deoxynojirimycin (DNJ) and (2R,3R,4R,5R)-2,5-bis (hydroxymethyl)-3,4-dihydroxypyrrolidine (DMDP), which are isolated from *C. communis*, have been proven to exhibit the capacity of α-glucosidase inhibition [59]. In recent years, pharmacological investigations of *C. benghalensis* have revealed that its methanolic extract exhibits significant antihyperglycemic activity in the alloxan-induced diabetic mouse model [52]. The above results suggest that *C. diffusa*,

which is one of the plant species in the *Commelina* genus, may also have a hypoglycemic property. Although *C. diffusa* has been reported to exhibit various biological activities and contain several phytochemical constituents, its secondary metabolites and antihyperglycemic activities have not yet been fully investigated. Therefore, further integrated phytochemical and pharmacological studies are needed to clarify the chemical composition and hypoglycemic potential of this plant species. On that basis, the thesis entitled "Chemical constituents and hypoglycemic effects of *Commelina diffusa* Burm.f." was carried out with the following objectives:

1. Extract, isolate, and determine the chemical structure of compounds from the extract fractions of *Commelina diffusa* Burm.f.

2. Evaluate *in vitro* the ability to inhibit α-glucosidase and α-amylase enzymes of the extract fractions and compounds isolated from *Commelina diffusa* Burm.f.

3. Evaluate the acute toxicity and hypoglycemic effect of the fraction with the best inhibitory activities against α-glucosidase and α-amylase enzymes in an *in vivo* model.

4. Study the α-glucosidase and α-amylase inhibitory effects of the compounds isolated from *Commelina diffusa* Burm.f. using the molecular docking method.

# Chapter II – Materials and Methods

## 2.1. Plant materials

The whole plant of *Commelina diffusa* Burm.f. was collected in October 2021 in Nam Dinh province, Vietnam, and identified by MSc. Nghiem Duc Trong, Hanoi University of Pharmacy. A voucher specimen (No. HNIP/18651/22) is deposited at the Department of Medicinal Materials – Traditional Medicine, University of Medicine and Pharmacy, Vietnam National University. The collected plants were washed to remove dirt, air-dried for seven days, and ground into a coarse powder.

## 2.2. Experimental animals

Healthy Swiss male mice with an average weight of 25 ± 2 g were provided by the National Institute of Hygiene and Epidemiology. All animals were kept in polycarbonate cages under a light-controlled environment (12 h light/dark cycles) at room temperature and were offered pelleted food and water *ad libitum*. The animals were maintained for a quarantine period of seven days to acclimate to the laboratory conditions before the experiment. All experimental procedures, including animal housing, monitoring, and administration of the experimental treatments throughout the study period, were employed at the Tue Tinh Institute of Medical and Pharmaceutical Research, Vietnam University of Traditional Medicine. At the end of the experimental period, the animals were sacrificed and dissected at Hanoi Medical University, where the required tissue and blood samples were collected. The study was conducted in accordance with the international regulations for the care and use of laboratory animals and complied with ethical principles for animal welfare.

## 2.3. Chemicals, solvents, and reagents

- Solvents used for extraction and isolation included methanol (MeOH), *n*-hexane, dichloromethane (DCM/$CH_2Cl_2$), ethyl acetate (EtOAc), acetone, and distilled water ($H_2O$). All solvents were of pharmaceutical grade and purchased from Xilong (China). The water used was purified by reverse osmosis.

- Reagents used for detecting traces of substances on thin-layer plates included $H_2SO_4$ 10% and cerium sulfate, which were of analytical grade.

- Chemicals used in the study to evaluate enzyme inhibition included α-amylase (from porcine pancreas) and α-glucosidase (from *Saccharomyces cerevisiae*) enzymes (Sigma-Aldrich, USA); starch, p-nitrophenyl-α-D-glucopyranoside (pNPG), potassium phosphate, sodium chloride, sodium carbonate, dinitrosalicylic acid, dimethyl sulfoxide (Merck, Germany); acarbose (Chemcruz, USA). Other chemicals were of pharmaceutical or analytical grades.

- Chemicals used in the *in vivo* study included streptozotocin (Sigma-Aldrich, Singapore), gliclazide 30 mg (Diamicron®MR, Les Laboratoires Servier, France), sodium citrate (Merck, Germany), fructose (Daesang Corporation, Korea), and diagnostic reagent kits for ALT, AST, total cholesterol, triglycerides, and high-density lipoprotein cholesterol (Erba, Germany).

- The normal-fat diet contained 28.05% protein, 12.14% fat, and 59.81% carbohydrate, while the high-fat diet contained 18.23% protein, 42.89% fat, and 38.88% carbohydrate.

### 2.4. Equipment and instruments

#### 2.4.1. Equipment for extraction, isolation, and structural elucidation

- Normal phase silica gel (230 – 400 mesh, Merck, Germany), reverse phase silica gel (Fuji Silysia Chemical Ltd., Japan), Diaion HP-20 (Mitsubishi Chem. Co., Japan), and Sephadex LH-20 (Merck, Germany) were used as adsorbents in column chromatography.

- DC-Alufolien 60 F254 (1.05715.0001, Merck, Germany) and RP18 F254s (Merck, Germany) pre-coated plates.

- Rotary evaporator N-1200BV-W (Eyela, Japan).

- Automatic collector DC-1200 (Eyela, Japan).

- Kofler micro hotstage (Wagner & Munz, Germany).

- Electron spray ionization mass spectrometry (ESI-MS) was measured on an AGILENT 1100 Series LC-MSD-Trap-SL.

- Magnetic resonance spectroscopy was measured on a Bruker Avance AM600 FT-NMR Spectrometer (Germany).

- Other instruments: Microcentrifuge, micropipettes, Pasteur pipettes, Pasteur pipette bulbs, Eppendorf tubes, Falcon tubes, UV lamp at 254 and 365 nm, TLC plate developing chamber, pencil, capillary tubes, NMR tubes with caps, Erlenmeyer flasks or breakers, metric ruler, and cotton wool.

### 2.4.2. Equipment for enzymatic inhibition assays

- Microplate absorbance spectrophotometer (xMark$^{TM}$, Bio-Rad, USA).

- UV-Vis Cary 60 spectrophotometer (Agilent, USA).

- Dry bath incubator MK2000-2E (China).

- Water bath Julabo TW12 (Germany).

- Ultrasonic cleaner (MRC, Israel).

- Analytical balance Sartorius PRACTUM64-1S (Germany).

- 96-well plates (Biologix, USA).

### 2.4.3. Equipment for *in vivo* hypoglycemic activity study

- Blood glucose monitoring device (On-Call EZ II, ACON Biotech, USA).

- Glucose monitor kits (On-Call EZ II, ACON Biotech, USA).

- Biochemical analyzer (Erba, Germany).

- Biological microscope (Optika, Italy).

### 2.4.4. Equipment for *in silico* study

Software and tools used for this study included:

- Molecular Operating Environment (MOE) 2015.10

- ICM Pro 3.8-6

- ChemDraw Professional 15.0

- Open Babel 3.1.1

- UCSF Chimera 1.18

- Discovery Studio 2021

- Microsoft Excel 2019

- pkCSM online tool (https://biosig.lab.uq.edu.au/pkcsm/).

- SwissADME online tool (https://www.swissadme.ch/index.php).

**2.5. Ligands and proteins**

- Ligands: Pure compounds isolated from different fractions of *C. diffusa*. Their structures were drawn using ChemDraw Professional 15.0. Subsequently, Avogadro 1.2.0 was utilized to create their three-dimensional structures. Finally, all ligands were transformed into dockable SDF format using Open Babel 3.1.1.

- Proteins: The X-ray crystal structures of α-glucosidase (AG) and α-amylase (AA) enzymes were downloaded from the Protein Data Bank (PDB – http://www.rcsb.org) with PDB codes of 3W37 and 2QV4 [103,104], respectively (Figures 2.1 and 2.2).

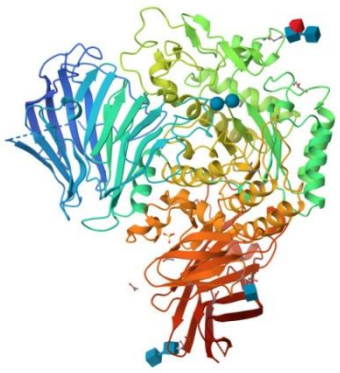


**Figure 2.1.** Three-dimensional structure of α-glucosidase enzyme (3W37), downloaded from PDB

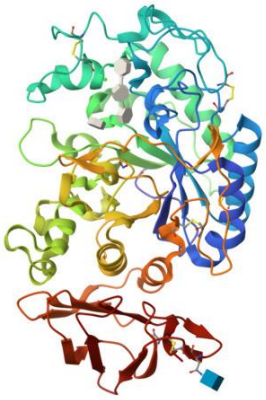


**Figure 2.2.** Three-dimensional structure of α-amylase enzyme (2QV4), downloaded from PDB

## 2.6. Methods

### 2.6.1. Studies on the chemical constituents of *C. diffusa*

#### 2.6.1.1. Preparation of plant extracts

After the preliminary process, the dry powder of *C. diffusa* (5 kg) was extracted with 40 L of methanol (MeOH) by maceration at room temperature within 24 hours, and the extraction was repeated three times. The combined solutions were filtered and concentrated with an evaporator under reduced pressure at 50°C. The total methanol extract (520 g) was then consecutively fractionated with *n*-hexane, ethyl acetate (EtOAc), and distilled water (W). The *n*-hexane extract (142.1 g), ethyl acetate extract (23.4 g), and aqueous extract (285.5 g) were obtained, evaporated to remove the solvents, and stored at 4 – 6°C for further use (Figure 2.3).

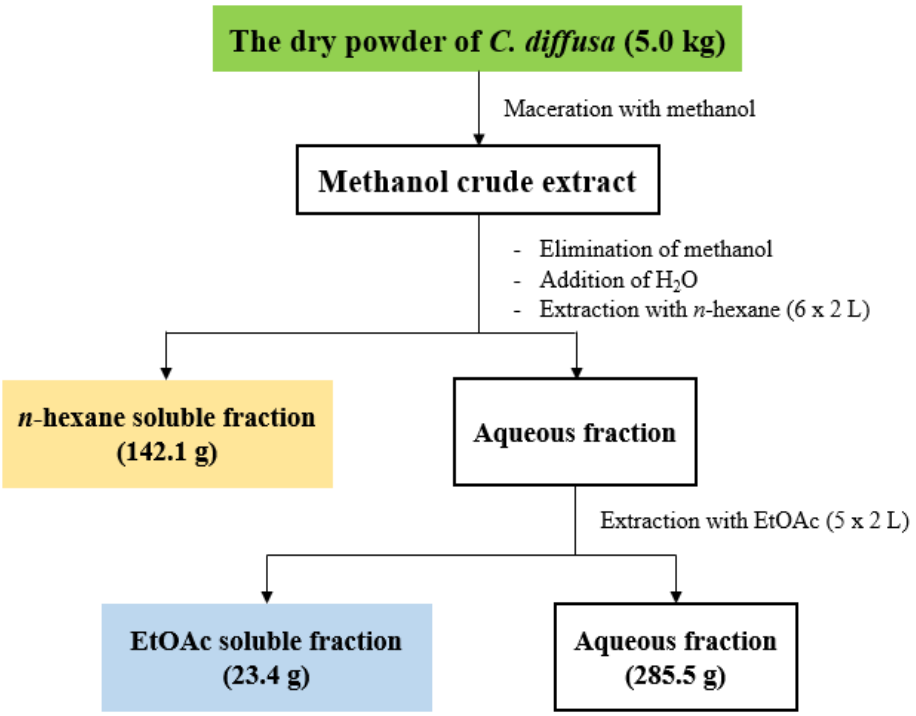



**Figure 2.3.** Extraction of plant materials

### 2.6.1.2. Isolation of pure compounds

Pure compounds from different fractions of *C. diffusa* were isolated by using thin-layer chromatography and column chromatography techniques, as mentioned below.

***Thin-layer chromatography***

Thin-layer chromatography (TLC) is based primarily on the phenomenon of absorption, in which the mobile phase is a solvent or a mixture of solvents. This phase moves across a stationary phase, which is an inert absorbent such as silica gel or alumina oxide. This stationary phase is coated into a thin, uniform layer on a flat substrate such as a glass plate, an aluminum plate, or a plastic plate [105].

In this study, TLC was performed on DC-Alufolien 60 F254 and RP18 F254 pre-coated plates. A solution in a volatile solvent of at least 10 mg/mL of the crude material was prepared for separation. Apply 2-5 µL of this solution to each of several TLC plates. Each plate was developed using different solvent compositions, and the plates were visualized with a UV lamp and/or various sprayer agents (Table 2.1). A solvent system

that put the compound of interest at Rf = 0.2 – 0.3 was chosen. In the case of crude samples, an initial solvent composition with the highest spot at about Rf = 0.5 was chosen, and a final solvent composition with the most retained spot at about Rf = 0.2.

**Table 2.1.** Sprayer agents of TLC tests

| **Reagent** | **Works well for** |
|---|---|
| UV light | Compounds with extended conjugation, like aromatic compounds |
| Sulfuric acid | General stain |
| Cerium sulfate | Good general stain, very good for alkaloids |
| Vanillin | Good general stain, very good for hydroxyl or carbonyl compounds |
| Dragendorff | Amines, even the ones that are low in reactivity |

***Column chromatography***

The column contained a stationary phase of the given packing material. The sample was applied to the top of the packing material. A solvent reservoir was located above, and fractions were collected below. The sizes and dimensions of the glass column were adjusted according to the amount of sample and the required separation capacity.

*Test sample:* Air-dried sample powder was extracted using a suitable solvent (depending on the nature of the work) and vacuum dried before loading into the column. The amount of the sample was measured to define the yield of the desired extract.

*Stationary phase:* Column chromatography with silica gel (Kieselgel 60, 70−230 mesh, and 230−400 mesh, Merck, Darmstadt, Germany) and Sephadex® LH-20 were used. The column chromatography was prepared using the wet packing method.

*Mobile phase:* The choice of solvent depended on the solubility characteristics of the mixture and TLC results. However, the volume of solvent could be dependent on the amount of the sample to be purified.

*Procedure:* The powdered sample mass was placed on the top of the pre-packed silica column, and the samples were covered with a layer of cotton. Then, solvents of different polarities were passed through the column at a uniform rate under gravity to fractionate the sample extract. Each fraction was collected separately in a test tube and numbered consecutively for further analysis on thin-layer chromatography (Figure 2.4).

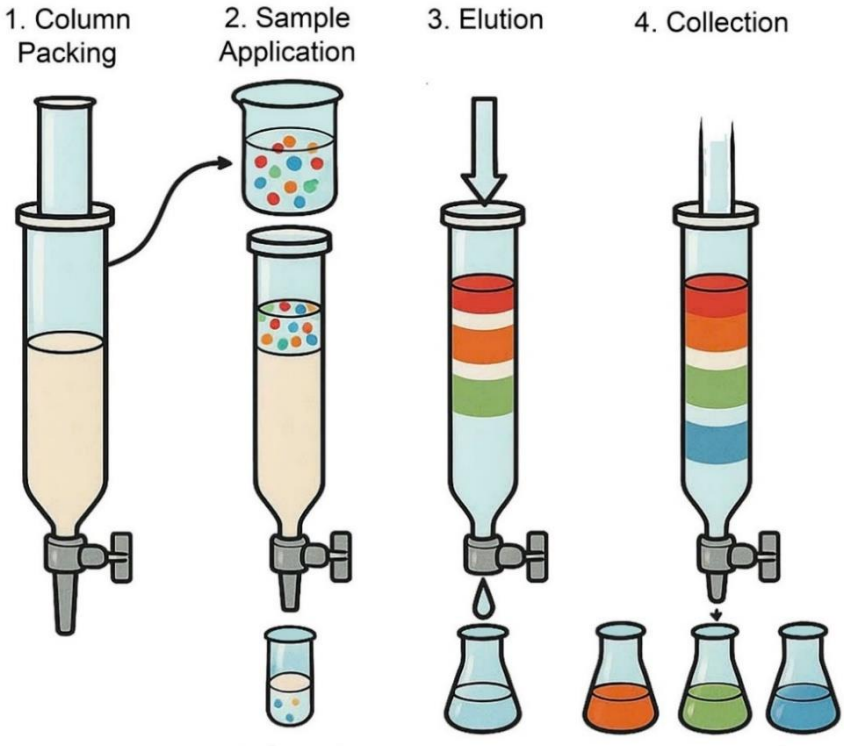



**Figure 2.4.** Procedure of column chromatography

### 2.6.1.3. Structural elucidation of isolated compounds

The isolated compounds were purified by repeated chromatographic separation, with the purification process monitored by TLC. The purity of these compounds was assessed based on their chromatographic behavior and spectroscopic consistency. Their

structures were elucidated through various spectral and chromatographic techniques, including UV, $^{1}$H-NMR, $^{13}$C-NMR, HSQC, HMBC, Mass spectroscopy, melting point, and comparing the data obtained from experiments with published data.

### 2.6.2. Studies on the hypoglycemic effects of *C. diffusa*

#### 2.6.2.1. Enzymatic inhibition assays

***α-Glucosidase enzymatic assay***

*Principle*

The *in vitro* α-glucosidase inhibition assay is a widely used method to evaluate the potential of compounds or extracts to delay carbohydrate digestion, thereby controlling postprandial blood glucose levels. In this method, *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG) is a synthetic substrate for the α-glucosidase enzyme. Upon enzymatic hydrolysis, *p*NPG is cleaved to release *p*-nitrophenol, a yellow-colored compound that can be quantified spectrophotometrically at 405 nm. The presence of an α-glucosidase inhibitor in the reaction mixture reduces the formation of *p*-nitrophenol, corresponding to a decrease in absorbance. The degree of inhibition is calculated by comparing the absorbance of testing samples with that of a control (enzyme without inhibitor). This assay enables the determination of inhibitory concentration ($IC_{50}$) values for potential antidiabetic agents [106].

*Procedure*

The α-glucosidase inhibitory activity of *C. diffusa* was measured using the method described by Moradi-Afrapoli et al. with slight modifications [107]. Phosphate buffer [100 mM potassium phosphate, pH 6.8] was utilized as a buffer solution, whereas *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG) 5 mM was used for enzyme activity measurement. The extracts, fractions, and isolated compounds were initially dissolved in dimethyl sulfoxide (DMSO) to prepare stock solutions at a concentration of 1 mg/mL and subsequently diluted to the required working concentrations for the assay. The α-glucosidase assay was conducted by mixing 50 µL of plant extracts or pure compounds

at different concentrations with 50 µL of the enzyme solution (0.2 U/mL) and incubating for 10 min at 37ºC. Subsequently, 100 µL of the substrate solution was added and incubated for 20 min at 37ºC. Finally, 80 µL of 0.2 M sodium carbonate was added to terminate the reaction. The absorbance was measured at 405 nm using a microplate spectrophotometer. For each sample, assays were run in triplicate. Acarbose was used as the positive control. The percentage of inhibition of the samples was calculated from the obtained absorbance by the following equation:

$$\%_{\text{Inhibition}} = \frac{A_C - A_T}{A_C} \times 100\%$$

Where: $A_C$ is the absorbance of the control;

$A_T$ is the absorbance of the testing samples.

Then, curves were constructed by plotting the percentage of inhibition against concentration in µg/mL. The equation of this curve allowed calculating the $IC_{50}$ corresponding to the sample concentration that inhibits 50% of enzyme activity.

***α-Amylase enzymatic assay***

*Principle*

The *in vitro* inhibition of α-amylase is commonly assessed using the dinitrosalicylic acid (DNSA) method, which quantifies the amount of reducing sugars released during starch hydrolysis. α-Amylase catalyzes the hydrolysis of α-1,4-glycosidic linkages in starch to produce smaller sugar units like maltose and glucose. In the presence of an α-amylase inhibitor, this enzymatic activity is reduced, leading to a lower concentration of reducing sugars. After incubation of the enzyme with the testing sample and starch substrate, the reaction is stopped by adding the DNSA reagent. Upon boiling, DNSA is reduced to 3-amino-5-nitrosalicylic acid, resulting in a reddish-brown color whose intensity is directly proportional to the amount of reducing sugars formed. The absorbance is typically measured at 540 nm using a spectrophotometer. The degree

of color development is directly proportional to the enzyme activity, and inhibition is calculated by comparing the absorbance of the testing samples to the control [108].

*Procedure*

The α-amylase assay was measured using a sodium phosphate buffer solution (6.7 mM NaCl, pH 6.9) and a substrate solution (1% starch in 20 mM buffer solution) [109]. For each test, 10 µL of the testing solutions containing plant extracts or pure compounds dissolved in DMSO at different concentrations were mixed with 10 µL of the enzyme solution (50 U/mL) in test tubes and then incubated for 10 min at 25ºC. After this preincubation, 10 µL of substrate solution was added to each sample, and the mixture was further incubated for 10 min at 25ºC. The reaction was terminated by adding 20 µL of DNSA and was heated in a water bath at 90 – 100ºC for 10 min. Finally, the reaction system was cooled to room temperature and diluted with 300 µL of distilled water. The absorbance was recorded at 540 nm using a UV-VIS spectrophotometer. The negative control was similarly prepared by replacing the testing solution with a buffer solution, while the blank was prepared by the addition of the enzyme solution during the boiling step. Acarbose was utilized as the positive control. The experiments were conducted in triplicate. The $IC_{50}$ values were calculated by regression analysis by measuring the inhibitory activity at different concentrations, as described in the procedure of the α-glucosidase enzymatic assay.

**2.6.2.2. Acute toxicity study**

The oral acute toxicity of the ethyl acetate fraction of *Commelina diffusa* Burm.f. (CD.EAF) was evaluated following the guidelines of the Organisation for Economic Cooperation and Development (OECD Guidelines 423/425) [110,111] for acute toxicity testing. Briefly, the mice were randomly divided into five groups, with 10 mice in each group, and fasted overnight. Each group was orally administered with testing samples at ascending doses of 125, 250, 500, 1000, and 2000 mg/kg. The general condition, behavioral signs of harmfulness, and mortality in each batch were strictly monitored

within 72 hours to determine the median lethal dose ($LD_{50}$) values. Animals that survived for 72 hours were further observed for seven days for any signs of delayed toxicity.

#### 2.6.2.3. *In vivo* hypoglycemic effects of *C. diffusa*

The *in vivo* study was conducted on an experimental model of a high-fat diet and streptozotocin-induced diabetic mice and divided into two stages with some modifications to the previously reported methods [112,113].

*The first stage*

After the acclimation period, mice were assigned randomly into five groups (I, II, III, IV, and V) of 10 mice each.

- Group I (vehicle control) (n = 10): Mice were fed a normal-fat diet (NFD).

- Groups II, III, IV, and V (diabetic control) (n = 40): Mice were fed a high-fat diet (HFD) for 8 weeks. The fructose solution (55%) was added to the food of mice on the high-fat diet.

After an eight-week treatment period, hyperglycemic mice in groups II, III, IV, and V were obtained by a single intraperitoneal injection of 100 mg/kg of streptozotocin (STZ) dissolved in 0.1 M sodium citrate buffer (pH 4.5). At the same time, mice from group I were administered citrate buffer solution as a vehicle control. Blood samples to evaluate glycemia were taken by cutting 1mm from the tip of the tails. Blood glucose level was measured by using a commercial glucometer and expressed as mmol/L. Three days after STZ injection, the mice exhibiting glycemia levels equal to or greater than 10 mmol/L were included in the study as stable diabetic animals.

*The second stage*

In the second stage, the experimental mouse groups were administered orally once daily for 14 days with the following treatment schedule. The CD.EAF was freshly suspended in 0.5% carboxymethyl cellulose solution to obtain homogeneous suspensions at the required concentrations for 100 mg/kg/day and 300 mg/kg/day doses,

which were thoroughly mixed immediately before administration. These doses were selected based on the acute toxicity findings and previous pharmacological literature [117].

- Group I (negative control group): NFD + distilled water

- Group II (diabetic control group): HFD + an injection of STZ 100 mg/kg + distilled water

- Group III (positive control group): HFD + an injection of STZ 100 mg/kg + gliclazide (80 mg/kg/day)

- Group IV (treated group): HFD + an injection of STZ 100 mg/kg + CD.EAF (100 mg/kg/day)

- Group V (treated group): HFD + an injection of STZ 100 mg/kg + CD.EAF (300 mg/kg/day)

Gliclazide was selected as the positive control for the *in vivo* experiment because it is an established oral antihyperglycemic agent and provided an appropriate pharmacological comparator for evaluating the glucose-lowering effect of CD.EAF in the diabetic mouse model.

To determine the antihyperglycemic activity of the CD.EAF, after overnight fasting, the fasting blood glucose level was evaluated just before treatment (day 0) and then at 7 and 14 days post-treatment.

To assess the effect of the CD.EAF on the serum lipid levels of high-fat diet and STZ-induced diabetic mice, on completion of the 14 days of treatment, the overnight fasted animals were anesthetized with halothane and sacrificed. The blood samples were collected by cardiac puncture into sterilized heparin tubes. The serum was separated from the blood by centrifuging at 3000 rpm for 15 minutes. The total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, aspartate aminotransferase, and alanine aminotransferase were examined using the respective commercial diagnostic kits. Besides, the livers and pancreases of three mice in each group were taken for macroscopic observations and histopathology evaluations.

### 2.6.3. Statistical analysis

The experimental results are reported as mean ± standard deviation (SD) and analyzed using Microsoft Excel 2016 and SPSS Statistics version 23.0 (IBM Corp., Armonk, NY, USA) using independent sample *t*-test and one-way analysis of variance (ANOVA), followed by Tukey's test. *P* values less than 0.05 are considered statistically significant.

### 2.6.4. Molecular docking

#### 2.6.4.1. Redock acarbose

The AG (PDB: 3W37) and AA (PDB: 2QV4) complexes contain a co-crystallized ligand called acarbose. Therefore, redocking acarbose is a way to assess the validity of the docking procedure. If the difference in the position of acarbose in the crystal before and after redocking is negligible, the suitability of the docking procedure can be concluded. The redocking procedure is carried out through the following steps:

*Ligand preparation:* This process was carried out with 3 ligand conformations:

(1) Separate the co-crystallized ligand acarbose in the crystals of 3W37 and 2QV4, respectively.

(2) Separate the co-crystallized ligand acarbose and prepare it again in the MOE 2015.10 software.

(3) Draw a new acarbose ligand using ChemDraw Professional 15.0 and prepare it again in MOE version 2015.10.

*Protein preparation:* The crystal structure of AG co-crystallized with acarbose (PDB: 3W37, resolution 1.70 Å) and the crystal structure of AA co-crystallized with acarbose (PDB: 2QV4, resolution 1.97 Å) were obtained from the Protein Data Bank. The water molecules and ligands were removed from the protein structure, while hydrogen was added. Subsequently, the polar hydrogens were optimized, and the Merck

Molecular Force Field (MMFF94) was attached. All these steps were performed using MOE 2015.10 software.

*Docking simulation:* Docking was performed using MOE software with grid box sizes of 60 x 60 x 70 Å for AG and 70 x 70 x 70 Å for AA, respectively, with a grid spacing of 0.375 Å. The grid centers (x,y,z) were set at coordinates (14.103, -27.101, -42.876) for AG and (16.11, 49.57, 25.06) for AA, respectively [114,115]. The results were analyzed using Discovery Studio and MOE software.

*Evaluation of results:* The docking results were evaluated through three criteria, including root mean square deviation (RMSD), interaction ability, and docking score.

- RMSD: This value measures the average deviation of atoms between two conformations. Normally, if RMSD values are equal to or less than 1.5 Å, the deviation can be considered negligible [116].

- Interaction ability: The interactions (hydrogen, π-π, ion, cation-π, Van der Waals interactions) between redocked acarbose and protein were analyzed using MOE software and compared with those of the original acarbose.

- Docking score: In the docking simulation, a lower binding energy is considered closer to the native state of the complex. The scoring function of the docking algorithm is an equation that aggregates the values of parameters along with coefficients according to a certain imposed theory. Each type of interaction is assigned to a value domain. The final result reflects the strength or weakness of the interactions of ligands with the receptor.

#### 2.6.4.2. Virtual screening of isolated compounds from *C. diffusa*

After being validated by the redocking results, this docking procedure was used to virtually screen the isolated compounds from *C. diffusa*. The potential compounds were entered into ICM as structural formulas in .sdf files. ICM applied the MMFF force field, minimized energy, and adjusted the charge at pH 7.0 for the compounds.

The protein docking results were selected based on three criteria:

(1) The docking score was better than the acarbose redocking result.

(2) The conformation had the lowest RMSD.

(3) Good binding with amino acids at the active site.

The results were analyzed using Discovery Studio 2021 software to find interactions of ligands with the crystal structures of AG and AA. This software provided a tool for two-dimensional (2D) and three-dimensional (3D) visualization of interactions between ligands and amino acids in the active site of proteins.

#### 2.6.4.3. Prediction of physicochemical parameters and ADMET properties of isolated compounds from *C. diffusa*

The combination of analyzing the physicochemical parameters, pharmacokinetics, and toxicology properties helps screen and predict the drug-likeness of a chemical compound. In this study, the physical and chemical characteristics of isolated compounds from *C. diffusa*, such as hydrogen bond donors, hydrogen bond acceptors, and octanol–water partition coefficient (LogP), were estimated using the SwissADME online tool (http://www.swissadme.ch/). Meanwhile, the pkCSM online tool (https://biosig.lab.uq.edu.au/pkcsm/) was applied to predict the ADMET parameters with input data as SMILES formulas of isolated compounds.

# Chapter III – Results

## 3.1. Isolation and structural elucidation of pure compounds from *C. diffusa*

### 3.1.1. Isolation of pure compounds

The *n*-hexane residue (65.0 g) was passed through the chromatography column with silica gel adsorbent using a solvent system of *n*-hexane-$CH_2Cl_2$ (15:1). The eluate was collected in tubes and examined by thin-layer chromatography to afford three fractions (H1-H3). The fraction H1 (3.6 g) was chromatographed over silica gel with an elution system of *n*-hexane-$CH_2Cl_2$ (10:1) to obtain four subfractions (H1.1-H1.4). Subfraction H1.3 (502.2 mg) was eluted by silica gel CC with *n*-hexane-EtOAc (7:3) to give compound **1** (21.3 mg). Subfraction H1.4 (758.4 mg) over silica gel CC with a mixture of *n*-hexane-EtOAc (9:1) was purified to afford compound **2** (26.0 mg). Fraction H2 (1.1 g) was subjected to silica gel CC and eluted with a gradient solvent system of *n*-hexane-EtOAc (10:1 to 5:1) to give two subfractions (H2.1 and H2.2). Chromatography of subfraction H2.2 (382.6 mg) over silica gel and elution with *n*-hexane-EtOAc (20:1) provided compound **3** (27.5 mg). The procedure for isolating pure compounds from the *n*-hexane fraction of *C. diffusa* is shown in Figure 3.1.

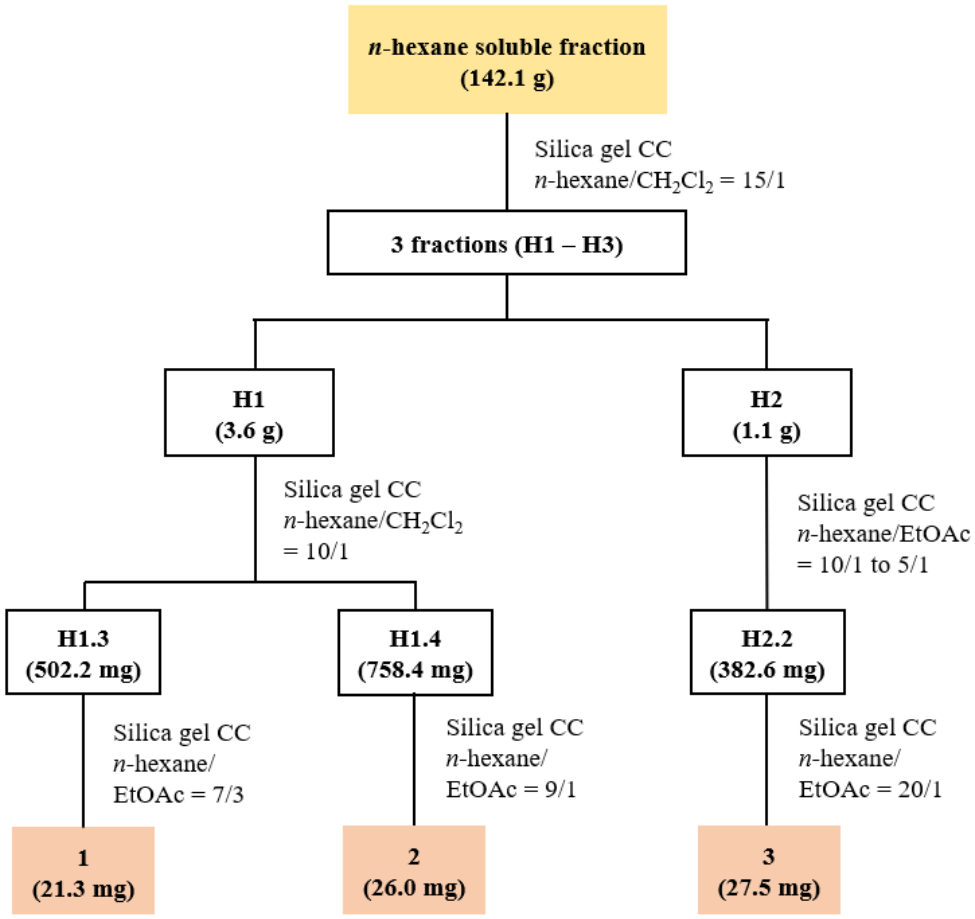
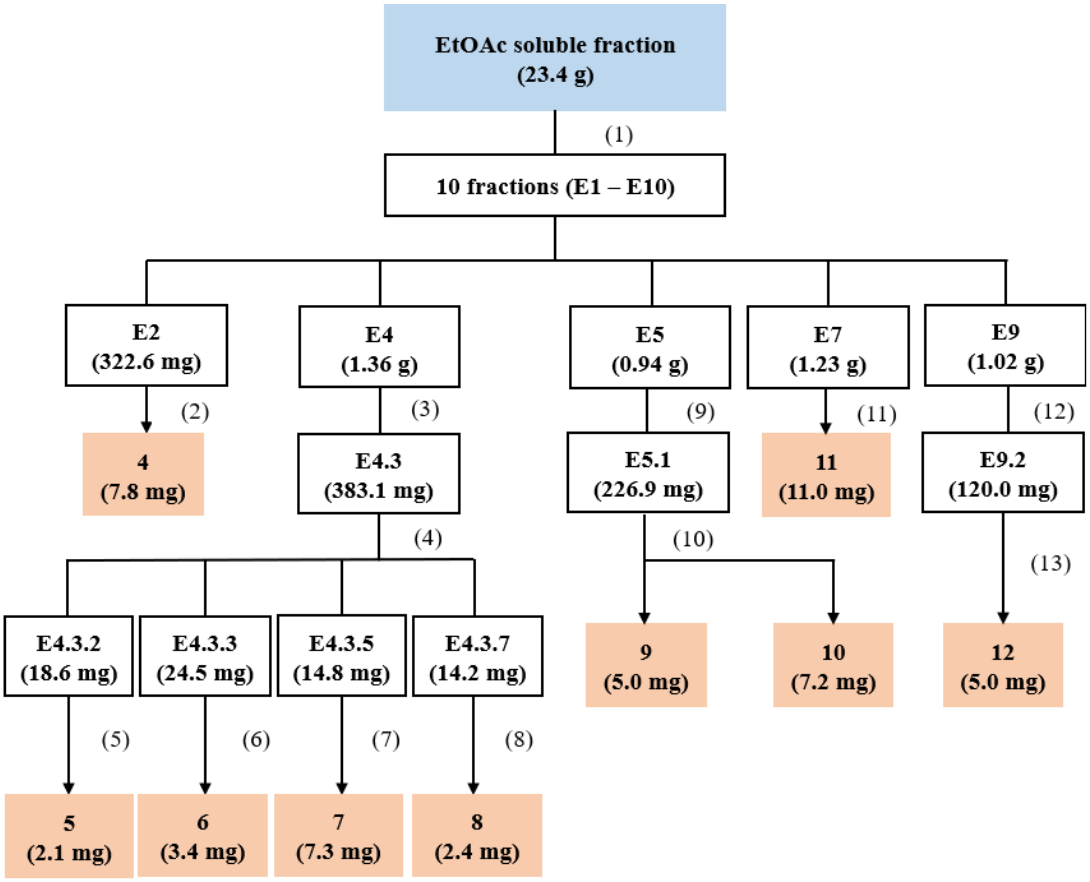



**Figure 3.1.** Isolation process of pure compounds from the *n*-hexane fraction of *C. diffusa*

The ethyl acetate residue (23.4 g) was subjected to silica gel column chromatography (CC), eluting with a gradient solvent system ($CH_2Cl_2$-MeOH, 1:0 to 0:1) to obtain ten fractions (E1-E10). Fraction E2 (322.6 mg) was subjected to silica gel CC, using a mixture of *n*-hexane-EtOAc (4:1), and further purified by Sephadex LH-20 CC with MeOH to afford compound **4** (7.8 mg). Chromatography of fraction E4 (1.36 g) over silica gel and elution with a gradient system of *n*-hexane-EtOAc (2:1 to 1:4) obtained four subfractions (E4.1-E4.4). Subfraction E4.3 (383.1 mg) was further separated by silica gel CC with *n*-hexane-EtOAc (1:1 to 1:2) to provide eight subfractions (E4.3.1-E4.3.8). Subfractions E4.3.2 (18.6 mg), E4.3.3 (24.5 mg), and E4.3.5 (14.8 mg) were purified by Sephadex LH-20 CC with MeOH to afford compounds **5** (2.1 mg), **6** (3.4 mg), and **7** (7.3 mg), respectively. Purification of

subfraction E4.3.7 (14.2 mg) over Sephadex LH-20 CC with MeOH-$H_2O$ (9:1) afforded compound **8** (2.4 mg). Fraction E5 (0.94 g) was eluted by silica gel CC with a mixture of $CH_2Cl_2$-MeOH (9:1) to give three subfractions (E5.1-E5.3). Subfraction E5.1 (226.9 mg) was subjected to silica gel CC, eluting with *n*-hexane-EtOAc (1:4), and further purified by Sephadex LH-20 CC with MeOH to afford compounds **9** (5.0 mg) and **10** (7.2 mg). Fraction E7 (1.23 g) was separated by silica gel CC, eluting with a mixture of $CH_2Cl_2$-MeOH (9:1), followed by a Sephadex LH-20 CC with MeOH-$H_2O$ (4:1) to afford compound **11** (11.0 mg). Chromatography of fraction E9 (1.02 g) over silica gel and elution with a mixture of $CH_2Cl_2$-MeOH-$H_2O$ (5:1:0.1) provided four subfractions (E9.1-E9.4). Purification of subfraction E9.2 (120.0 mg) over silica gel CC with a mixture of $CH_2Cl_2$-MeOH-$H_2O$ (5:1:0.1) gave compound **12** (5.0 mg). The isolation process of pure compounds from the ethyl acetate fraction of *C. diffusa* is described in Figure 3.2.




| No | Stationary phase | Mobile phase |
|---|---|---|
| 1 | Silica gel | $CH_2Cl_2$:MeOH (1:0 to 0:1) |
| 2 | Silica gel | *n*-hexane:EtOAc (4:1) |
| | Sephadex LH-20 | MeOH 100% |
| 3 | Silica gel | *n*-hexane:EtOAc (2:1 to 1:4) |
| 4 | Silica gel | *n*-hexane:EtOAc (1:1 to 1:2) |
| 5 – 7 | Sephadex LH-20 | MeOH 100% |
| 8 | Sephadex LH-20 | MeOH:$H_2O$ (9:1) |
| 9 | Silica gel | $CH_2Cl_2$:MeOH (9:1) |
| 10 | Silica gel | *n*-hexane:EtOAc (1:4) |
| | Sephadex LH-20 | MeOH 100% |
| 11 | Silica gel | $CH_2Cl_2$:MeOH (9:1) |
| | Sephadex LH-20 | MeOH:$H_2O$ (4:1) |
| 12 – 13 | Silica gel | $CH_2Cl_2$:MeOH:$H_2O$ (5:1:0.1) |

**Figure 3.2.** Isolation process of compounds from the ethyl acetate fraction of *C. diffusa*

The aqueous residue (285.5 g) was subjected to Diaion HP-20 CC, using distilled water to eliminate free monosaccharides and mineral salts. Then, it was eluted with a gradient system of MeOH-$H_2O$ (0:1 to 1:0) to provide three fractions (W1-W3). Fraction W2 (11.1 g) was separated by silica gel CC with a mixture of $CH_2Cl_2$-MeOH-$H_2O$ (5:1:0.1) to obtain nine subfractions (W2.1-W2.9). Subfraction W2.2 (812.8 mg) was applied to RP-18 CC with a gradient solvent system of MeOH-$H_2O$ (1:4 to 4:1) to obtain six subfractions (W2.2.1-W2.2.6). Sephadex LH-20 CC further separated subfraction W2.2.4 (54.5 mg) with a mixture of MeOH-$H_2O$ (3:1) to afford compound **13** (12.1 mg). Subfraction W2.6 (437.5 mg) was chromatographed over RP-18, eluting with MeOH-$H_2O$ (1:2) to give five subfractions (W2.6.1-W2.6.5). Purification of subfraction W2.6.2 (39.2 mg) over Sephadex LH-20 CC with MeOH-$H_2O$ (4:1) afforded compound **14** (7.0 mg). The detailed process of isolating compounds from the aqueous residue of *C. diffusa* is demonstrated in Figure 3.3.

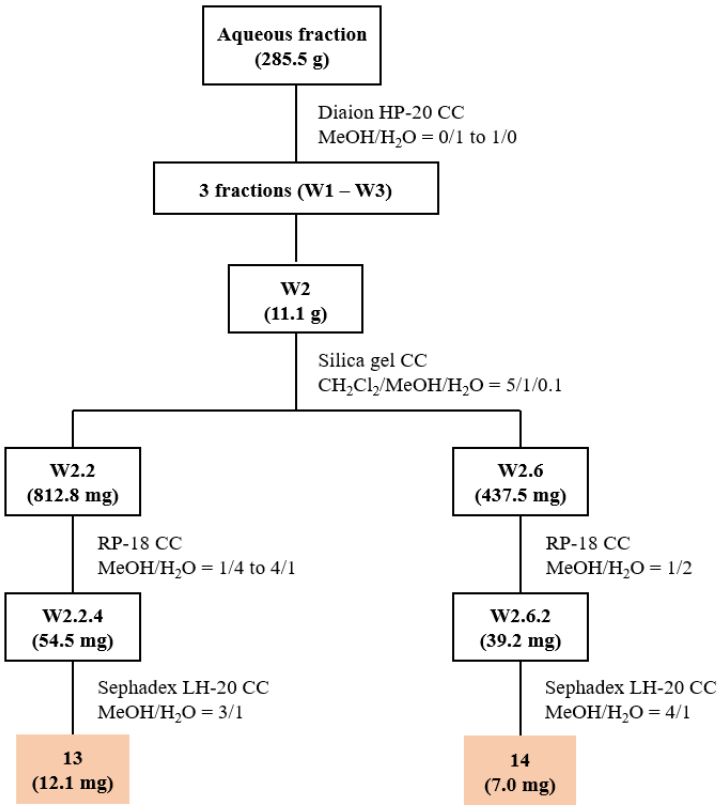



**Figure 3.3.** Isolation process of pure compounds from the aqueous residue of *C. diffusa*

### 3.1.2. Structure elucidation of isolated compounds

Compound **1**: Corosolic acid.

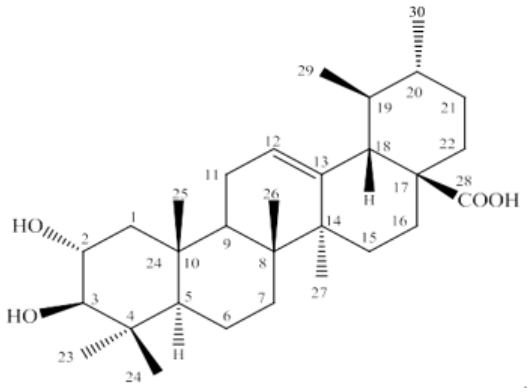



**Figure 3.4.** Chemical structure of compound **1**

Compound **1** was isolated as a white powder. The $^{1}$H-NMR spectrum of compound **1** presented a total of seven methyl groups with singlet signals at $\delta_H$ 0.71 (H-29), 0.75 (H-30), 0.82 (H-25), 0.88 (H-24), 0.91 (H-26), 0.92 (H-27), and 1.03 (H-23). In addition, the $^{1}$H-NMR spectrum also revealed signals of two hydroxy groups attached to the tertiary carbon at $\delta_H$ 4.24 (1H, d, $J$ = 4.8 Hz, H-2) and 4.35 (1H, d, $J$ = 10.8 Hz, H-3), along with one signal of a double bond at $\delta_H$ 5.14 (1H, brs, H-12). The $^{13}$C-NMR and DEPT spectra of this compound showed the presence of 30 carbons, including seven methyl groups at $\delta_C$ 16.5 (C-25), 16.9 (C-26), 17.1 (C-30), 17.2 (C-24), 21.1 (C-29), 23.3 (C-27), 28.8 (C-23), eight methylene groups at $\delta_C$ 18.0 (C-6), 22.9 (C-11), 23.8 (C-16), 27.6 (C-15), 30.2 (C-21), 32.5 (C-7), 36.3 (C-22), 47.1 (C-1), eight methine groups at $\delta_C$ 38.4 (C-19), 38.5 (C-20), 46.9 (C-9), 52.2 (C-18), 54.6 (C-5), 67.2 (C-2), 82.1 (C-3), 124.5 (C-12), and seven quaternary carbon groups at $\delta_C$ 37.6 (C-10), 39.0 (C-4), 39.3 (C-8), 41.7 (C-14), 47.0 (C-17), 138.3 (C-13), 178.2 (C-28). The spectral data of compound **1** were completely identical to those of corosolic acid in reference [117] (Table S1). Therefore, it can be confirmed that compound **1** was corosolic acid.

Compound **2**: 3*α*-acetyl-20*S*,24*S*-epoxydammarane-25-ol.

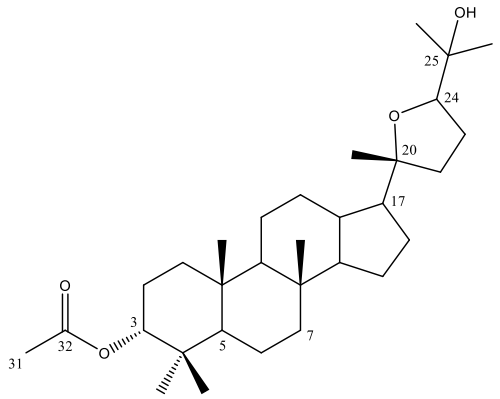



**Figure 3.5.** Chemical structure of compound **2**

Compound **2** was obtained as colorless needle-shaped crystals with a melting point range of 148 – 150°C. The $^{1}$H-NMR spectrum of compound **2** showed proton signals of two methine groups attached to oxygen at $\delta_H$ 4.48 (1H, m, H-3) and 3.72 (1H, t, $J$ = 9.0 Hz, H-24). The $^{1}$H-NMR spectrum also represented proton signals of eight methyl groups at $\delta_H$ 0.85 (3H, s, H-28), 0.87 (3H, s, H-19), 0.88 (3H, s, H-29), 0.90 (3H, s, H-30), 0.96 (3H, s, H-18), 1.11 (3H, s, H-17), 1.13 (3H, s, H-21), 1.20 (3H, s, H-26), and a proton signal of the methyl group attached to the electron-withdrawing group at $\delta_H$ 2.07 (3H, s, Ac-Me). In addition, the presence of proton signals of methylene and methine groups in the $\delta_H$ range from 1.2 to 1.9 ppm was confirmed. The $^{13}$C-NMR spectrum revealed that compound **2** had 32 carbons in total, including nine methyl groups, ten methylene groups, six methine groups, and seven quaternary carbon groups. Two methine carbon signals linked to oxygen at $\delta_C$ 80.9 (C-3), 83.3 (C-24), and two quaternary carbon signals linked to oxygen at $\delta_C$ 83.3 (C-24), 86.4 (C-20) suggested that compound **2** was a triterpenoid with a dammarane skeleton. The interactions on the HMBC spectrum of H-3 ($\delta_H$ 4.48)/C-28 ($\delta_C$ 28.0), C-2 ($\delta_C$ 23.5), C-4 ($\delta_C$ 36.9), C-32 ($\delta_C$ 171.0), and OCO<u>Me</u> ($\delta_H$ 2.07)/C-32 ($\delta_C$ 171.0), C-3 ($\delta_C$ 80.9) demonstrated the presence of an acetoxy group at the C-3 position. In addition, the HMBC spectrum also showed the interactions of H-28 ($\delta_H$ 0.85)/C-28 ($\delta_C$ 28.0), C-3 ($\delta_C$ 80.9), C-4 ($\delta_C$ 36.9), C-5 ($\delta_C$ 50.7), H-17 ($\delta_H$ 1.11)/C-20 ($\delta_C$ 86.4), C-17 ($\delta_C$ 49.5), H-26 ($\delta_H$ 1.20)/C-26

($\delta_C$ 27.5), C-24 ($\delta_C$ 83.3), C-25 ($\delta_C$ 71.4), and H-24 ($\delta_H$ 3.72)/C-26 ($\delta_C$ 27.5), C-27 ($\delta_C$ 24.3). The above results, combined with the spectrum of the published data [118] (Table S2), determined compound **2** as 3α-acetyl-20*S*,24*S*-epoxydammarane-25-ol.

Compound **3**: 11-oxo-α-amyrin acetate.

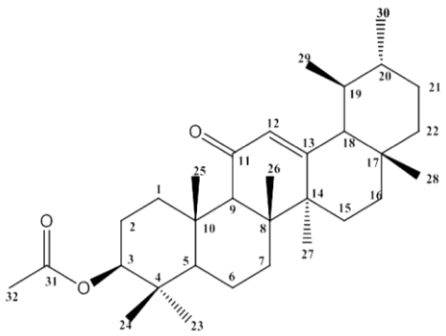


**Figure 3.6.** Chemical structure of compound **3**

Compound **3** was isolated as a white amorphous solid. The $^1$H-NMR spectrum of this compound showed the presence of eight methyl groups at $\delta_H$ 0.81 (3H, d, $J$ = 2.4 Hz, H-29), 0.82 (3H, s, H-28), 0.88 (3H, s, H-23), 0.89 (3H, s, H-24), 0.95 (3H, s, H-30), 1.17 (3H, s, H-26), 1.19 (3H, s, H-25), 1.29 (3H, s, H-27), an acetoxy group at $\delta_H$ 2.05 (3H, s, H-32), and an olefinic proton at $\delta_H$ 5.54 (1H, s, H-12). The $^{13}$C-NMR and HSQC spectra suggested that compound **3** contained 32 carbons in total, including nine methyl groups, eight methylene groups, five methine groups, six saturated quaternary carbons, an acetoxy group at $\delta_C$ 21.3 (COO**C**H$_3$) and 171.0 (**C**OOCH$_3$), an oxymethine group at $\delta_C$ 80.7 (C-3), and a carbonyl group at $\delta_C$ 199.6 (C-11). In the HMBC spectrum, the interactions between H-3 ($\delta_H$ 4.52) with C-23 ($\delta_C$ 28.1), C-24 ($\delta_C$ 16.7), and C-31 ($\delta_C$ 171.0), and between H-32 ($\delta_H$ 2.05) with C-31 ($\delta_C$ 171.0) allowed the determination of the position of the acetoxy group at C-3. In addition, the position of the carbonyl group at C-11 was proven based on the interactions between H-9 ($\delta_H$ 2.35) with C-8 ($\delta_C$ 45.1), C-10 ($\delta_C$ 36.8), C-11 ($\delta_C$ 199.6), C-25 ($\delta_C$ 18.5), and C-26 ($\delta_C$ 16.6). The α configuration of the acetoxy group at the C-3 position was determined through the split form of the H-3 proton as a doublet-doublet transition with a small interaction constant ($J$ = 5.4 Hz) and a large interaction constant ($J$ = 14.4 Hz). Based on the analysis of these results and a comparison with the

spectral data from the literature [119] (Table S3), the structure of compound **3** was identified as 11-oxo-α-amyrin acetate, also known as neoilexonol acetate.

Compound **4**: Stigmasterol

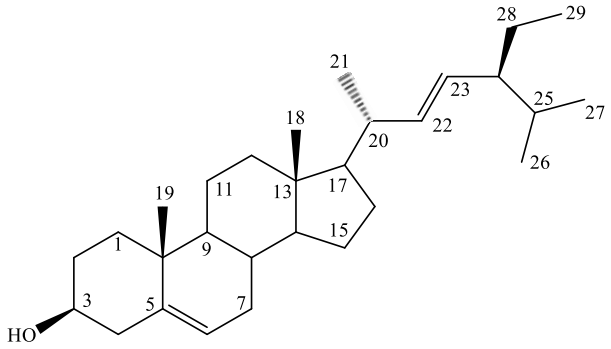



**Figure 3.7.** Chemical structure of compound **4**

Compound **4** was isolated as white needle crystals with a melting point range of 169 – 170°C. The $^{1}$H-NMR spectrum of compound **4** showed the presence of two singlet methyl groups at $\delta_H$ 0.85 (3H, s, H-18), 1.02 (3H, s, H-19), three methyl doublet groups at $\delta_H$ 0.92 (3H, d, $J$ = 6.0 Hz, H-21), 0.83 (3H, d, $J$ = 7.2 Hz, H-26), 0.81 (3H, d, $J$ = 6.8 Hz, H-27), and a triplet methyl group at $\delta_H$ 0.79 (3H, t, $J$ = 9.6 Hz, H-29). In addition, the $^{1}$H-NMR spectrum also showed the signal of olefinic protons at $\delta_H$ 5.35 (1H, brs, H-6), 5.15 (1H, m, H-22), 5.03 (1H, m, H-23), indicating the presence of two double bonds at C-5/C-6 and C-22/C-23. Besides, there was also a characteristic proton signal of the hydroxyl group on the sterol framework at $\delta_H$ 3.51 (1H, m, H-3). The $^{13}$C-NMR and DEPT spectra revealed that compound **4** had 29 carbons in the molecule, including six methyl groups, nine methylene groups, eleven methine groups, and three quaternary carbons. There were carbon signals of double bonds at $\delta_C$ 140.7 (C-5), 121.7 (C-6), 138.3 (C-22), 129.3 (C-23), a carbon signal of an oxymethin group at $\delta_C$ 71.8 (C-3), together with the carbon signal of the six methyl groups at $\delta_C$ 12.0 (C-18), 19.1 (C-19), 19.8 (C-21), 21.2 (C-26), 18.9 (C-27), 12.2 (C-29), respectively. The spectral data for compound **4** were compared with the corresponding values in the literature [120] (Table S4). The agreement between the data allowed the confirmation of compound **4** as stigmasterol.

Compound **5**: Lyratol F.

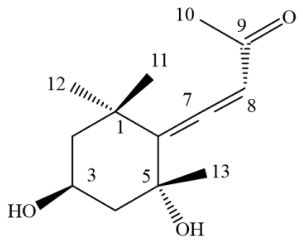



**Figure 3.8.** Chemical structure of compound **5**

Compound **5** was isolated as a colorless powder. The $^{1}$H-NMR spectra showed the presence of signals of four singlet methyl groups at $\delta_H$ 2.21 (3H, s, H-10), 1.41 (3H, s, H-13), 1.41 (3H, s, H-12), 1.12 (3H, s, H-11), along with a signal of an olefinic proton at $\delta_H$ 5.85 (1H, s, H-8), and a signal of an oxymethine group at $\delta_H$ 4.23 (1H, m, H-3). Combining the analysis of the $^{13}$C-NMR and HSQC spectral suggested that compound **5** had 13 carbons including one carbonyl carbon at $\delta_C$ 211.5 (C-9), three unsaturated carbons at $\delta_C$ 200.9 (C-7), 120.0 (C-6), 101.1 (C-8), two quaternary carbons at $\delta_C$ 72.5 (C-5), 37.0 (C-1), two methylene groups at $\delta_C$ 49.7 (C-2), 49.9 (C-4), one oxymethine group at $\delta_C$ 64.4 (C-3), and four methyl groups at $\delta_C$ 26.5 (C-10), 32.3 (C-11), 29.3 (C-12), 30.8 (C-13). The interactions on the HMBC spectrum of H-8 with C-9 and C-7 indicated the binding site of the acetyl group at C-8. Besides, the molecular formula of this compound was determined as $C_{13}H_{20}O_3$ based on the pseudo-molecular positive ion peak signal at *m/z* 246.9 $[M+Na]^+$ on the ESI-MS spectrum. Based on analysis of the obtained results and comparison with previously published reference data [121] (Table S5), compound **5** was identified as lyratol F.

Compound **6**: 4-hydroxybenzoic acid.

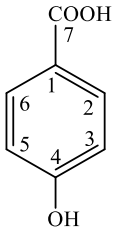



**Figure 3.9.** Chemical structure of compound **6**

Compound **6** was isolated as a colorless solid. The $^{1}$H-NMR spectrum of this compound had two doublet signals of four protons at $\delta_H$ 7.89 (2H, d, $J$ = 8.4 Hz, H-2/H-6), 6.83 (2H, d, $J$ = 8.4 Hz, H-3/H-5), which indicated a para-substituted benzene aromatic ring. Meanwhile, the $^{13}$C-NMR spectrum showed the presence of 7 carbon signals in total, including six carbon atoms of a benzene ring (four methines, two quaternary carbons) and a hydroxyl group at $\delta_C$ 163.3 (C-4). Besides, the molecular formula of this compound was determined as $C_7H_6O_3$ based on the pseudo-molecular positive ion peak signal at *m/z* 136.7 $[M-H]^-$ on the ESI-MS spectrum. Comparing the above results with published literature data [122] (Table S6), compound **6** was identified as 4-hydroxybenzoic acid.

Compound **7**: Methyl gallate.

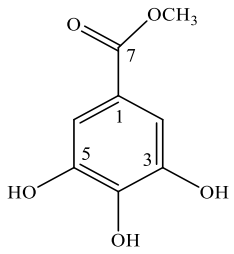



**Figure 3.10.** Chemical structure of compound **7**

Compound **7** was obtained as a white solid. The $^{1}$H-NMR spectrum showed singlet proton signals of a benzene aromatic ring at $\delta_H$ 7.06 (1H, s, H-2), 7.06 (1H, s, H-6), along with a methoxy group at $\delta_H$ 3.83 (3H, s, 7-$OCH_3$). On the $^{13}$C-NMR and DEPT spectra, there were eight carbon signals in total, including six carbons of the aromatic ring at $\delta_C$ 121.5 (C-1), 110.0 (C-2), 146.5 (C-3), 139.8 (C-4), 146.5 (C-5), 110.0 (C-6), one carbon of carbonyl group (>C=O) at $\delta_C$ 169.0 (C-7), and one carbon of methoxy group at $\delta_C$ 52.3 ($OCH_3$). The ESI-MS spectrum showed a pseudo-molecular ion peak signal at *m/z* 182.7 $[M-H]^-$, which indicated the molecular formula of compound **7** as $C_8H_8O_5$. According to the analysis of spectral data and spectral comparison with published literature [123] (Table S7), compound **7** was identified as methyl gallate.

Compound **8**: *N-trans*-feruloyltyramine.

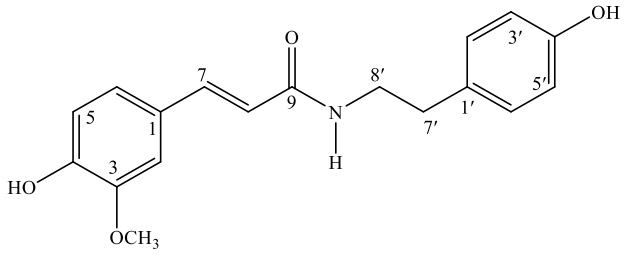



**Figure 3.11.** Chemical structure of compound **8**

Compound **8** was isolated and purified as an amorphous solid. The $^{1}$H-NMR spectrum showed that compound **8** contained two spin systems, including ABX and $A_2B_2$. The ABX spin system was recorded at $\delta_H$ 7.14 (1H, d, *J* = 1.8 Hz, H-2), 7.05 (1H, dd, *J* = 2.4, 8.4 Hz, H-6), 6.82 (1H, d, *J* = 8.4 Hz, H-5) and $A_2B_2$ spin system was appeared at $\delta_H$ 7.08 (1H, d, *J* = 8.4 Hz, H-2'), 7.08 (1H, d, *J* = 8.4 Hz, H-6'), 6.75 (1H, d, *J* = 8.4 Hz, H-3'), 6.75 (1H, d, *J* = 8.4 Hz, H-5'). In addition, there were signals of the olefinic proton, which characterized a double bond outside the benzene ring at $\delta_H$ 7.46 (1H, d, *J* = 15.6 Hz, H-7), 6.43 (1H, d, *J* = 15.6 Hz, H-8). The *trans* configuration of the double bond was validated by a large interaction constant with *J* = 15.6 Hz. The feruloyl moiety was determined through $^{13}$C-NMR and HSQC spectral data with the resonance signals at $\delta_C$ 131.3 (C-1), 111.6 (C-2), 149.3 (C-3), 149.9 (C-4), 116.5 (C-5), 123.2 (C-6), 142.0 (C-7), 118.8 (C-8), 169.2 (C-9). Similar to compounds **9** and **10**, compound **8** also had specific signals of the tyramine group at $\delta_H$ 7.08 (1H, d, *J* = 8.4 Hz, H-2')/$\delta_C$ 130.7 (C-2'), $\delta_H$ 7.08 (1H, d, *J* = 8.4 Hz, H-6')/$\delta_C$ 130.7 (C-6'), $\delta_H$ 6.75 (1H, d, *J* = 8.4 Hz, H-3')/ $\delta_C$ 116.3 (C-3'), $\delta_H$ 6.75 (1H, d, *J* = 8.4 Hz, H-5')/$\delta_C$ 116.3 (C-5'), $\delta_H$ 3.48 (2H, t, *J* = 7.2 Hz, H-8')/$\delta_C$ 35.8 (C-8'), and $\delta_H$ 2.77 (2H, t, *J* = 7.2 Hz, H-7')/$\delta_C$ 42.5 (C-7'). The interactions on the HMBC spectrum of H-3-$OCH_3$ ($\delta_H$ 3.90)/C-3 ($\delta_C$ 149.3), H-7' ($\delta_H$ 2.77)/C-1' ($\delta_C$ 128.3), C-2' ($\delta_C$ 130.7), C-6' ($\delta_C$ 130.7), and H-8' ($\delta_H$ 3.48)/C-9 ($\delta_C$ 169.2) revealed the location of the methoxyl group at C-3 and the amide linkage between the C-9 of the feruloyl moiety and the C-8' of the tyramine moiety. The molecular formula of compound **8** was determined as $C_{18}H_{19}NO_4$ based on the pseudo-

molecular ion peak signal on the ESI-MS spectrum at *m/z* 311.9 $[M-H]^-$. Compound **8** was identified as *N-trans*-feruloyltyramine by detailed analysis of the spectral data and spectral comparison with the literature [124] (Table S8).

Compound **9**: *N-trans-p*-coumaroyl-3',4'-dihydroxyphenylethylamine.

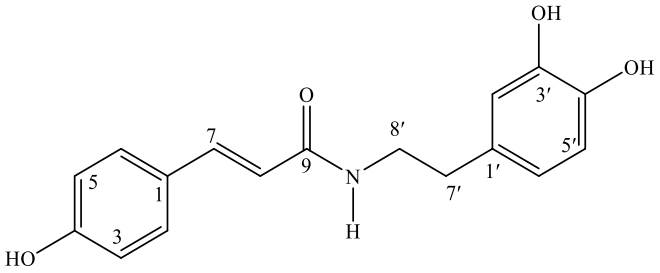



**Figure 3.12.** Chemical structure of compound **9**

Compound **9** was obtained as an amorphous solid. The $^1$H-NMR spectrum showed two spin systems that were typical signals for the presence of benzene aromatic rings including the ABX spin system at $\delta_H$ 6.72 (1H, d, *J* = 8.4 Hz, H-5'), 6.69 (1H, d, *J* = 1.8 Hz, H-2'), 6.58 (1H, dd, *J* = 2.4, 8.4 Hz, H-6'), and the $A_2B_2$ spin system at $\delta_H$ 7.41 (1H, d, *J* = 8.4 Hz, H-2), 7.41 (1H, d, *J* = 8.4 Hz, H-6), 6.81 (1H, d, *J* = 8.4 Hz, H-3), 6.81 (1H, d, *J* = 8.4 Hz, H-5). Besides, there were also signals of a pair of doublets at $\delta_H$ 7.47 (1H, d, *J* = 15.6 Hz, H-7), 6.41 (1H, d, *J* = 15.6 Hz, H-8) with a large separation constant (*J* = 15.6 Hz), which characterized the presence of a double bond outside the benzene ring. Hence, the configuration of the double bond was determined as *trans*. The signals on the $^{13}$C-NMR and HSQC spectra revealed that the structure of compound **9** was made up of two *N-trans*-p-coumaroyl moieties along with a tyramine moiety. The interactions between H-7' with C-1, C-2', C-6', and H-8' with C-9, C-1' suggested the presence of an amide bond between C-9 and C-8'. In addition, the molecular formula of compound **9** was determined to be $C_{17}H_{17}NO_4$ based on the pseudo-molecular ion peak signal on the ESI-MS spectrum at *m/z* 297.9 $[M-H]^-$. The detailed analysis of spectral data and spectral comparison with the literature [125] (Table S9) allowed the determination of compound **9** as *N-trans-p*-coumaroyl-3',4'-dihydroxyphenylethylamine.

Compound **10**: 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide.

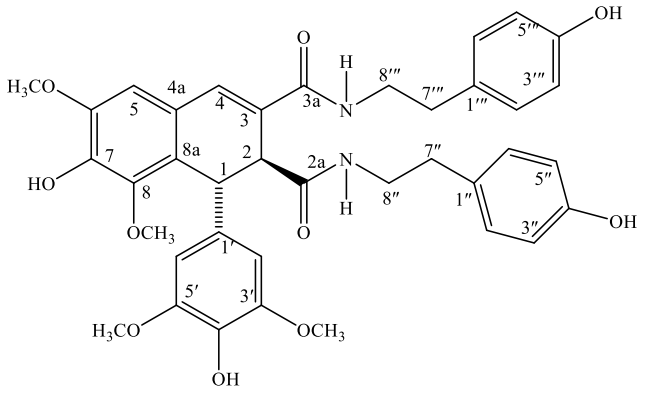



**Figure 3.13.** Chemical structure of compound **10**

Compound **10** was isolated as an amorphous solid. The $^1$H-NMR spectrum had the presence of two $A_2B_2$ spin systems at $\delta_H$ 6.85 (1H, d, $J$ = 8.4 Hz, H-2"), 6.85 (1H, d, $J$ = 8.4 Hz, H-6"), 6.65 (1H, d, $J$ = 8.4 Hz, H-3"), 6.65 (1H, d, $J$ = 8.5 Hz, H-5") and 6.96 (1H, d, $J$ = 8.4 Hz, H-6'"), 6.96 (1H, d, $J$ = 8.4 Hz, H-2'"), 6.68 (1H, d, $J$ = 8.4 Hz, H-3'"), 6.68 (1H, d, $J$ = 8.4 Hz, H- 5'"). Besides, on the $^1$H-NMR spectrum of this compound, there were proton signals of four methylene groups at $\delta_H$ 3.43 (1H, m, H-8a"), 3.35 (1H, m, H-8b"), 3.35 (1H, m, H-8a'"), 3.23 (1H, m, H-8b'"), 2.70 (2H, t, $J$ = 7.2 Hz, H-7"), 2.56 (2H, m, H -7'"). These data suggested that compound **10** had two tyramine moieties in the molecule. Three singlet signals were also confirmed at $\delta_H$ 6.79 (1H, s, H-5), 6.35 (1H, s, H-2'), 6.35 (1H, s, H-6') together with the signal of the olefinic proton at δH 7.30 (1H, s, H-4). The binding sites of the two tyramine moieties were demonstrated on the HMBC spectrum by interactions of H-8" with C-2a, and H-8'" with C-3a. In addition, the positions of four methoxyl groups were determined at C-6, C-8, C-3', and C-5', respectively. The chemical shift of two methine groups at $\delta_H$ 4.97 (1H, brs, H-1), 3.72 (1H, overlapped, H-2) together with reference data indicated that these two protons were in a *trans* form, similar to lignanamides isolated from *Procelia macrocarpa* species [126]. The molecular formula was determined as $C_{38}H_{40}N_2O_{10}$ based on the ESI-MS spectral signal at *m/z* 683.2 $[M-H]^-$. By analyzing 1D, 2D-NMR spectra

and comparing with previously published reference data (Table S10), compound **10** was identified as 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide.

Compound **11**: Daucosterol.

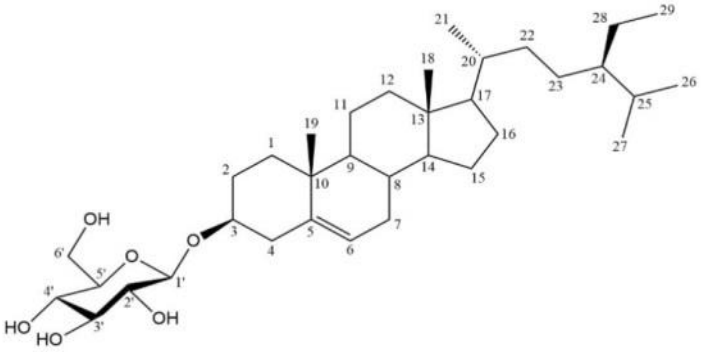



**Figure 3.14.** Chemical structure of compound **11**

Compound **11** was isolated as a white, amorphous powder that turned pink with cerin sulfate. The $^1$H-NMR spectrum of compound **11** showed six resonance signals of the methyl group at $\delta_H$ 0.62 (3H, s, H-18), 0.84 (3H, m, H-27), 0.86 (3H, s, H-26), 0.88 (3H, m, H-29), 0.92 (3H, m, H-19), 0.95 (3H, m, H-21). A proton at C-3 appeared as a multiplet at $\delta_H$ 4.25 (1H, m). In addition, an anomer proton signal at $\delta_H$ 4.99 (1H, d, $J$ = 7.8 Hz, H-1') showed that compound **11** had a $\beta$-glucopyranose residue with a large interaction constant. The $^{13}$C-NMR and DEPT spectra showed that compound **11** contained 35 carbons in the structure, where the resonance signals of 29 carbon atoms of the $\beta$-sitosterol framework included six methyl groups at $\delta_C$ 12.0 (C-18), 19.3 (C-19), 18.9 (C-21), 19.8 (C-26), 19.1 (C-27), 11.8 (C-29); eleven methylene groups at $\delta_C$ 37.3 (C-1), 30.0 (C-2), 39.8 (C-4), 32.0 (C-7), 21.1 (C-11), 39.1 (C-12), 24.3 (C-15), 28.4 (C-16), 34.1 (C-22), 26.3 (C-23), 23.2 (C-28); nine methine groups at $\delta_C$ 78.1 (C-3), 121.8 (C-6), 31.9 (C-8), 50.2 (C-9), 56.7 (C-14), 56.1 (C-17), 36.2 (C-20), 45.9 (C-24), 29.3 (C-25), and three quaternary carbons at δC 140.8 (C-5), 36.8 (C-10), 42.3 (C-13). In addition, six resonance signals proved the presence of a $\beta$-glucopyranose residue at $\delta_C$ 102.3 (C-1'), 75.0 (C-2'), 78.2 (C-3'), 71.4 (C-4'), 78.1 (C-5'), 62.5 (C-6'). According

to the obtained spectral data, combined with the spectra of the published data [127] (Table S11), it can be concluded that compound **11** was daucosterol.

Compound **12**: Quercitrin.

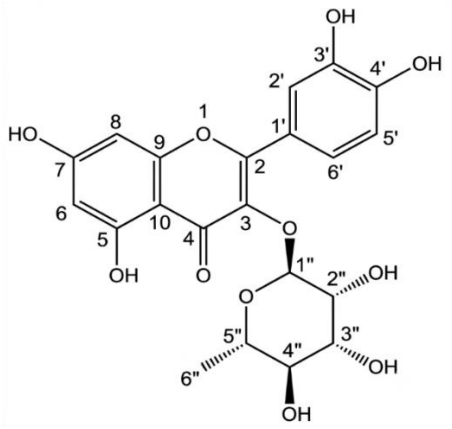



**Figure 3.15.** Chemical structure of compound **12**

Compound **12** was obtained as a yellow, amorphous powder. The $^{1}$H-NMR spectrum of compound **12** showed signals of three aromatic protons at $\delta_H$ 7.36 (1H, d, $J$ = 2.4 Hz, H-2'), 7.33 (1H, dd, $J$ = 2.4, 8.4 Hz, H-6'), 6.94 (1H, d, $J$ = 8.4 Hz, H-5'), which suggested the presence of an ABX aromatic ring system. Signals of two aromatic protons at $\delta_H$ 6.40 (1H, d, $J$ = 2.4 Hz, H-8) and 6.23 (1H, d, $J$ = 2.4 Hz, H-6) also suggested the presence of an AX aromatic ring system in the structure. These data were specific to a flavonoid compound. In addition, the $^{1}$H-NMR spectrum which appeared as an anomeric proton signal at $\delta_H$ 5.37 (1H, d, $J$ = 1.2 Hz, H-1") along with the proton signals of oxymethine groups at $\delta_H$ 4.23 (1H, dd, $J$ = 1.8, 3.6 Hz, H-2"), 3.77 (1H, dd, $J$ = 3.6, 9.6 Hz, H-3"), 3.35 (1H, m, H-4"), 3.37 (1H, m, H-5"), and doublet of the methyl group at $\delta_H$ 0.96 (3H, d; $J$ = 6.0 Hz, H-6") suggested the structure of an α-L-rhamnopyranoside sugar moiety. The $^{13}$C-NMR and DEPT spectra of compound **12** showed signals of 21 carbons, including one methyl group, ten methine groups, and ten quaternary carbons. Two aromatic ring systems (ABX and AX) were confirmed by the signals of five methine groups at $\delta_C$ 117.0 (C-2'), 116.4 (C-5'), 123.0 (C-6'), 99.8 (C-6), 94.7 (C-8). Meanwhile, the appearance of a carbonyl group signal at $\delta_C$ 179.7 (C-4) along

with the carbon signals of the oxygen-bonded aromatic ring at $\delta_C$ 136.2 (C-3), 163.2 (C-5), 165.9 (C-7), 146.4 (C-3'), 149.8 (C-4') allowed us to determine the structure of a flavonol framework. Five signals of methine bound to oxygen at $\delta_C$ 103.6 (C-1"), 71.9 (C-2"), 72.1 (C-3"), 73.3 (C-4"), 72.1 (C-5"), and a methyl signal at $\delta_C$ 17.6 (C-6") confirmed the presence of a rhamnopyranoside group. Thus, compound **12** can be identified as a flavonol monoglycoside. The NMR spectral data of compound **12** were similar to those reported in the reference literature for quercitrin [128] (Table S12). Moreover, the ESI-MS demonstrated a pseudo-molecular ion peak at *m/z* 446.9 $[M-H]^-$, which revealed a molecular formula of $C_{21}H_{20}O_{11}$. Therefore, it can be confirmed that compound **12** is quercitrin (quercetin-3-O-α-L-rhamnopyranoside).

Compound **13**: Benzyl-O-β-D-glucopyranoside.

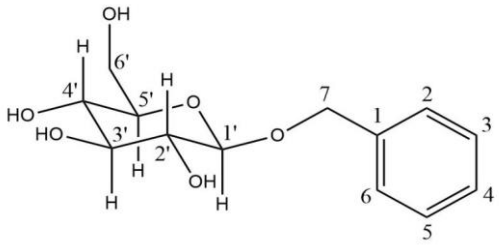



**Figure 3.16.** Chemical structure of compound **13**

Compound **13** was isolated as a white powder. The $^1$H-NMR spectrum of compound **13** revealed the presence of a benzene ring at $\delta_H$ 7.29 (1H, t, *J* = 8.4 Hz, H-4), 7.35 (2H, t, *J* = 9.0 Hz, H-3/H-5), 7.44 (2H, d, *J* = 9.0 Hz, H-2/H-6). In addition, the $^1$H-NMR spectrum also demonstrated proton signals of a methylene group at $\delta_H$ 4.69 (1H, d, *J* = 14.4 Hz, H-7α), 4.95 (1H, d, *J* = 14.4 Hz, H-7β), an anomeric proton of sugar at $\delta_H$ 4.38 (1H, d, *J* = 9.6 Hz, H-1'), along with protons of the sugar molecule resonating at $\delta_H$ 3.27 – 3.91 (m, H-Glc). The $^{13}$C-NMR and DEPT spectra showed signals of 13 carbons, including one quaternary carbon at $\delta_C$ 139.1 (C-1), five methine groups bearing double bonds of the aromatic ring at $\delta_C$ 128.7 (C-4), 129.2 (C-2/ C-6), 129.3 (C-3/C-5), two methylene groups at $\delta_C$ 62.8 (C-6'), 71.8 (C-7), and five methine groups at $\delta_C$ 71.7 (C-4'), 75.1 (C-2'), 78.0 (C-3'), 78.1 (C-5'), 103.3 (C-1'). The $^1$H-NMR and $^{13}$C-NMR

spectral data suggested that compound **13** had a benzene ring and a sugar moiety. Besides, the HMBC spectrum showed that the anomeric proton of the sugar molecule at $\delta_H$ 4.38 (1H, d, *J* = 9.6 Hz, H-1') correlated with $\delta_C$ 71.8 (C-7). Therefore, it could be concluded that the benzene ring was attached at the C-1' position of the sugar moiety. Furthermore, the ESI-MS spectral data showed a pseudo-molecular ion peak at *m/z* 287.2 $[M-H+H_2O]^-$. Therefore, the molecular weight of this compound was 270, corresponding to the molecular formula of $C_{13}H_{18}O_6$. From the above spectral data and combined with reference [129] (Table S13), it was possible to confirm that compound **13** was benzyl-7-O-β-D-glucopyranoside.

Compound **14**: Isoschaftoside.

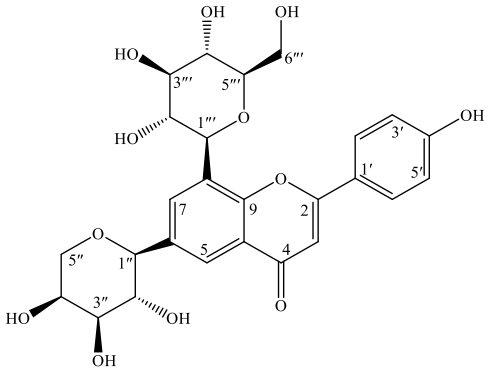



**Figure 3.17.** Chemical structure of compound **14**

Compound **14** was obtained as a yellow powder. The $^1$H-NMR spectrum showed the characteristic signals of the $A_2B_2$ spin system at $\delta_H$ 8.03 (2H, d, *J* = 9.0 Hz, H-2'/H-6'), 6.90 (2H, d, *J* = 9.0 Hz, H-3'/H-5') together with a singlet signal at 6.80 (1H, s, H-3). In addition, there were two doublet signals at $\delta_H$ 4.76 (1H, d, *J* = 10.2 Hz, H-1'''), 4.72 (1H, d, *J* = 9.6 Hz, H-1'') representing the presence of two sugar moieties of the C-linked glycosides. The $^{13}$C-NMR and HSQC spectra confirmed that compound **14** had 26 carbons including 15 carbon signals of the apigenin framework at $\delta_C$ 182.2 (C-4), 164.1 (C-7), 161.2 (C-2), 160.8 (C-4'), 158.2 (C-5), 155.0 (C-9), 129.0 (C-2'/C-6'), 121.5 (C-1'), 115.8 (C-3'/C-5'), 108.0 (C-6), 105.1 (C-8), 103.7 (C-3), 102.6 (C-10), and 11 carbons of two sugar moieties at $\delta_C$ 81.9 (C-5'''), 78.8 (C-3'''), 74.2 (C-1''), 73.8 (C-3''),

73.2 (C-1'''), 70.9 (C-2'''), 70.5 (C-4'''), 70.0 (C-5''), 69.6 (C-2''), 68.4 (C-4''), 61.2 (C-6'''). The interactions on the HMBC spectrum of H-1'' ($\delta_H$ 4.72)/C-5 ($\delta_C$ 158.2), C-6 ($\delta_C$ 108.0), and H-1''' ($\delta_H$ 4.76)/C-8 ($\delta_C$ 105.1), C-9 ($\delta_C$ 155.0) suggested the positions of the sugar moieties in the molecular formula. Additionally, the molecular formula of this compound was determined as $C_{26}H_{28}O_{14}$ based on the pseudo-molecular ion peak signal on the ESI-MS spectrum at *m/z* 565.0 $[M+H]^+$. According to the above data and spectral comparison with published reference data [130] (Table S14), compound **14** was identified as isoschaftoside.

### 3.2. Evaluation of the hypoglycemic effects of *C. diffusa*

#### 3.2.1. Enzymatic inhibition of the extracts and fractions of *C. diffusa*

*C. diffusa* extracts and fractions were screened for their antihyperglycemic capacity by evaluating the inhibitory effects on α-glucosidase and α-amylase enzymes according to the method described in section 2.6.2.1. The tested concentrations of the extracts and fractions from *C. diffusa* included 12.5, 25, 50, 100, and 200 µg/mL. Acarbose was used as the positive control. The percentage of inhibition of testing samples at different concentrations and their $IC_{50}$ values are presented in Tables 3.1 and 3.2.

**Table 3.1.** $IC_{50}$ values of *C. diffusa* extracts and fractions against the α-glucosidase enzyme

| **Extracts/ Fractions** | **% inhibition at different concentrations (µg/mL)** | | | | | **$IC_{50}$ (µg/mL)** |
|---|---|---|---|---|---|---|
| | **12.5** | **25** | **50** | **100** | **200** | |
| **MeOH** | 25.17 ± 0.78 | 45.39 ± 0.84 | 59.23 ± 1.42 | 71.83 ± 0.48 | 80.44 ± 1.25 | 36.23 ± 0.46 |
| ***n*-hexane** | 6.43 ± 0.98 | 17.57 ± 1.35 | 28.05 ± 1.13 | 38.94 ± 1.17 | 58.46 ± 1.62 | 152.19 ± 1.39 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **EtOAc** | 40.26 ± 1.90 | 51.14 ± 1.63 | 66.53 ± 1.29 | 80.32 ± 2.58 | 97.19 ± 2.33 | 21.88 ± 2.47 |
| **$H_2O$** | 10.29 ± 1.04 | 18.66 ± 1.66 | 35.14 ± 0.73 | 52.37 ± 1.34 | 67.07 ± 0.94 | 93.59 ± 1.13 |
| **Acarbose** | 8.14 ± 0.69 | 15.79 ± 2.02 | 29.90 ± 1.39 | 41.45 ± 1.24 | 60.71 ± 1.56 | 135.73 ± 1.79 |

**Table 3.2.** $IC_{50}$ values of *C. diffusa* extracts and fractions against the α-amylase enzyme

| **Extracts/ Fractions** | **% inhibition at different concentrations (µg/mL)** | | | | | **$IC_{50}$ (µg/mL)** |
|---|---|---|---|---|---|---|
| | **12.5** | **25** | **50** | **100** | **200** | |
| **MeOH** | 32.92 ± 1.08 | 45.69 ± 1.96 | 63.59 ± 1.75 | 72.84 ± 2.60 | 89.36 ± 2.47 | 29.20 ± 1.95 |
| ***n*-hexane** | 8.63 ± 0.32 | 16.04 ± 1.19 | 33.73 ± 0.87 | 41.84 ± 1.22 | 58.22 ± 1.05 | 138.25 ± 1.15 |
| **EtOAc** | 34.95 ± 1.28 | 48.52 ± 0.99 | 67.47 ± 1.57 | 76.13 ± 1.14 | 93.10 ± 1.30 | 25.46 ± 1.32 |
| **$H_2O$** | 24.51 ± 1.89 | 31.96 ± 2.13 | 46.09 ± 2.75 | 65.28 ± 2.64 | 76.59 ± 2.43 | 52.81 ± 2.66 |
| **Acarbose** | 17.91 ± 1.44 | 22.64 ± 1.83 | 37.15 ± 2.30 | 57.10 ± 2.05 | 66.59 ± 1.59 | 83.33 ± 2.02 |

According to Tables 3.1 and 3.2, in both assays, the potential antihyperglycemic effects of *C. diffusa* extracts and fractions were in the same ascending order: *n*-hexane <

$H_2O$ < MeOH < EtOAc. In general, the MeOH extracted displayed enzymatic inhibitory activities with $IC_{50}$ values of 36.23 ± 0.46 µg/mL against α-glucosidase and 29.20 ± 1.95 µg/mL against α-amylase, which were significantly higher than those of the positive control, acarbose, with $IC_{50}$ values of 135.73 ± 1.79 µg/mL for the former assay and 83.33 ± 2.02 µg/mL for the latter assay, respectively. Among fractions, the ethyl acetate fraction (CD.EAF) was the most active fraction with the lowest $IC_{50}$ values of 21.88 ± 2.47 µg/mL in the α-glucosidase method and 25.46 ± 1.32 µg/mL in the α-amylase method, respectively. Therefore, this fraction was chosen to evaluate the acute toxicity and *in vivo* antihyperglycemic activity assays.

### 3.2.2. Acute toxicity of CD.EAF

The acute toxicity of CD.EAF was evaluated following the procedure expressed in section 2.6.2.2 at ascending doses of 125, 250, 500, 1000, and 2000 mg/kg. The detailed results are shown in Table 3.3.

**Table 3.3.** Acute toxicity of CD.EAF

| Doses (mg/kg) | Mortality | Toxicity observed |
|---|---|---|
| 125 | 0/10 | None |
| 250 | 0/10 | None |
| 500 | 0/10 | None |
| 1000 | 0/10 | None |
| 2000 | 0/10 | None |

As seen from Table 3.3, there was no mortality in any of the mouse groups treated with the CD.EAF at doses from 125 mg/kg to 2000 mg/kg. When using a maximum dose of 2000 mg/kg, mice were still healthy, ate, excreted, and moved naturally. After the

testing period, CD.EAF did not generate any visible toxicity in mice. Therefore, the $LD_{50}$ index of CD.EAF was thus above 2000 mg/kg.

### 3.2.3. Hypoglycemic effects of CD.EAF on the high-fat diet and streptozotocin-induced type 2 diabetic mice

#### 3.2.3.1. Effects of HFD on the body weight of mice

The body weight changes of mice fed with HFD and NFD were estimated at different time points: Before studying, after 4 weeks, after 6 weeks, and after 8 weeks. Table 3.4 demonstrates the effects of HFD on the body weight of the tested animals.

**Table 3.4.** Effects of HFD on the body weight of mice

| **Time points** | **Body weight (g)** | |
|---|---|---|
| | **Group I: NFD** | **Groups II, III, IV, V: HFD** |
| Before studying | 25.86 ± 2.64 | 25.34 ± 2.20 |
| After 4 weeks | 28.65 ± 2.12$^{*}$ | 41.02 ± 6.98$^{***###}$ |
| After 6 weeks | 29.78 ± 3.26$^{*}$ | 43.86 ± 9.21$^{***###}$ |
| After 8 weeks | 34.03 ± 3.31$^{*}$ | 49.01 ± 8.84$^{***###}$ |

*Data are represented as mean ± SD. $^{*}p < 0.05$ and $^{***}p < 0.001$ compared with the time point "Before studying", $^{###}p < 0.001$ compared with group I. NFD: Normal-fat diet, HFD: High-fat diet.*

After 8 weeks, the body weight of the mice group fed with NFD increased remarkably ($p < 0.05$), while a dramatic rise was seen in the body weight of mice in the groups fed with HFD ($p < 0.001$) as compared with that before studying. Furthermore, at all evaluation time points, the body weight of the mice groups treated with HFD was significantly higher than that of the group treated with NFD ($p < 0.001$).

### 3.2.3.2. Effects of HFD on serum glucose level

The impact of HFD on the blood glucose levels of treated mice at three evaluated time points is expressed in Table 3.5.

**Table 3.5.** Effects of HFD on blood glucose levels

| **Time points** | **Blood glucose levels (mmol/L)** | | ***p* (compared with group I)** |
|---|---|---|---|
| | **Group I: NFD** | **Groups II, III, IV, V: HFD** | |
| Before studying | 5.61 ± 0.69 | 5.79 ± 0.82 | > 0.05 |
| After 8 weeks | 5.23 ± 0.54 | 6.41 ± 1.29$^{*}$ | < 0.01 |
| 72 hours after injection | 5.83 ± 0.74 | 17.68 ± 4.51$^{***\#\#\#}$ | < 0.001 |

*Data are represented as mean ± SD. $^{*}p < 0.05$ and $^{***}p < 0.001$ compared with the time point "Before studying", $^{\#\#\#}p < 0.001$ compared with the time point "After 8 weeks". NFD: Normal-fat diet, HFD: High-fat diet.*

After 72 hours of STZ injection, the serum glucose concentrations of HFD-treated mouse groups exhibited a dramatic increase ($p < 0.001$) as compared with time points "Before studying" and "After 8 weeks" (Table 3.5). Meanwhile, there was no significant change in the blood glucose levels of group I (treated with NFD) at any time point ($p > 0.05$). Besides, after 8 weeks of treatment and after 72 hours of injection, the blood glucose concentrations of HFD-fed mice increased significantly compared with those of NFD-fed mice ($p < 0.01$ and $p < 0.001$, respectively).

### 3.2.3.3. Effect of CD.EAF on blood glucose levels

The hypoglycemic activity of CD.EAF at two tested doses of 100 mg/kg/day and 300 mg/kg/day in the type 2 diabetic mouse model induced by a high-fat diet and streptozotocin injection is demonstrated in Table 3.6.

**Table 3.6.** Effect of CD.EAF on blood glucose concentrations

| Groups | Blood glucose concentrations (mmol/L) | | |
|---|---|---|---|
| | Day 0 | Day 7 | Day 14 |
| Group I | 5.31 ± 0.71 | 5.38 ± 0.56 | 4.94 ± 0.33 |
| Group II | 16.01 ± 4.96*** | 15.74 ± 5.01*** | 15.81 ± 4.21*** |
| Group III | 17.32 ± 2.11*** | 13.50 ± 4.12*** | 12.68 ± 3.20***# |
| Group IV | 16.78 ± 3.15*** | 15.84 ± 3.36*** | 12.88 ± 3.23***# |
| Group V | 17.16 ± 5.16*** | 14.48 ± 2.72*** | 12.62 ± 3.16***# |

*Data are represented as mean ± SD, n = 10. ***$p < 0.001$ compared with group I, #$p < 0.05$ compared with group II. Group I: Normal control; Group II: Diabetic control; Group III: Gliclazide 80 mg/kg/day; Group IV: CD.EAF 100 mg/kg/day; Group V: CD.EAF 300 mg/kg/day.*

Based on the results presented in Table 3.6, diabetic mice had significant increases in blood glucose levels when compared with normoglycemic mice ($p < 0.001$). After 14 days of treatment, the repeated daily administration of the CD.EAF at both doses of 100 mg/kg/day and 300 mg/kg/day showed significant antihyperglycemic activities as compared with the diabetic control group ($p < 0.05$). Similarly, the blood glucose concentrations of the mice group treated with the standard drug (gliclazide 80 mg/kg/day) were reduced markedly compared with those at the beginning of the testing period ($p < 0.05$). Besides, there was no significant difference in serum glucose concentrations between the mouse groups treated with the CD.EAF and gliclazide ($p > 0.05$).

### 3.2.3.4. Effect of CD.EAF on blood lipid indexes

The lipid profile (total cholesterol, triglyceride, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol ) of mice in five tested groups after the treatment period is exhibited in Table 3.7.

**Table 3.7.** Effect of CD.EAF on lipid profile

| Groups | Serum lipid levels (mmol/L) | | | |
|---|---|---|---|---|
| | TC | TG | HDL-C | LDL-C |
| Group I | 1.54 ± 0.23 | 0.92 ± 0.18 | 0.39 ± 0.06 | 0.79 ± 0.20 |
| Group II | 2.43 ± 0.48*** | 1.12 ± 0.15* | 0.54 ± 0.06*** | 1.44 ± 0.36*** |
| Group III | 2.10 ± 0.21*** | 0.98 ± 0.18 | 0.48 ± 0.04*** | 1.20 ± 0.15*** |
| Group IV | 2.32 ± 0.26*** | 1.12 ± 0.16* | 0.42 ± 0.06 | 1.43 ± 0.32*** |
| Group V | 2.55 ± 0.52*** | 1.09 ± 0.30 | 0.46 ± 0.08* | 1.61 ± 0.32*** |

*Data are represented as mean ± SD, n = 10. *$p < 0.05$ and ***$p < 0.001$ compared with group I. TC: Total cholesterol, TG: Triglyceride, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol. Group I: Normal control; Group II: Diabetic control; Group III: Gliclazide 80 mg/kg/day; Group IV: CD.EAF 100 mg/kg/day; Group V: CD.EAF 300 mg/kg/day.*

As shown in Table 3.7, the concentrations of TC, TG, HDL-C, and LDL-C significantly increased in the diabetic mice compared to the normal mice ($p < 0.001$). No significant difference was observed in all serum lipid indices between the two groups treated with the CD.EAF and the diabetic control group ($p > 0.05$). Therefore, the CD.EAF in both 100 mg/kg/day and 300 mg/kg/day doses did not affect the lipid profile of STZ-treated mouse groups. In the group treated with gliclazide, there were downward trends in TC, TG, HDL-C, and LDL-C levels when compared with the diabetic control group; however, no significant difference was seen ($p > 0.05$).

### 3.2.3.5. Effect of CD.EAF on serum AST and ALT levels

The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels of the tested animals after 14 days of treatment are presented in Table 3.8.

**Table 3.8.** Effects of CD.EAF on blood AST and ALT levels

| Groups | AST levels (UI/L) | ALT levels (UI/L) |
|---|---|---|
| Group I | 127.58 ± 29.82 | 31.51 ± 8.62 |
| Group II | 138.14 ± 26.93 | 35.84 ± 8.65 |
| Group III | 174.18 ± 35.46$^{**\#}$ | 41.55 ± 12.44 |
| Group IV | 120.64 ± 15.42 | 33.69 ± 10.10 |
| Group V | 121.92 ± 24.30 | 32.12 ± 5.89 |

*Data are represented as mean ± SD, n = 10. $^{**}p < 0.01$ compared with group I, $^{\#}p < 0.05$ compared with group II. AST: Aspartate aminotransferase, ALT: Alanine aminotransferase. Group I: Normal control; Group II: Diabetic control; Group III: Gliclazide 80 mg/kg/day; Group IV: CD.EAF 100 mg/kg/day; Group V: CD.EAF 300 mg/kg/day.*

It can be seen from Table 3.8 that the diabetic mice had higher levels of both AST and ALT compared to the normal mice, but these differences were not significant ($p > 0.05$). While the AST level of mice treated with gliclazide increased remarkably compared to normoglycemic mice and hyperglycemic mice ($p < 0.01$ and $p < 0.05$, respectively), no significant change was seen in the ALT level of this group ($p > 0.05$). Regarding groups IV and V, the repeated daily administration of CD.EAF at both tested doses did not significantly alter the ALT levels ($p > 0.05$). Similarly, although the AST levels of these two groups tended to decrease compared to the diabetic control group, these alterations were not significant ($p > 0.05$).

### 3.2.3.6. Macroscopic observations of the liver and the pancreas

***Macroscopic observations of the liver***

The results of macroscopic observations of the livers of mice in each group are exhibited in Figure 3.18.

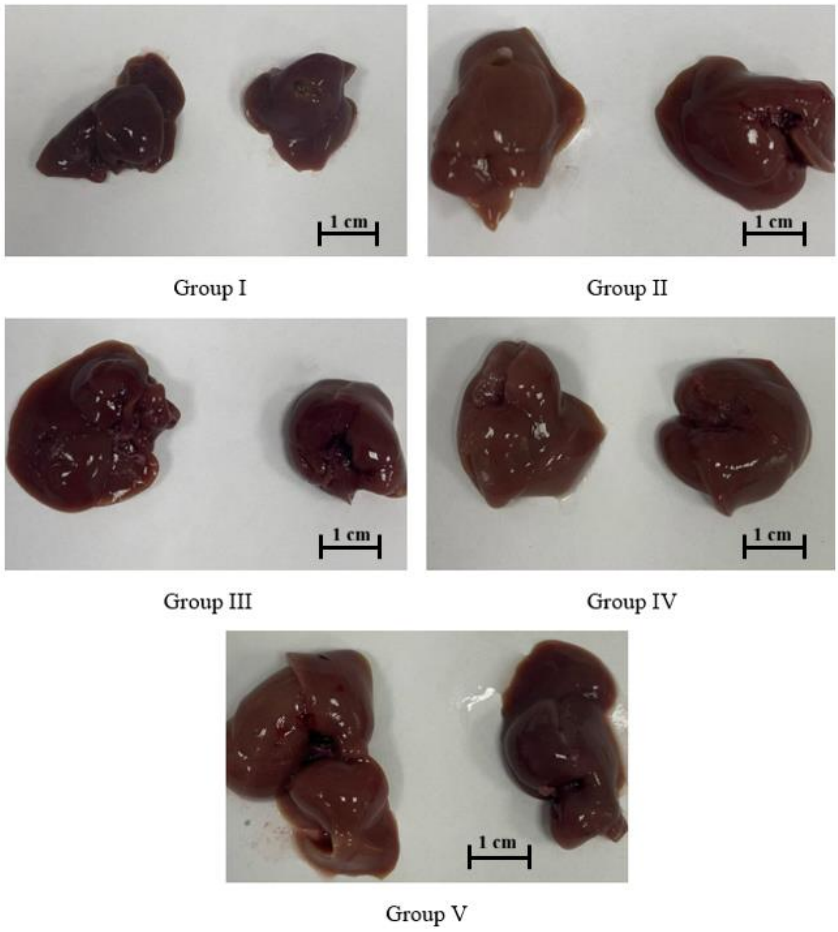



**Figure 3.18.** Effect of CD.EAF on the liver macroscopy of the treated mice. Group I: Normal control; Group II: Diabetic control; Group III: Gliclazide 80 mg/kg/day; Group IV: CD.EAF 100 mg/kg/day; Group V: CD.EAF 300 mg/kg/day.

As demonstrated in Figure 3.18, the macroscopic images of the liver of normal mice were dark pink, uniform in color, and the tissue density was soft and uniform. On the other hand, the liver of mice in the diabetic control group was mostly discolored with loose tissue density compared to the normal control group. Meanwhile, the liver macroscopy of the mice treated with gliclazide and CD.EAF at both doses of

100 mg/kg/day and 300 mg/kg/day were lighter pink, less regular in color, and had less loose tissue density than the normoglycemic mice.

***Macroscopic observations of the pancreas***

The observation results of the pancreas macroscopy of the five tested animal groups are described in Figure 3.19.

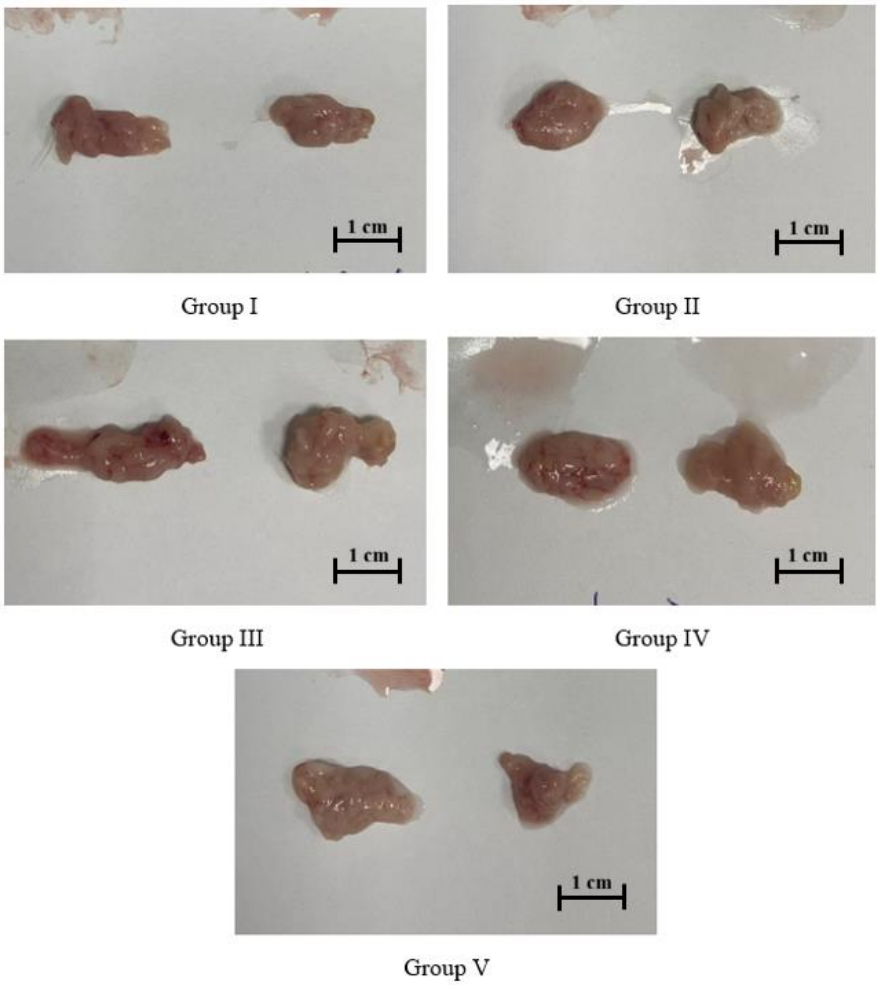



**Figure 3.19.** Effect of CD.EAF on the pancreas macroscopy of the treated mice. Group I: Normal control; Group II: Diabetic control; Group III: Gliclazide 80 mg/kg/day; Group IV: CD.EAF 100 mg/kg/day; Group V: CD.EAF 300 mg/kg/day.

It can be seen from Figure 3.19 that the macroscopic images of the pancreas of mice in all five groups were light pink and had a firm, elastic density. Furthermore, no congestion or gross lesions were observed.

### 3.2.3.7. Histopathology of the liver and the pancreas

***Histopathological assessment of the liver***

Figure 3.20 presents the histopathological images of the liver of five treated mouse groups.

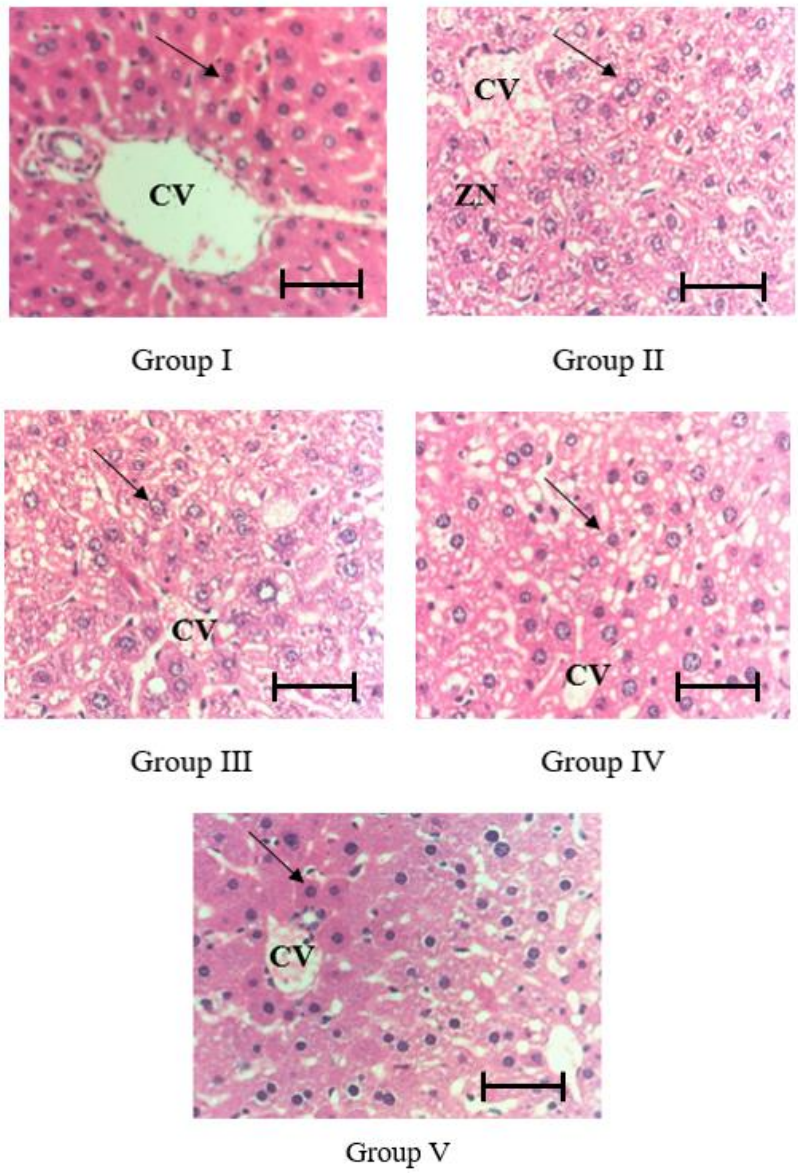



**Figure 3.20.** Effects of the CD.EAF on the liver histology of the treated mice (HE x 400). CV: Central venule, ZN: Zonal necrosis. Group I: Normal control; Group II: Diabetic control; Group III: Gliclazide 80 mg/kg/day; Group IV: CD.EAF 100 mg/kg/day; Group V: CD.EAF 300 mg/kg/day. The black arrows indicate hepatocytes. Scale bar: 50 µm.

It can be seen from Figure 3.20 that normoglycemic mice had a normal portal triad and central venule. Their blood vessels were rich in red blood cells and had few fat particles, while their hepatocytes scattered a few foci of inflammatory cells. By contrast, the portal triad and central venule of hyperglycemic mice (group II) contained many inflammatory cells. Many red blood cells were in the blood vessels, and fat particles adhered to the vessel wall. The hepatocytes of this group, which had crypt degeneration and fatty degeneration in many areas, and some cells had lost their nuclei, contained several large inflammatory foci. Similarly, the histopathological images of the liver of the group treated with gliclazide (group III) shared the same expressions as the diabetic control group. At the same time, clear improvements were observed in the histopathological results of the groups treated with the CD.EAF at both tested doses. In particular, in group IV (CD.EAF 100 mg/kg/day), the portal triad and central venule possessed some inflammatory cells. The blood vessels consisted of many red blood cells, and the hepatocytes contained several areas of crypt degeneration, some of which had lost their nuclei and had nuclear degeneration. In group V (CD.EAF 300 mg/kg/day), there were fewer inflammatory cells in the portal triad and central venule, while several fibrin fibers and fat particles were found in the blood vessels. The hepatocytes included a few foci of fatty degeneration, and the nuclei in some hepatocytes were replaced by fat particles.

***Histopathological assessment of the pancreas***

The histopathological results of the pancreas from five groups of treated mice are demonstrated in Figure 3.21.

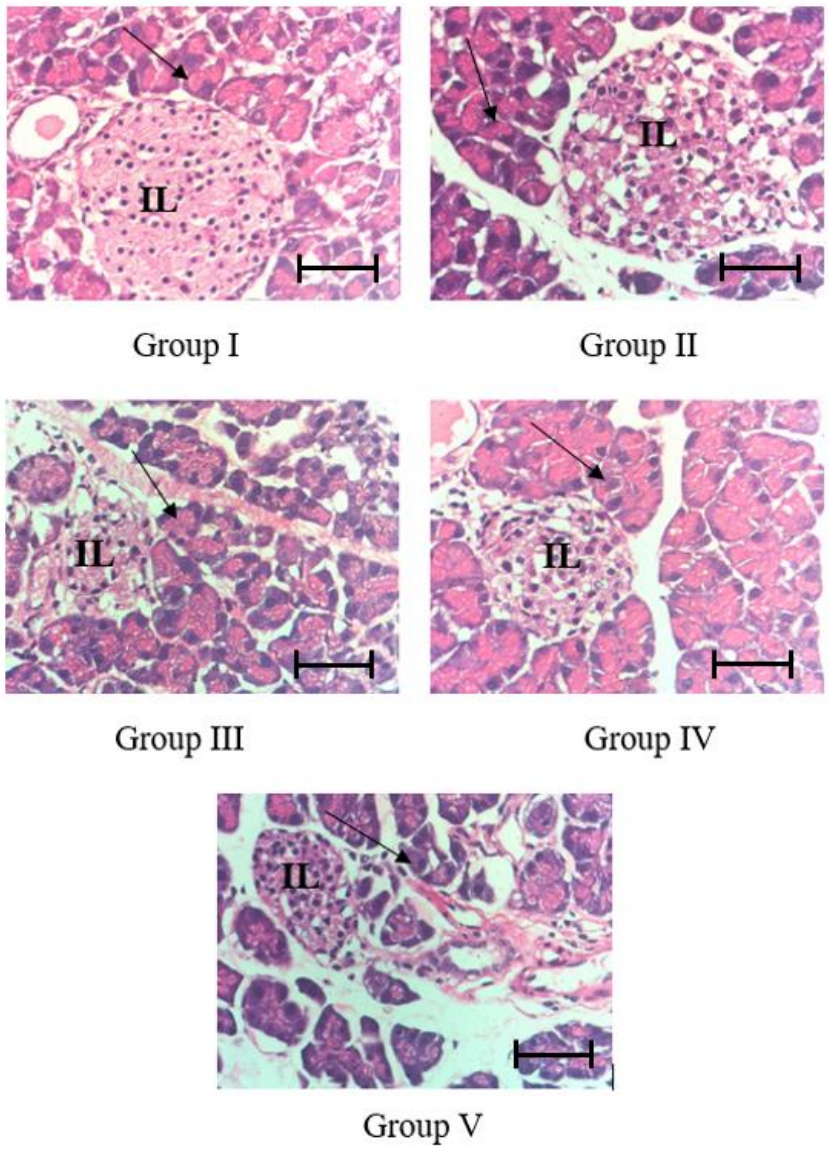



**Figure 3.21.** Effects of the CD.EAF on the pancreas histology of the treated mice (HE x 400). IL: Islet of Langerhans. Group I: Normal control; Group II: Diabetic control; Group III: Gliclazide 80 mg/kg/day; Group IV: CD.EAF 100 mg/kg/day; Group V: CD.EAF 300 mg/kg/day. The black arrows indicate acinar cells. Scale bar: 50 µm.

As shown in Figure 3.21, mice in the normal group exhibited a healthy pancreas structure, with normal-sized islets and pancreatic cells. On the other hand, the pancreatic islets of the diabetic control group were uneven in size; some were small. Furthermore, the acinar cells degenerated, and a few inflammatory cells were found around blood vessels. Although maintaining a normal pancreas structure, the pancreatic islets of mice

treated with gliclazide proliferated in size and number. Meanwhile, the histopathological images of the pancreas tissue from the animal group treated with CD.EAF at doses of 100 mg/kg/day and 300 mg/kg/day indicated that the pancreatic islets had minimal proliferation, appeared irregular in size, and many exhibited fatty degeneration.

### 3.2.4. Enzymatic inhibition of isolated compounds from *C. diffusa*

#### 3.2.4.1. Inhibitory effect of isolated compounds from *C. diffusa* against α-glucosidase enzyme

The isolated compounds from *C. diffusa* were evaluated for their inhibitory effects on α-glucosidase enzyme at different concentrations of 31.25, 62.5, 125, 250, and 500 µg/mL, following the method described in section 2.6.2.1. Acarbose was used as the positive control. The percentage inhibition of testing samples at various concentrations and their $IC_{50}$ values are illustrated in Table 3.9.

**Table 3.9.** The percentage inhibition at each concentration and the corresponding $IC_{50}$ values of isolated compounds from *C. diffusa* against the α-glucosidase enzyme

| Compounds | % inhibition at different concentrations (µg/mL) | | | | | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|---|---|
| | 31.25 | 62.5 | 125 | 250 | 500 | |
| **1** | 21.47 ± 1.69 | 43.15 ± 2.15 | 55.02 ± 2.97 | 72.89 ± 1.24 | 94.86 ± 3.25 | 93.68 ± 3.07 |
| **2** | 10.97 ± 1.14 | 19.72 ± 1.96 | 41.13 ± 2.36 | 52.24 ± 2.10 | 60.89 ± 2.77 | 247.03 ± 5.63 |
| **3** | 19.45 ± 1.56 | 26.52 ± 1.32 | 39.47 ± 2.01 | 57.99 ± 2.39 | 67.03 ± 1.88 | 193.55 ± 2.48 |
| **4** | 2.73 ± 0.53 | 6.84 ± 1.37 | 14.09 ± 1.04 | 25.75 ± 1.82 | 31.61 ± 2.05 | > 500 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **5** | 24.49 ± 1.68 | 36.16 ± 2.02 | 50.17 ± 2.35 | 62.41 ± 2.61 | 81.83 ± 2.07 | 119.22 ± 3.09 |
| **6** | 1.55 ± 0.09 | 5.03 ± 0.11 | 8.26 ± 0.31 | 14.48 ± 1.04 | 19.85 ± 0.70 | > 500 |
| **7** | 23.31 ± 1.00 | 38.09 ± 1.75 | 51.75 ± 1.99 | 60.13 ± 1.64 | 79.60 ± 2.13 | 121.16 ± 2.28 |
| **8** | 48.23 ± 0.92 | 60.07 ± 1.28 | 72.67 ± 1.72 | 80.30 ± 2.59 | 91.11 ± 2.20 | 32.71 ± 0.35 |
| **9** | 55.89 ± 1.46 | 68.20 ± 1.30 | 74.44 ± 2.10 | 90.13 ± 1.89 | 95.08 ± 2.26 | 19.63 ± 0.94 |
| **10** | 40.10 ± 0.95 | 60.36 ± 1.17 | 74.99 ± 1.81 | 85.52 ± 1.60 | 94.97 ± 2.44 | 41.98 ± 0.57 |
| **11** | 1.86 ± 0.25 | 4.99 ± 0.61 | 10.62 ± 1.13 | 17.20 ± 1.67 | 22.12 ± 2.03 | > 500 |
| **12** | 17.61 ± 2.02 | 33.19 ± 1.76 | 40.78 ± 2.74 | 54.29 ± 2.22 | 69.20 ± 2.61 | 184.89 ± 3.40 |
| **13** | 5.89 ± 1.11 | 27.22 ± 0.92 | 37.55 ± 1.60 | 61.87 ± 1.74 | 75.19 ± 1.55 | 175.31 ± 2.16 |
| **14** | 43.77 ± 1.75 | 65.31 ± 1.60 | 78.15 ± 2.08 | 89.42 ± 2.25 | 99.54 ± 2.14 | 34.45 ± 1.22 |
| **Acarbose** | | | | | | 135.73 ± 1.79 |

It can be seen from Table 3.9 that the α-glucosidase enzymatic inhibition activity of all investigated compounds increased in a concentration-dependent manner. Among the isolated compounds, *N-trans-p*-coumaroyl-3',4'-dihydroxyphenylethylamine (**9**) exhibited the lowest $IC_{50}$ value (19.63 ± 0.94 μg/mL), indicating the highest potency, approximately 7-fold more effective than the reference compound, acarbose ($IC_{50}$ = 135.73 ± 1.79 μg/mL). *N-trans*-feruloyltyramine (**8**), 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**), and isoschaftoside (**14**) also possessed strong inhibition with the $IC_{50}$ values of 32.71 ± 0.35 μg/mL, 41.98 ± 0.57 μg/mL, and 34.45 ± 1.22 μg/mL, respectively, which were about three times better than acarbose. Similarly, corosolic acid (**1**), lyratol F (**5**), and methyl gallate (**7**) displayed greater inhibitory activity than the reference drug. By contrast, stigmasterol (**4**), 4-hydroxybenzoic acid (**6**), and daucosterol (**11**) showed no inhibitory effect at the tested concentrations, with the $IC_{50}$ values exceeding 500 μg/mL. Meanwhile, 3*α*-acetyl-20*S*,24*S*-epoxydammarane-25-ol (**2**), 11-oxo-α-amyrin acetate (**3**), quercitrin (**12**), and benzyl-O-β-D-glucopyranoside (**13**) expressed moderate to weak activity, with the $IC_{50}$ values ranging from 175.31 ± 2.16 μg/mL to 247.03 ± 5.63 μg/mL.

### 3.2.4.2. Inhibitory effect of isolated compounds from *C. diffusa* against α-amylase enzyme

The inhibitory activities of the pure compounds isolated from *C. diffusa* against α-amylase enzyme were performed at five ascending concentrations (31.25, 62.5, 125, 250, and 500 μg/mL) using the method outlined in section 2.6.2.1. Acarbose was also utilized as the positive control. The percentage inhibition at each concentration and the corresponding $IC_{50}$ values of the testing samples are demonstrated in Table 3.10.

**Table 3.10.** The percentage inhibition at each concentration and the corresponding $IC_{50}$ values of isolated compounds from *C. diffusa* against the α-amylase enzyme

| Compounds | % inhibition at different concentrations (µg/mL) | | | | | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|---|---|
| | 31.25 | 62.5 | 125 | 250 | 500 | |
| **1** | 12.49 ± 2.66 | 20.32 ± 2.30 | 37.97 ± 1.83 | 51.58 ± 2.16 | 63.30 ± 1.57 | 245.41 ± 2.83 |
| **2** | 26.95 ± 1.43 | 39.98 ± 2.97 | 57.22 ± 2.52 | 65.11 ± 3.02 | 82.06 ± 3.69 | 100.64 ± 3.59 |
| **3** | 15.32 ± 1.39 | 24.12 ± 1.90 | 40.54 ± 2.21 | 51.97 ± 2.03 | 61.26 ± 1.96 | 242.35 ± 4.26 |
| **4** | 5.87 ± 0.84 | 13.64 ± 1.54 | 26.10 ± 1.63 | 34.28 ± 2.37 | 46.68 ± 2.19 | > 500 |
| **5** | 32.47 ± 1.42 | 42.93 ± 1.77 | 58.59 ± 1.58 | 70.27 ± 1.79 | 77.12 ± 1.86 | 86.10 ± 1.24 |
| **6** | 2.66 ± 0.73 | 4.90 ± 1.36 | 11.38 ± 1.91 | 15.72 ± 1.67 | 22.51 ± 2.07 | > 500 |
| **7** | 20.76 ± 0.81 | 31.91 ± 1.72 | 41.42 ± 2.11 | 56.85 ± 1.54 | 72.94 ± 1.89 | 165.42 ± 2.31 |
| **8** | 11.70 ± 1.84 | 16.39 ± 1.65 | 25.31 ± 2.28 | 37.22 ± 2.14 | 45.06 ± 2.60 | > 500 |
| **9** | 44.18 ± 1.22 | 58.46 ± 1.62 | 73.79 ± 1.57 | 80.65 ± 1.91 | 89.29 ± 1.69 | 38.24 ± 0.62 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **10** | 50.75 ± 0.90 | 63.01 ± 1.32 | 72.51 ± 1.46 | 79.14 ± 1.69 | 88.83 ± 2.11 | 26.15 ± 0.71 |
| **11** | 1.84 ± 0.43 | 5.90 ± 0.79 | 12.31 ± 1.07 | 20.79 ± 1.25 | 29.02 ± 1.67 | > 500 |
| **12** | 30.71 ± 1.50 | 41.68 ± 1.83 | 56.30 ± 2.13 | 66.81 ± 2.03 | 76.26 ± 2.86 | 96.57 ± 3.18 |
| **13** | 22.31 ± 1.39 | 40.57 ± 1.88 | 46.34 ± 2.01 | 60.23 ± 2.30 | 71.17 ± 2.52 | 139.54 ± 2.91 |
| **14** | 40.07 ± 2.31 | 61.41 ± 2.50 | 75.16 ± 1.92 | 91.62 ± 2.18 | 98.25 ± 2.04 | 41.58 ± 2.47 |
| **Acarbose** | | | | | | 83.33 ± 2.02 |

Table 3.10 indicates that all tested compounds exhibited α-amylase inhibitory activity in a concentration-dependent manner. Among them, 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-*N*$^{1}$,*N*$^{2}$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**) had the highest inhibitory effective with an $IC_{50}$ value of 26.15 ± 0.71 μg/mL, followed closely by *N-trans-p*-coumaroyl-3',4'-dihydroxyphenylethylamine (**9**) (38.24 ± 0.62 μg/mL) and isoschaftoside (**14**) (41.58 ± 2.47 μg/mL). These compounds were significantly more potent than the reference drug, acarbose ($IC_{50}$ = 83.33 ± 2.02 μg/mL). Besides, lyratol F (**5**), quercitrin (**12**), and 3*α*-acetyl-20*S*,24*S*-epoxydammarane-25-ol (**2**) also demonstrated notable inhibitory activity, with the $IC_{50}$ values of 86.10 ± 1.24 μg/mL, 96.57 ± 3.18 μg/mL, and 100.64 ± 3.59 μg/mL, respectively. In contrast, stigmasterol (**4**), 4-hydroxybenzoic acid (**6**), *N-trans*-feruloyltyramine (**8**), and daucosterol (**11**) had $IC_{50}$ values exceeding 500 μg/mL, indicating negligible activity compared to acarbose. At the same time, the remaining

compounds exhibited moderate to weak activity, with $IC_{50}$ values ranging from 139.54 ± 2.91 µg/mL to 245.41 ± 2.83 µg/mL.

### 3.3. Molecular docking results

#### 3.3.1. Evaluation of the docking model

Before screening the compounds, the co-crystallized ligand needs to be re-docked into the active site of the target proteins to calculate the root mean square deviation (RMSD) and assess the reliability of the docking parameters. The results show that the structure of the substrate after being re-docked into the active site of the two targets had a high degree of overlap with the co-crystal substrate (Figure 3.22). The RMSD values for 2QV4 and 3W37 were 0.6915 Å and 0.8638 Å, respectively. Both values were less than 1.5 Å, confirming the accuracy and validity of the molecular docking protocol used in this study.

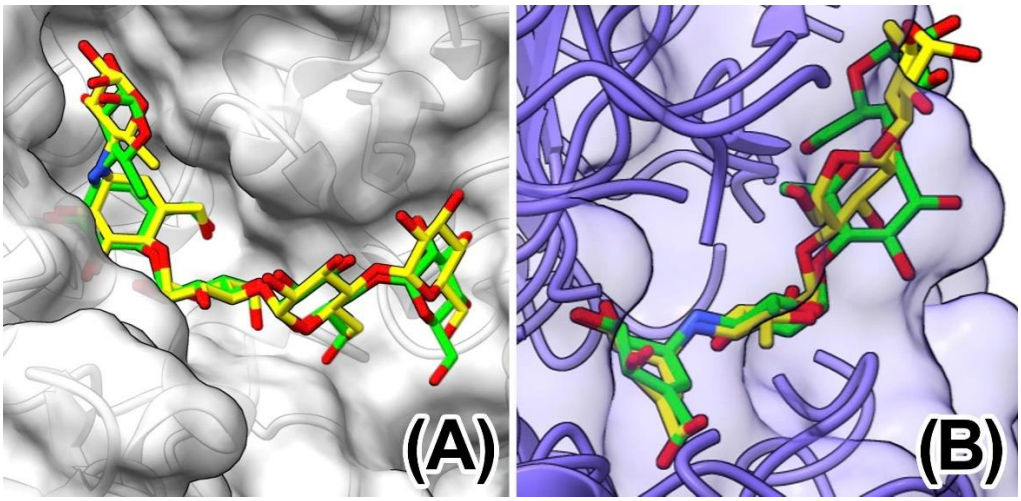



**Figure 3.22.** Re-dock results of the co-crystallised ligand into 2QV4 (A) and 3W37 (B). The native ligands are in green, and the re-docked ligands are in yellow.

The interaction model between the co-crystallised ligand (acarbose) and the α-amylase protein (2QV4) indicated that acarbose interacted with 2QV4 through hydrogen bonds with Asp197, Glu233, Asp300, Arg195, His101, His201, His305, Gln63, Thr183, Tyr262, Trp59, Ala106, Ala198, and Asn105 amino acids, and a Pi-Alkyl interaction with Leu162 residues in the active site of the target protein (Figure 3.23A).

Regarding the α-glucosidase protein (3W37), acarbose can form hydrogen bonds with the amino acids Asn237, Ala234, Arg552, Asp232, Asp357, Asp568, and His626, two Pi-Alkyl interactions with the residues Trp329 and Phe601, as well as a Pi-Sigma interaction with the Trp432 amino acid in its active site (Figure 3.23B).

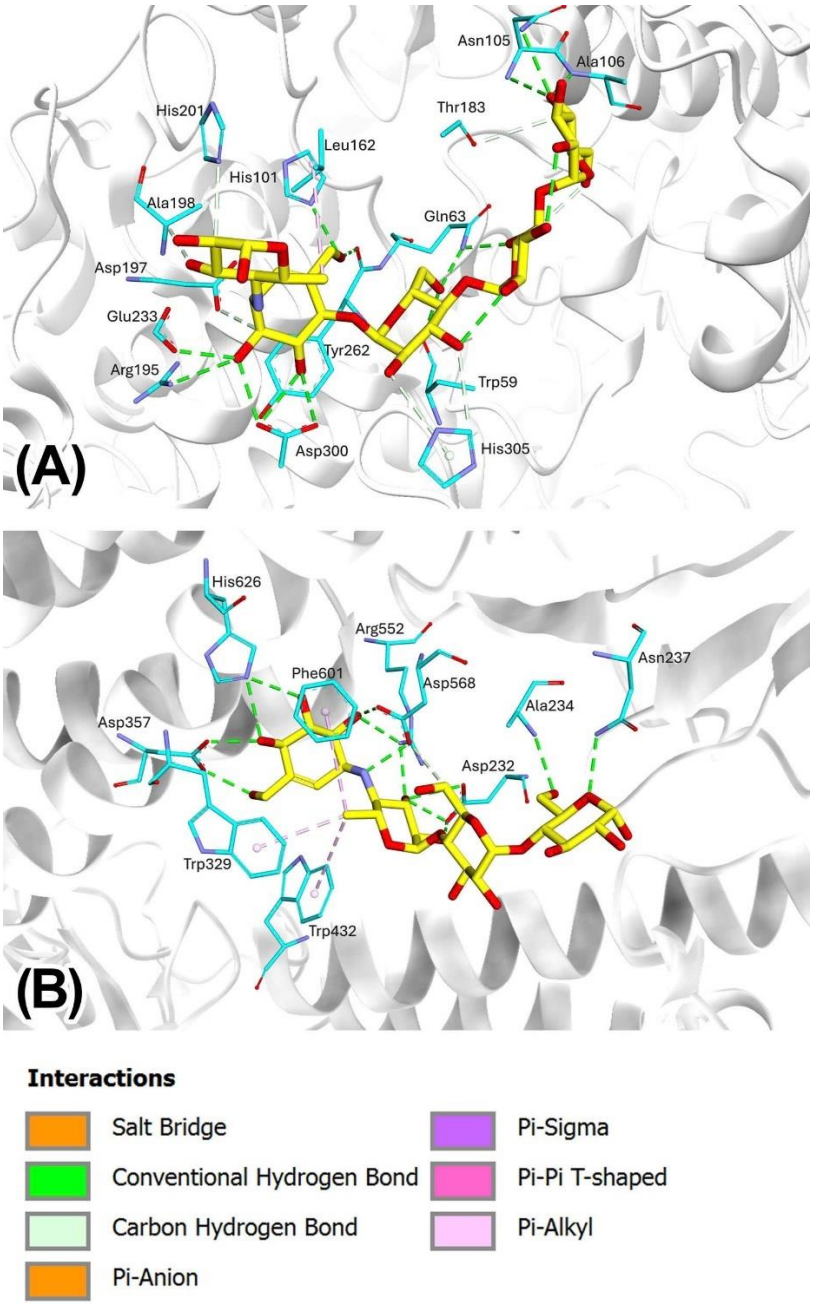



**Figure 3.23.** The interactions of the co-crystallised ligand with 2QV4 (A) and 3W37 (B)

### 3.3.2. Molecular docking of isolated compounds to the target proteins

After validation of the docking procedure using the re-docking method, isolated compounds from *C. diffusa* were docked to screen for their inhibitory activities against α-amylase and α-glucosidase enzymes (PDB IDs: 2QV4 and 3W37, respectively). Because of having reports for stigmasterol (**4**) and daucosterol (**11**) [131,132], this study performed docking simulations with the remaining compounds. Table 3.11 displays the docking scores (ΔG) of the investigated compounds compared to acarbose.

**Table 3.11.** The docking results of the isolated compounds from *C. diffusa* and acarbose

| No | Name of compounds | Docking scores with 2QV4 (kCal/mol) | Docking scores with 3W37 (kCal/mol) |
|---|---|---|---|
| 1 | Corosolic acid (**1**) | -4.8203 | **-8.2521** |
| 2 | 3*α*-acetyl-20*S*,24*S*-epoxydammarane-25-ol (**2**) | -6.5709 | -7.9084 |
| 3 | 11-oxo-α-amyrin acetate (**3**) | -5.8941 | -7.5878 |
| 4 | Lyratol F (**5**) | -4.7191 | -5.2207 |
| 5 | 4-hydroxybenzoic acid (**6**) | -4.6280 | -3.0380 |
| 6 | Methyl gallate (**7**) | -5.5996 | -4.4932 |
| 7 | *N-trans*-feruloyltyramine (**8**) | -6.0174 | -6.4995 |
| 8 | *N-trans-p*-coumaroyl-3',4'-dihydroxyphenylethylamine (**9**) | -6.5822 | -7.0217 |

| | | | |
|---|---|---|---|
| 9 | 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**) | **-8.0445** | **-9.6058** |
| 10 | Quercitrin (**12**) | -6.9666 | -7.3192 |
| 11 | Benzyl-O-β-D-glucopyranoside (**13**) | -6.4342 | -6.1337 |
| 12 | Isoschaftoside (**14**) | **-8.1662** | **-8.4492** |
| 13 | Acarbose | -7.5615 | -8.0445 |

Based on Table 3.11, the binding energies of the reference drug (acarbose) with 2QV4 and 3W37 were -7.5615 kCal/mol and -8.0445 kCal/mol, respectively. Among the listed compounds, 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**) and isoschaftoside (**14**) demonstrated the highest inhibitory effect on the α-amylase enzyme, with docking scores lower than acarbose (-8.0445 kCal/mol and -8.1662 kCal/mol, respectively). For the α-glucosidase enzyme, corosolic acid (**1**), 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**), and isoschaftoside (**14**) exhibited the strongest binding affinity toward the targeted enzyme, with docking scores in the range of -8.2521 – -9.6058 kCal/mol. These results revealed that 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**) and isoschaftoside (**14**) could effectively inhibit both α-amylase and α-glucosidase enzymes. Hence, we explored the possible binding interactions between these compounds and the targeted enzymes, which are shown in Figures 3.24 and 3.25.

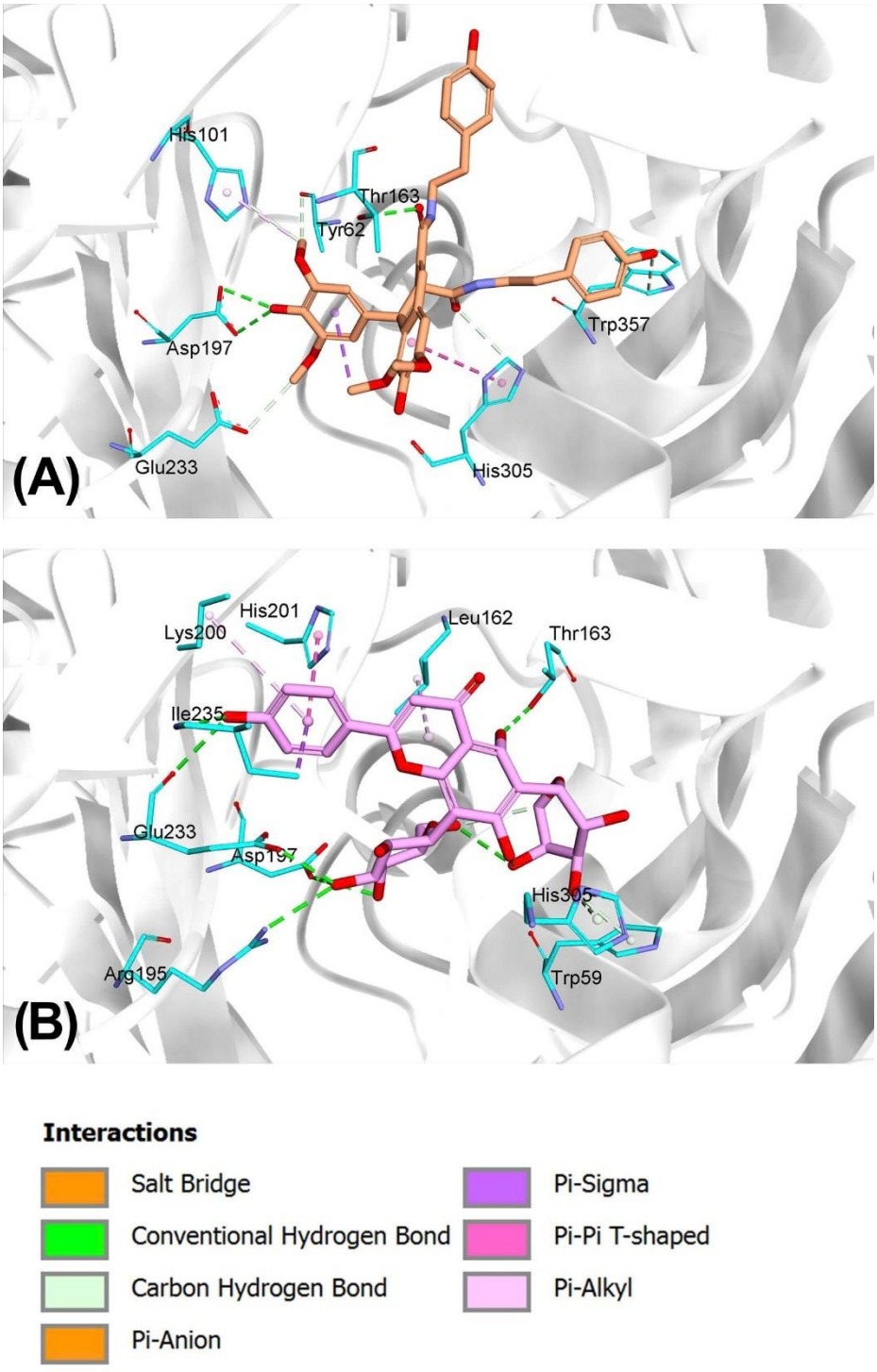



**Figure 3.24.** 3D interactions between compounds and the α-amylase enzyme (PDB: 2QV4). (A) 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide; (B) Isoschaftoside.

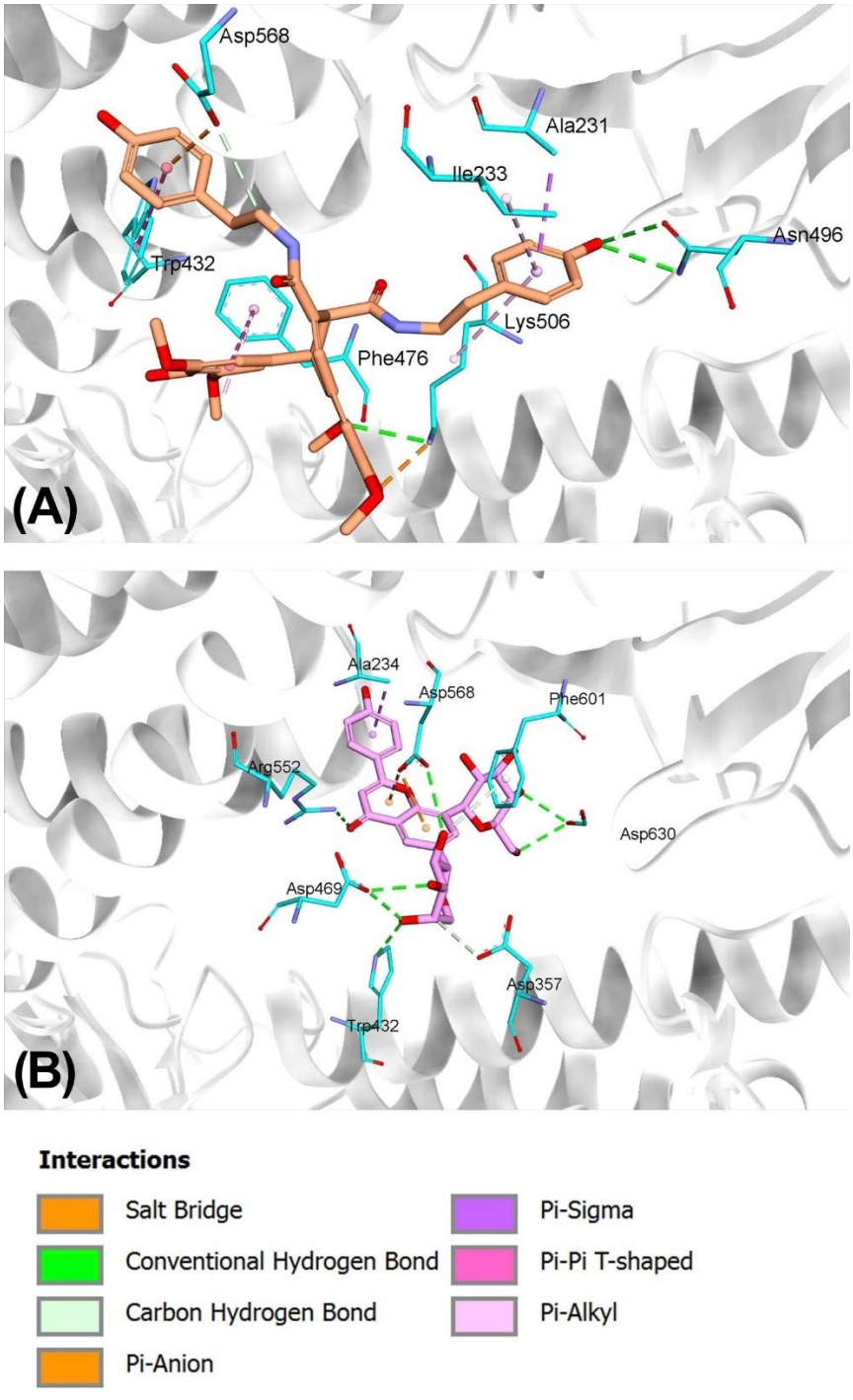



**Figure 3.25.** 3D interactions between compounds and the α-glucosidase enzyme (PDB: 3W37). (A) 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide; (B) Isoschaftoside.

In the evaluation of the α-amylase enzyme, 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**) formed hydrogen bonds with following acid amines Asp197, Glu233, Thr163, Tyr62, Trp357, a Pi-Alkyl interaction with residue His101, and a Pi-Pi T-shaped interaction with residue His305. Meanwhile, isoschaftoside (**14**) interacted with the targeted enzyme via hydrogen bonds, Pi-Sigma, Pi-Pi T-shaped, and Pi-Alkyl interactions. In particular, hydrogen bonds were formed with Asp197, Glu233, Arg195, Trp59, His305, and Thr163 residues; a Pi-Sigma interaction was formed with the Ile235 residue; a Pi-Pi T-shaped interaction was formed with the His201 residue; two Pi-Alkyl interactions were formed with Lys200 and Leu162 residues. In the analysis of the α-glucosidase enzyme, 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**) showed hydrogen bond with Asn496, Asp568 acid amines, Pi-Anion interaction with Trp432 acid amine, Pi-Sigma interactions with Ala231, Ile233, Lys506 acid amines, and Pi-Alkyl interaction with Phe476 acid amine. Besides, hydrogen bonds were seen between isoschaftoside (**14**) and Arg552, Asp357, Asp469, Asp568, Asp630, Trp432, and Phe601 acid amines. Furthermore, this compound can form a Pi-Sigma interaction with residue Ala234.

### 3.3.3. Drug likeness prediction

The drug-like properties of the isolated compounds from *C. diffusa* were evaluated using Lipinski's Rule of Five. The SwissADME online tool (http://www.swissadme.ch/) was used to calculate four physicochemical parameters specified in this rule, including molecular weight (MW), number of hydrogen bond donors (HBD), number of hydrogen bond acceptors (HBA), and octanol–water partition coefficient (LogP). Table 3.12 presents the drug likeness prediction results of 14 isolated compounds.

**Table 3.12.** Drug likeness prediction of isolated compounds from *C. diffusa* according to Lipinski's Rule of Five

| Name of compounds | MW | HBD | HBA | LogP | Drug-likeness |
|---|---|---|---|---|---|
| Corosolic acid (**1**) | 472.7 | 3 | 4 | **6.06** | Yes |
| 3*α*-acetyl-20*S*,24*S*-epoxydammarane-25-ol (**2**) | **502.8** | 1 | 4 | **7.31** | No |
| 11-oxo-α-amyrin acetate (**3**) | 482.7 | 0 | 3 | **7.77** | Yes |
| Stigmasterol (**4**) | 412.7 | 1 | 1 | **7.80** | Yes |
| Lyratol F (**5**) | 224.3 | 2 | 3 | 1.59 | Yes |
| 4-hydroxybenzoic acid (**6**) | 138.1 | 2 | 3 | 1.09 | Yes |
| Methyl gallate (**7**) | 184.1 | 3 | 5 | 0.59 | Yes |
| *N-trans*-feruloyltyramine (**8**) | 313.3 | 3 | 4 | 2.37 | Yes |
| *N-trans-p*-coumaroyl-3',4'-dihydroxyphenylethylamine (**9**) | 299.3 | 4 | 4 | 2.07 | Yes |
| 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**) | **684.7** | **6** | 10 | 4.30 | No |
| Daucosterol (**11**) | **576.8** | 4 | 6 | **5.85** | No |
| Quercitrin (**12**) | 448.4 | **7** | **11** | 0.49 | No |

| | | | | | |
|---|---|---|---|---|---|
| Benzyl-O-β-D-glucopyranoside (**13**) | 270.3 | 4 | 6 | -1.15 | Yes |
| Isoschaftoside (**14**) | **564.5** | **10** | **14** | -2.40 | No |

According to Lipinski's Rule of Five, compounds are considered "drug-like" with good oral bioavailability if they meet at least three of Lipinski's Rule of Five criteria, including molecular weight (MW) less than 500 Da, no more than 5 hydrogen bond donors (HBD), no more than 10 hydrogen bond acceptors (HBA), and octanol/water partition coefficient (logP) not exceeding more than 5 [133]. The results from Table 3.13 indicated that five compounds isolated from *C. diffusa* violated more than one of the specified criteria, which were 3*α*-acetyl-20*S*,24*S*-epoxydammarane-25-ol (**2**), 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**), daucosterol (**11**), quercitrin (**12**), and isoschaftoside (**14**).

### 3.3.4. Analysis of ADMET properties

In this study, the ADMET profile of isolated compounds from *C. diffusa* was computed using the pkCSM online tool. The predictions for ADMET property parameters of the 14 isolated compounds are summarized in Tables 3.13 and 3.14.

**Table 3.13.** Absorption, distribution, and metabolism properties of isolated compounds from *C. diffusa*

| **Name of compounds** | **Absorption** | | **Distribution** | | | **Metabolism** | |
|---|---|---|---|---|---|---|---|
| | **Caco2** | **HIA** | **VDss** | **BBB** | **CNS** | **CYP2D6** | **CYP3A4** |
| Corosolic acid (**1**) | 0.64 | 100 | -1.28 | -0.47 | -1.51 | No | No |
| 3*α*-acetyl-20*S*,24*S*-epoxydammarane-25-ol (**2**) | 1.19 | 97.22 | 0.07 | 0.17 | -1.19 | No | No |
| 11-oxo-α-amyrin acetate (**3**) | 1.42 | 100 | 0.02 | -0.09 | -2.14 | No | No |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Stigmasterol (**4**) | 1.21 | 94.97 | 0.18 | 0.77 | -1.65 | No | No |
| Lyratol F (**5**) | 1.26 | 94.31 | -0.13 | -0.05 | -2.90 | No | No |
| 4-hydroxybenzoic acid (**6**) | 1.15 | 83.96 | -1.56 | -0.33 | -3.21 | No | No |
| Methyl gallate (**7**) | -0.06 | 76.64 | 0.36 | -1.05 | -3.38 | No | No |
| *N-trans*-feruloyltyramine (**8**) | 0.93 | 90.23 | 0.13 | -0.72 | -2.55 | No | Yes |
| *N-trans-p*-coumaroyl-3',4'-dihydroxyphenylethylamine (**9**) | 1.00 | 87.66 | -0.11 | -0.72 | -2.65 | No | No |
| 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**) | 0.36 | 86.59 | -0.71 | -1.23 | -3.44 | No | Yes |
| Daucosterol (**11**) | 0.47 | 79.68 | -1.16 | -0.79 | -3.02 | No | No |
| Quercitrin (**12**) | 0.05 | 52.71 | 1.52 | -1.50 | -4.16 | No | No |
| Benzyl-O-β-D-glucopyranoside (**13**) | 0.16 | 41.71 | -0.27 | -0.72 | -3.85 | No | No |
| Isoschaftoside (**14**) | -1.18 | 45.39 | 1.30 | -1.67 | -4.84 | No | No |

*Caco2: Caco2 permeability (log Papp in $10^{-6}$ cm/s), HIA: Human intestinal absorption (% Absorbed), VDss: Volume of distribution (log L/kg), BBB: Blood-brain barrier permeability (logBB), CNS: Central nervous system permeability (logPS), CYP2D6: CYP2D6 inhibitor, CYP3A4: CYP3A4 inhibitor.*

**Table 3.14.** Excretion and toxicity properties of isolated compounds from *C. diffusa*

| Name of compounds | Excretion | | Toxicity | | | | |
|---|---|---|---|---|---|---|---|
| | **Clr** | **OCT2** | **AMES** | **hERG** | **$LD_{50}$** | **HT** | **SS** |
| Corosolic acid (**1**) | 0.09 | No | No | No | 2.51 | Yes | No |
| 3*α*-acetyl-20*S*,24*S*-epoxydammarane-25-ol (**2**) | 0.11 | No | No | No | 2.15 | No | No |
| 11-oxo-α-amyrin acetate (**3**) | -0.04 | No | No | No | 2.09 | No | No |
| Stigmasterol (**4**) | 0.62 | No | No | No | 2.54 | No | No |
| Lyratol F (**5**) | 1.27 | No | No | No | 1.53 | No | Yes |
| 4-hydroxybenzoic acid (**6**) | 0.59 | No | No | No | 2.26 | No | No |
| Methyl gallate (**7**) | 0.64 | No | Yes | No | 1.90 | No | No |
| *N-trans*-feruloyltyramine (**8**) | 0.27 | No | No | No | 1.87 | Yes | No |
| *N-trans-p*-coumaroyl-3',4'-dihydroxyphenylethylamine (**9**) | 0.26 | No | No | No | 1.45 | No | No |
| 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**) | 0.40 | No | No | No | 2.53 | No | No |
| Daucosterol (**11**) | 0.69 | No | No | No | 2.57 | No | No |
| Quercitrin (**12**) | 0.36 | No | No | No | 2.59 | No | No |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Benzyl-O-β-D-glucopyranoside (**13**) | 0.23 | No | Yes | No | 2.01 | No | No |
| Isoschaftoside (**14**) | -0.11 | No | No | No | 2.50 | No | No |

*Clr: Total clearance (log ml/min/kg), OCT2: Renal organic cation transporter 2 substrate, AMES: AMES toxicity, hERG: Human ether-à-go-go related gene cardiac potassium channel inhibitor, $LD_{50}$: Oral rat acute toxicity (mol/kg), HT: Hepatic toxicity, SS: Skin sensitisation.*

The ADMET evaluation of 14 compounds isolated from *C. diffusa* revealed generally favorable pharmacokinetic and safety profiles. The absorption characteristics were assessed via Caco-2 cell permeability (log Papp in $10^{-6}$ cm/s) and human intestinal absorption (HIA, %). Most compounds demonstrated moderate to high intestinal absorption, with HIA values ranging from 41.71% (Benzyl-O-β-D-glucopyranoside) to 100% (Corosolic acid and 11-oxo-α-amyrin acetate). At the same time, there was a significant difference in Caco-2 permeability among the compounds, with 11-oxo-α-amyrin acetate showing the highest permeability (1.42) and isoschaftoside having the lowest permeability (−1.18). The distribution parameters included volume of distribution at steady state (VDss, log L/kg), which ranged from −1.56 (4-hydroxybenzoic acid) to 1.52 (Quercitrin), suggesting varied tissue distribution potential among the compounds. Furthermore, blood-brain barrier permeability (logBB) and central nervous system (CNS) permeability (logPS) values were generally negative, suggesting limited brain access, which may help reduce neurological side effects. For metabolic predictions, the data show that none of the compounds inhibited CYP2D6, and only two compounds (*N-trans*-feruloyltyramine and 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide) were predicted to be CYP3A4 inhibitors. Regarding excretion properties, the total clearance (Clr, log ml/min/kg) ranged from -0.11 (Isoschaftoside) to 1.27 (Lyratol F), while none of the compounds were substrates for renal organic cation transporter 2 (OCT2). In the assessments of toxicity, the obtained results indicated a low overall risk of all isolated compounds. The AMES test predicted mutagenic potential for only two

compounds, including methyl gallate and benzyl-O-β-D-glucopyranoside, while the other compounds were non-mutagenic. Notably, none of the compounds were predicted to inhibit the hERG cardiac potassium channel. The isolated compounds from *C. diffusa* performed at moderate acute toxicity levels, with predicted oral rat acute toxicity ($LD_{50}$, mol/kg) values ranging from 1.45 (*N-trans-p*-coumaroyl-3′,4′-dihydroxyphenylethylamine) to 2.59 (Quercitrin). Besides, hepatotoxicity was predicted for only two compounds (Corosolic acid and *N-trans*-feruloyltyramine), while skin sensitization was noted solely for lyratol F.

# Chapter IV – Discussion

## 4.1. Chemical constituents of *C. diffusa*

Repeated column chromatography resulted in the isolation and purification of 14 pure compounds from *C. diffusa* in total. Specifically, three compounds were isolated from the *n*-hexane fraction, nine compounds from the ethyl acetate fraction, and two compounds from the aqueous fraction. After determining their chemical structures through the results of melting point, mass spectrometry, nuclear magnetic resonance spectroscopy, and comparison with published data of related compounds, these compounds were identified as corosolic acid (**1**), 3*α*-acetyl-20*S*,24*S*-epoxydammarane-25-ol (**2**), 11-oxo-α-amyrin acetate (**3**), stigmasterol (**4**), lyratol F (**5**), 4-hydroxybenzoic acid (**6**), methyl gallate (**7**), *N-trans*-feruloyltyramine (**8**), *N-trans-p*-coumaroyl-3',4'-dihydroxyphenylethylamine (**9**), 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**), daucosterol (**11**), quercitrin (**12**), benzyl-O-β-D-glucopyranoside (**13**), and isoschaftoside (**14**). To the best of our knowledge, compounds **1-3**, **5**, **8-10**, and **13** were isolated for the first time from *C. diffusa* and had not previously been reported from the genus *Commelina*. In addition, compounds **7**, **11**, **12**, and **14** were also reported for the first time from *C. diffusa*. In a previous study, compound **4** was found in the aerial parts of *C. diffusa* at a content of 2.61 mg/100 g using the gas chromatography method [134]. Besides, Chumroenphat et al. [135] discovered, by the use of HPLC, the presence of compound **6** in *C. diffusa* with a concentration of 46.65 ± 0.53 μg/g. The present phytochemical investigation substantially expands the known chemical profile of *C. diffusa* and provides additional chemotaxonomic information for the genus *Commelina*. The significance of these findings is further supported by the biological evaluation conducted in this study. Several newly reported compounds exhibited inhibitory activity against both α-glucosidase and α-amylase, with compounds **9**, **10**, and **14** showing particularly strong inhibitory effects. These findings indicate that the newly identified constituents not only contribute to a better understanding of the phytochemical

composition of *C. diffusa*, but also represent promising candidates for further investigation of their potential antihyperglycemic effects. However, the observed enzyme-inhibitory activities were considered preliminary evidence. Therefore, further mechanistic, pharmacological, toxicological, and *in vivo* studies are needed to clarify the biological effects and assess the therapeutic potential of these individual compounds.

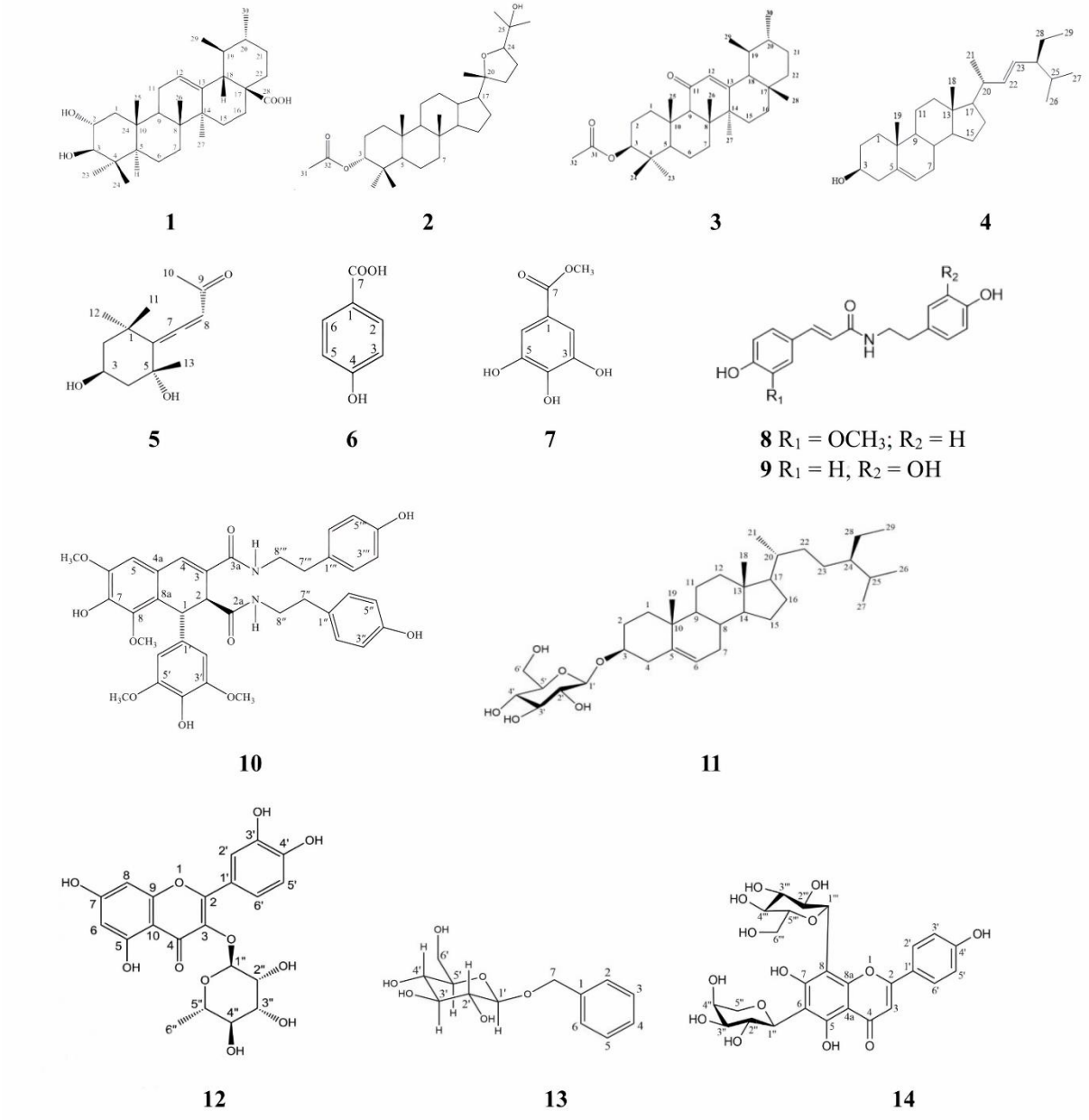



**Figure 4.1.** Chemical structures of isolated compounds (**1**–**14**) from *C. diffusa*

Corosolic acid (**1**), also identified as 2α-hydroxyursolic acid, is a pentacyclic triterpenoid that was primarily isolated from the leaves of *Lagerstroemia speciosa* L. (banaba), a medicinal plant traditionally used in Southeast Asia for the treatment of diabetes. It has also been identified in other plant species such as *Actinidia chinensis*, *Eriobotrya japonica*, *Canna indica*, and *Ursolia ulmaceae* [136]. Due to its structural relationship to ursolic acid, corosolic acid has received significant scientific interest. This compound is particularly well-known for its potent antidiabetic effects, which are mediated through multiple mechanisms, including enhancing glucose uptake by activating the translocation of GLUT4 in cells, improving insulin sensitivity, and modulating key enzymes involved in carbohydrate metabolism [137]. Besides that, corosolic acid exhibits antimicrobial, anti-obesity, anticancer, antioxidant, anti-inflammatory, and hepatoprotective properties [137,138]. Recent studies have proven its ability to inhibit tumor cell proliferation, induce apoptosis, and suppress metastasis in various cancer models [139]. Additionally, the anti-inflammatory activity of this compound is mediated through inhibition of nuclear factor kappa B and pro-inflammatory cytokines, such as TNF-α and IL-6. Its antioxidant and anti-inflammatory actions contribute to its protective effects against oxidative stress-related diseases [136,138]. Despite possessing several significant pharmacological effects, corosolic acid has low bioavailability due to its poor water solubility, which is a key factor limiting its application as a therapeutic drug [140].

3α-acetyl-20S,24S-epoxydammarane-25-ol (**2**), also known as cabraleadiol monoacetate, is the acetylation product of cabraleadiol. This compound is a dammarane-type triterpenoid, which is isolated from various plant and lichen sources. In Vietnam, it has been extracted from the bark of *Aglaia lawii* (Meliaceae), a traditional medicinal plant used for treating bacterial infections and tumors [141]. According to a study conducted by Kathirgamanathai and colleagues, this compound, isolated from the hexane fraction of *Pyxine consocians*, demonstrated the ability to kill Aedes aegypti mosquito larvae at a concentration of 10 ppm, resulting in a 90% mortality rate after 24 hours [142]. Furthermore, cabraleadiol monoacetate may perform weak cytotoxic activity

against CV-1 normal kidney fibroblasts, B16-F10 melanoma, MCF-7 breast cancer, and P-388 murine leukemia cell lines [143,144].

11-oxo-α-amyrin acetate (**3**), also known as neoilexonol acetate, is an ester of oleanolic acid with the reactive site at C-3 [145]. In Vietnam, Bui Xuan Hao and his colleagues isolated this compound from the ethyl acetate fraction of the leaves and stems of *Streptocaulon juventas* [119]. In a study by Muhammad S. Ali, 11-oxo-α-amyrin acetate isolated from the flowers and fruits of *Alstonia scholaris* (L.) R. Br. (Apocynaceae) showed significant antibacterial activity against *S. typhi* and *P. mirabilis* [146]. Interestingly, an *in silico* study revealed that this compound can inhibit 22.8% of the specific binding of [$^3$H]-DPDPE to HEKhDOR cell membranes expressing δ-opioid receptors and 23.0% of the specific binding of [$^3$H]-DAMGO to HEKhMOR cell membranes expressing μ-opioid receptors at a concentration of 10 μM [147].

Stigmasterol (**4**) is a phytosterol found abundantly in edible and medicinal plants, including *Abelmoschus esculentus* (okra), *Glycine max* (soybean), *Solanum tuberosum* (potato), *Brassica napus* (rapeseed), *Solanum lycopersicum* (tomato), *Canna indica* L., and *Calotropis gigantea*. Structurally similar to cholesterol, stigmasterol is a precursor to the biosynthesis of progesterone and other steroid hormones [148]. Various pharmacological studies have demonstrated a broad spectrum of its bioactivities, including anticancer, anti-osteoarthritic, anti-inflammatory, antidiabetic, immunomodulatory, antiparasitic, antifungal, antibacterial, antioxidant, and neuroprotective effects [149]. In cancer therapy, stigmasterol modulates key intracellular signaling pathways. It targets the Akt/mTOR and JAK/STAT pathways in ovarian and gastric cancers and inhibits angiogenesis in human cholangiocarcinoma through downregulation of TNF-α and VEGFR-2 signaling. Additionally, the combination of this stigmasterol and sorafenib enhances caspase-3 activation and reduces Bcl-2 expression in breast cancer cells. In skin cancer, stigmasterol may exert chemoprotective effects by reducing lipid peroxidation and DNA damage thanks to its antioxidant activity [150]. Stigmasterol can regulate reactive oxygen species (ROS), inhibit acetylcholinesterase,

and attenuate dopamine depletion, which results in its neuroprotective effect. The anti-inflammatory properties of this compound involve the suppression of iNOS and COX-2, reduction of pro-inflammatory mediator liberation, and promotion of anti-inflammatory cytokines [151]. Meanwhile, its antidiabetic effects are achieved through the reduction of fasting glucose, serum insulin, and the improvement of glucose tolerance. Moreover, recent studies have proven that stigmasterol demonstrates antiparasitic activity against *Trypanosoma congolense* and *Leishmania major*. It also exhibits antifungal effects against *Candida albicans*, *C. tropicalis*, and certain viruses at low concentrations [148,151].

Lyratol F (**5**) is a monoterpene, which was primarily isolated from the chloroform extract of the whole plant of *Solanum lyratum* Thunb, a perennial herbaceous plant from the Solanaceae family [121]. Besides, this compound can be found naturally in other species, such as *Illicium merrillianum*, *Croton euryphyllus*, and *Magnolia maudiae* [152,153]. Preliminary cytotoxicity screening revealed that lyratol F performed significant cytotoxicity against two cancer cell lines, including P-388 (mouse lymphocytic leukemia) and HT-29 (human colon adenocarcinoma) cells, with $ED_{50}$ values of 2.7 μg/ml and 1.9 μg/ml, respectively [154]. Currently, data on the specific pharmacological activities of lyratol F are scarce, as it is a relatively understudied compound compared to other monoterpenes.

4-hydroxybenzoic acid (**6**) is an aromatic hydroxy acid that serves as a key intermediate in the synthesis of various bioproducts, with potential applications in the cosmetic, food, and pharmaceutical industries [155]. In nature, it is widely distributed in various plant-based foods and medicinal herbs, such as *Oryza sativa* (rice hulls), *Daucus carota* (carrot), *Vitis vinifera* (grape), *Dendrocalamus asper* (bamboo), *Elaeis guineensis* (oil palm), *Areca catechu* (betel palm), *Mespilus germanica* (medlar), *Pterocarpus santalinus* (red sandalwood), and *Commelina communis* (Asiatic dayflower) [156]. This compound has a broad range of pharmacological activities, making it a promising candidate for therapeutic applications. The well-known pharmacological characteristic

of 4-hydroxybenzoic acid was its antibacterial properties against both Gram-negative and Gram-positive bacteria, especially in gastrointestinal infections (e.g., *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, etc) [122,156]. As a phenolic compound, it scavenges free radicals to reduce oxidative stress, which leads to its role in the protection of oxidative damage in cellular models and the prevention of chronic diseases [157]. A previous study demonstrated that this compound was capable of dose-dependent reduction of the serum glucose level in streptozotocin-treated diabetic mice by increasing peripheral glucose consumption [122]. Furthermore, the antiepileptic potential of 4-hydroxybenzoic acid is mediated through the enhancement of the activation of Kv1.4 potassium channels, resulting in the lowering of membrane excitation [158]. Besides, 4-hydroxybenzoic acid also has various functional biological properties, including neuroprotective, anticancer, antiaging, antiviral, anti-inflammatory, anticoagulant, antiplatelet aggregation, and lifespan extension activities [159-161].

Methyl gallate (**7**), also known as methyl 3,4,5-trihydroxybenzoate, is a gallotannin present in many medicinal and food plants, such as *Galla chinensis* (Chinese gallnut), *Mangifera indica* (mango), *Paeonia lactiflora* (peony), and *Commelina communis*, a plant belonging to the *Commelina* genus. It has been proven to possess anti-allergic, antioxidant, anti-inflammatory, antitumor, anti-aging, antidiabetic, antimalarial, antimicrobial, anti-leishmanial, antiurolithiatic, antiplatelet, hepatoprotective, and nephroprotective activities [162]. Its anti-inflammatory potential was exhibited by modifying the production of some inflammatory mediators, including IL-6 (interleukin-6), IL-1β (interleukin-1β), and the activation and migration of leukocytes. Furthermore, this compound could inhibit the activity of cyclooxygenase-2 (COX-2) and 5-lipoxygenase in bone marrow-derived mast cells [163]. The antimicrobial properties of methyl gallate are exhibited in both Gram-positive and Gram-negative bacteria, such as *Burkholderia cenocepacia*, *Achromobacter xylosoxidans*, and *Stenotrophomonas maltophilia*, by disrupting microbial cell membranes and inhibiting biofilm formation [164]. Methyl gallate is a potent free radical scavenger, neutralizing reactive oxygen species (ROS) and protecting cells from oxidative stress. Its strong antioxidant potential,

along with its lipid peroxidation inhibitory effect, contributes to preventing oxidative stress-related diseases like cardiovascular disorders and non-alcoholic fatty liver disease [165]. In oncology research, methyl gallate has been shown to induce apoptosis and inhibit proliferation in cancer cell lines, such as HepJ5, Hep3B, and Mahlavu cells, by modulating pathways like PI3K/Akt and upregulating pro-apoptotic proteins (e.g., Bad, Bax, Bcl-2) [166]. Recent studies have revealed that methyl gallate exhibits promising antidiabetic effects by enhancing insulin sensitivity and glucose metabolism. One of the primary mechanisms involves the inhibition of carbohydrate-digesting enzymes, particularly α-glucosidase, leading to delayed glucose absorption and attenuated postprandial hyperglycemia. Additionally, it enhances insulin sensitivity and improves glucose uptake in peripheral tissues, partly through modulation of the insulin signaling pathway, including increased phosphorylation of IRS-1 and Akt [164,167,168]. Another study has indicated that methyl gallate can significantly improve hyperuricemia nephropathy by reducing uric acid production, enhancing uric acid excretion, and mitigating renal damage associated with NLRP3 inflammasome activation, which is associated with the pathogenesis of hyperuricemic nephropathy symptoms. It is a direct inhibitor of the NLRP3 inflammasome, selectively suppressing its activation in macrophages without affecting the activity of other inflammasomes such as AIM2 or NLRC4 [169]. Moreover, methyl gallate expresses its antiurolithiatic activity by inhibiting the formation of calcium oxalate crystals [170]. This compound also possesses anti-leishmanial properties with a mechanism of promoting the production of ROS, NO, IL-12, and TNF-α, while decreasing the level of IL-6 and arginase activity [171].

*N-trans*-feruloyltyramine (**8**) is a phenolic amide that is primarily isolated from the roots of *Solanum melongena* L. [172]. In nature, it is also distributed in various edible and medicinal plants, such as *Tinospora cirspa*, *Corydalis pallida*, *Datura metel*, *Synsepalum dulcificum* Daniell, *Tetragonia tetragonioides*, *Piper nigrum*, *Cannabis sativa*, and *Polygonum sachalinensis* [172,173]. Recent studies have shown that it possesses a wide range of pharmacological activities, such as antioxidant, anticancer, anti-melanogenesis, antidiabetic, antibacterial, anticholinesterase, anti-

inflammatory, antiproliferative, neuroprotective, and slight analgesic properties [173,174]. It was reported that *N-trans*-feruloyltyramine expressed its anti-inflammatory activity in lipopolysaccharide-stimulated RAW 264.7 macrophages by downregulating the mRNA expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), leading to a significant reduction in the generation of nitric oxide and prostaglandin $E_2$ ($PGE_2$) without affecting the expression of tumor necrosis factor-alpha (TNF-α). In addition, it can inhibit the nuclear translocation of activator protein-1 (AP-1) and concurrently suppress both the expression and phosphorylation of c-Jun N-terminal kinase (JNK), suggesting its regulatory role in the AP-1/JNK signaling pathway [173]. Another study has revealed that *N-trans*-feruloyltyramine demonstrates antiproliferative effects on three cancer cell lines, including HepG2 (hepatocellular carcinoma), A2780 (ovarian cancer), and Panc1 (pancreatic cancer) cells [175,176]. Additionally, it exhibits neuroprotective potential by inhibiting the activities of key pathological targets of Alzheimer's disease, including β-site amyloid precursor protein cleaving enzyme 1, monoamine oxidase-B, amyloid-β aggregation, and Tau protein phosphorylation, with $IC_{50}$ values ranging from 1.2 to 11.6 μg/mL [177]. The anti-melanogenesis property of *N-trans*-feruloyltyramine is mediated through the reduction of melanin synthesis induced by α-melanocyte-stimulating hormone in B16F10 melanoma cells [178]. This compound also has an antidiabetic function relevant to the management of type 2 diabetes mellitus by enhancing insulin sensitivity, stimulating insulin secretion, modulating the activity of key enzymes related to glucose metabolism, and improving dysregulated lipid metabolism [179].

*N-trans-p*-coumaroyl-3',4'-dihydroxyphenylethylamine (**9**) is a phenolic amide within the phenylpropanoid family, which was first isolated from *Atraphaxis spinosa* L. var. *sinaica* Boiss. (Polygonaceae) [180]. This compound can also be found naturally in two other plant species, including *Solanum rostratum* Dunal and *Croton megalocarpoides* Friis & M. G. Gilbert [181,182]. It was demonstrated that *N-trans-p*-coumaroyl-3',4'-dihydroxyphenylethylamine exposed the cytotoxic activity on leukemic P388 cells with $IC_{50}$ values of 3.8 μg/mL [180]. At present, information regarding the

specific pharmacological activities of *N-trans-p*-coumaroyl-3',4'-dihydroxyphenylethylamine remains limited.

1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**) is a rare lignanamide, which is only found in *Procelia macrocarpa*, *Piper wallichii*, and *Corydalis saxicola* [126,183,184]. This compound exhibited potent antioxidant activity with an $IC_{50}$ value of 31.38 ± 0.97 μmol/L in the DPPH assay and the inhibitory effect against acetylcholinesterase (AChE) enzyme with an inhibition rate of 28.57% at the concentration of 100 μmol/L [183]. Besides, Zhang et al. reported the anticancer effect of 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide against three tumor cell lines, including MGC-803, T24, and Spca-2, with $IC_{50}$ values of 55.16 ± 0.78 μM, 48.15 ± 1.09 μM, and 43.89 ± 1.57 μM, respectively [184].

Daucosterol (**11**), also identified as β-sitosterol-β-D-glucoside or eleutheroside A, is a phytosterol glycoside found in many medicinal plants, such as *Fallopia cillinerve*, *Hyssopus cuspidatus*, *Centaurea resupinata*, *Ononis mitissima*, *Prangos ferulacea*, *Astragalus tanae*, *Dioscorea batatas*, and *Commelina communis*. In Vietnam, this compound is present at high concentrations in the plant extracts of *Archidendron clypearia*, *Sanchezia nobilis*, and *Canna edulis* [185-187]. Daucosterol is composed of sitosterol attached to β-D-glucopyranosyl at position 3 through a glycosidic bond; therefore, it has properties and pharmacological effects similar to β-sitosterol [188]. Recent studies have reported the antioxidant, anticancer, neuroprotective, anti-inflammatory, immunomodulatory, anti-osteoporosis, hypoglycemic, and lipid-lowering effects of daucosterol [185]. It expresses the anti-inflammatory effect by inhibiting the production of colitis-induced reactive oxygen species and the upregulation of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, and interferon-γ, while modulating T-cell populations and reducing macrophage infiltration [189]. An *in vivo* study in an alcohol-induced hepatic injury mouse model has reported that daucosterol provides

hepatic protection by exerting both anti-inflammatory and antioxidant effects, which are critical in the management of chronic inflammatory conditions. It modulates the p38/NF-κB/NLRP3 inflammasome signaling pathway, suppresses the nuclear translocation of NF-κB, and inhibits NLRP3 inflammasome activation through the reduction of p38 phosphorylation [190]. The mechanisms of the anticancer property of this molecule operate from the subcellular to the molecular level by elevating pro-apoptotic proteins such as Bax, reducing the Bcl-2/Bax ratio, and upregulating the PTEN gene to suppress the PI3K/Akt signaling pathway. These actions lead to the loss of mitochondrial membrane potential and the release of cytochrome C in breast cancer cells, thereby promoting apoptosis [185,191]. Besides, daucosterol may exhibit antidiabetic effects by significantly lowering fasting blood glucose levels, ameliorating glucose intolerance, and alleviating pancreatic islet damage. It also reduces the HOMA-IR values and increases ISI, reflecting improved insulin sensitivity [192].

Quercitrin (**12**) is a flavonol glycoside, which is widely distributed in plants, particularly in medicinal species such as *Quercus tinctoria* (oak bark), *Fagopyrum tataricum* (buckwheat), *Polygonum hydropiper*, *Albizia julibrissin*, *Hypericum japonicum*, and *Amomum villosum*, as well as in various fruits and vegetables [193,194]. For the genus *Commelina*, this compound has only been isolated from two species, including *Commelina erecta* and *Commelina communis* [195,196]. Due to its structural relationship with quercetin, quercitrin exhibits a broad spectrum of pharmacological properties, including antioxidant, anti-inflammatory, antitumor, neuroprotective, antifibrosis, antimicrobial, antidepressant, and cardioprotective activities. The antioxidant and anti-inflammatory effects of quercitrin are expressed through the reduction of nitric oxide, pro-inflammatory factors (IL-6, IL-1β, and TNF-α), and ROS levels in lipopolysaccharide-induced RAW264.7 macrophages [197]. A previous study has proven that these mechanisms contribute to the neuroprotective effects of this molecule. Additionally, quercitrin can markedly reduce the malondialdehyde (MDA) levels, attenuate tissue plasminogen activator activity, improve antioxidant enzyme activities, and inhibit the induction of cytochrome P450 2E1 (CYP2E1) in mice with

brain dysfunction induced by $CCl_4$. It also inhibits the activities of monoamine oxidase (MAO), acetylcholinesterase, and the N-methyl-D-aspartate receptor 2B subunit (NR2B) [198]. According to Cincin ZB and colleagues, quercitrin treatment led to an enhancement in caspase-3 activity, disruption of mitochondrial membrane potential, and an increase in apoptotic cell population in DLD-1 colon cancer cells, as well as A549 and NCI-H358 non-small cell lung cancer cells, in a time- and dose-dependent manner [199,200]. It was reported that a single dose of quercitrin at 10 mg/kg could inhibit the PI3K/AKT/NF-κB inflammatory pathways and enhance the neuroplasticity signaling (pCREB/BDNF/PSD95/Synapsin1) in the hippocampus of lipopolysaccharide-treated depression-like behavioral mice, which resulted in the antidepressant function of this compound [201]. Meanwhile, quercitrin exerts therapeutic activities in polycystic ovary syndrome combined with insulin resistance by improving the activation of peptidase M20 domain-containing 1, thereby activating the PI3K/Akt signaling pathway, enhancing adipocyte catabolism, and alleviating both reproductive and metabolic abnormalities [202].

Benzyl-O-β-D-glucopyranoside (**13**) is a phenolic glycoside composed of a benzyl moiety linked to a β-D-glucose unit via a glycosidic bond, which is naturally found in various medicinal plant species. Benzyl-O-β-D-glucopyranoside exhibits several pharmacological activities, although research on this compound is primarily in the preclinical stage. Because of its phenolic structure, this molecule has antioxidant activities, which scavenge free radicals and potentially protect against oxidative stress-related conditions [203]. A previous study indicated that benzyl-O-β-D-glucopyranoside isolated from the fruits of *Prunus mume* showed strong inhibitory effects on osteoclast differentiation by reducing the activation of tartrate-resistant acid phosphatase in pre-osteoclastic RAW264.7 cells [204]. Benzyl-O-β-D-glucopyranoside is considered a promising agent for the management of type 2 diabetes and obesity due to its significant inhibitory activity against protein tyrosine phosphatase 1B (PTP1B) [205]. This enzyme is an intracellular enzyme found in insulin-targeted tissues, such as the liver, muscle, and fat, which plays a major role as a negative regulator of insulin signaling by inactivating

the insulin receptor. In addition, because of its negative regulation of the leptin signaling pathway that causes metabolic disorders and obesity, inhibition of the PTP1B enzyme may also reduce obesity, a major risk factor for diabetes [206]. Moreover, benzyl-O-β-D-glucopyranoside also demonstrated the capacity to protect pancreatic islets in zebrafish larvae injured by alloxan, a type 1 diabetes animal model, by increasing the pancreatic islet size to 82.0% [207].

Isoschaftoside (**14**) is a C-glycosylflavonoid, which is widely identified in medicinal plants, such as *Viola yedoensis*, *Abrus cantoniensis*, *Desmodium uncinatum*, and *Passiflora incarnata* [208-210]. Recent studies have revealed that this compound may exert various pharmacological activities, making it a promising phytotherapeutic candidate. It was proven that isoschaftoside effectively decreased intracellular lipid deposition caused by palmitic acid in HepG2 cells *in vitro* and attenuated high-fat diet-induced hepatic steatosis, thereby reversing the progression of nonalcoholic fatty liver disease in the tested mice. These effects might be attributed to the capacity of this compound to modulate the autophagy pathway by inhibiting the expression of autophagy substrates, including light-chain 3-II and sequestosome 1 [209]. The anti-inflammatory activity of isoschaftoside is related to the inhibition of nitric oxide generation and the expression of pro-inflammatory factors, including IL-1β, COX-2, TNF-α, and iNOS. Meanwhile, the mechanism of its neuroprotective property involves the suppression of hypoxia-inducible factor 1-alpha, resulting in the modulation of the microglial metabolic reprogramming pathway [211]. A previous study indicated that isoschaftoside exhibited its antioxidant and anti-aging activities by suppressing the production of reactive oxygen species, which was related to the reduction of *RAC2* and *LINC00294* genes in senescent cells. Additionally, treatment with isoschaftoside enhanced mitochondrial function, resulting in the restoration of cellular metabolism and the amelioration of glycolytic dependence, thereby decreasing several senescence-associated phenotypes and contributing to the functional rejuvenation of senescent cells [212].

## 4.2. Hypoglycemic activities of *C. diffusa*

### 4.2.1. *In vitro* assays against α-glucosidase and α-amylase enzymes

Extracts of *Commelina diffusa* and their secondary metabolites were evaluated for their antihyperglycemic potential through inhibition assays against two digestive enzymes, including α-amylase and α-glucosidase. α-Amylase is a key enzyme that is secreted by the salivary glands and pancreas, and initiates the hydrolysis of dietary starch and glycogen into smaller oligosaccharides [42]. Subsequently, α-glucosidase, which is located in the brush-border membrane of the small intestine, catalyzes the cleavage of α-D-(1,4)- glycosidic bonds in oligosaccharides, thereby releasing glucose for intestinal absorption [45]. Consequently, inhibiting the activity of both enzymes is a well-established therapeutic strategy to delay carbohydrate digestion and reduce postprandial glucose absorption, resulting in glycemic control [41].

According to several pharmacological investigations, some extracts of plants from the *Commelina* genus demonstrate insulin-mimetic and antihyperglycemic activities. In 2004, Ji-Youn Youn et al. [66] evaluated the *in vitro* and *in vivo* blood glucose stabilizing effects of the extract of *C. communis* species and found that *C. communis* extract showed dose-dependent α-glucosidase inhibition and amelioration of maltose or starch-induced hyperglycemia in diabetic mice. Furthermore, prolonged administration of *C. communis* could stabilize blood glucose in the diabetic mouse model induced by streptozotocin. Besides, the polyhydroxyalkaloids, which are isolated from *C. communis*, are proven to exhibit the capacity of α-glucosidase inhibition [59]. In recent years, pharmacological investigations of *C. benghalensis* have revealed that the methanolic extract of this plant has significant antihyperglycemic activity in the alloxan-induced diabetic mouse model [64]. The evidence, as mentioned above, proposes that *C. diffusa*, a species within the *Commelina* genus, may effectively possess antihyperglycemic activities. The obtained results indicated that the ethyl acetate fraction of *C. diffusa* (CD.EAF) exhibited the strongest inhibitory activity against both α-glucosidase and α-amylase enzymes in a concentration-dependent manner. Their $IC_{50}$ values against

α-glucosidase and α-amylase enzymes were about 6-fold and 3-fold lower than those of the positive control, acarbose, respectively. Therefore, CD.EAF was selected to analyze the acute toxicity and the *in vivo* hypoglycemic effect.

In the evaluation of isolated compounds, all investigated substances demonstrated inhibitory effects on α-glucosidase and α-amylase enzymes in a concentration-dependent manner. Among them, *N-trans-p*-coumaroyl-3',4'-dihydroxyphenylethylamine (**9**), 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**), and isoschaftoside (**14**) had the strongest inhibitory potentials in both assays. This is the first report of the antidiabetic properties of compounds **9** and **10**, while compound **14** is proven to have an effective inhibitory effect on the α-glucosidase enzyme [213]. Similarly, this research indicated for the first time that 3*α*-acetyl-20*S*,24*S*-epoxydammarane-25-ol (**2**) and 11-oxo-α-amyrin acetate (**3**) possessed weak and moderate inhibitory activities against the two tested enzymes. Although stigmasterol (**4**), 4-hydroxybenzoic acid (**6**), and daucosterol (**11**) show no inhibitory effects on both α-glucosidase and α-amylase enzymes at the investigated concentrations, recent studies have proven that they exhibit antihyperglycemic ability through different mechanisms of action. Particularly, compound **4** could improve insulin resistance and oral glucose tolerance by decreasing the fasting plasma glucose levels and lipid parameters in KK-Ay mice with type 2 diabetes. These effects were related to the enhancement of the translocation and expression of glucose transporter 4 in L6 cells [214]. It was reported that oral treatment of compound **6** resulted in a dose-dependent reduction in plasma glucose levels without altering the plasma insulin concentrations or hepatic glycogen content in streptozotocin-induced diabetic mice. However, it significantly enhanced glucose uptake in the diaphragms of both normoglycemic and hyperglycemic mice. These findings suggested that the anti-diabetic effect of this compound was primarily mediated through increased peripheral glucose utilization rather than insulin modulation or hepatic glycogen storage [215]. Meanwhile, compound **11** exhibited hypoglycemic activity by a reduction in the homeostasis model assessment of insulin resistance index and an enhancement in the

insulin sensitivity index, resulting in an improvement in insulin sensitivity. It also optimized the gut microbiota diversity to improve insulin resistance in type 2 diabetic rats [192]. Although *N-trans*-feruloyltyramine (**8**) exhibited strong inhibitory activity against α-glucosidase, it demonstrated limited effectiveness in inhibiting α-amylase. Regarding the remaining substances, the inhibitory activity against the α-glucosidase enzyme of corosolic acid (**1**), lyratol F (**5**), and methyl gallate (**7**) was comparable to that of the positive control (acarbose), while lyratol F (**5**) and quercitrin (**12**) could be effective inhibitors of the α-amylase enzyme.

The differences in enzyme inhibitory activity among the isolated compounds suggest that structural features may influence their interactions with α-glucosidase and α-amylase enzymes. Compounds **9**, **10**, and **14**, which exhibited the strongest inhibitory activities against both enzymes, share structural characteristics that may favor interactions with the enzyme active sites, whereas compounds with less favorable structural features showed weaker inhibition. However, because the number of isolated compounds evaluated was limited, these observations were regarded as preliminary structure–activity relationships rather than definitive SAR conclusions. Hence, further studies using structurally related analogues are required to clarify the structural determinants of enzyme inhibition.

### 4.2.2. Acute toxicity of CD.EAF

Acute toxicity studies of medicinal plants are conducted to evaluate potential adverse effects, assess their clinical relevance, and determine dose-dependent toxicological responses, including mortality. In these evaluations, the median lethal dose ($LD_{50}$) is the dose required to cause death in 50% of the test animal population, which is a key parameter to indicate the toxic potential of pharmacological substances [216]. Our study assessed the $LD_{50}$ value of CD.EAF according to the OECD guidelines 423/425 [114,115]. These approaches are designed to provide toxicity information on pharmaceuticals, cosmetics, food, and industrial products across various administration routes, such as oral, dermal, and inhalation. Its advantages include requiring fewer

animals, exploring a wide dose range, simplicity, cost-effectiveness, and reproducibility, which align partially with the 3Rs principle (reduction, refinement, replacement). However, the main drawbacks of this method are the potential for inaccuracy in complex cases, ethical concerns, and limited non-lethal data, such as organ-specific toxicity or behavioral changes [217,218].

In our present study, oral treatment of CD.EAF at the highest dose of 2000 mg/kg did not produce any observable signs of toxicity or mortality in mice, indicating a high safety characteristic of this fraction with an estimated $LD_{50}$ value greater than 2000 mg/kg. This result was aligned with previous findings on the acute toxicity of the methanolic extract of *C. diffusa*, which similarly showed no lethal effects at 2000 mg/kg. Specifically, although a significant reduction was seen in physical activity between 0.5 and 4 hours post-administration, all animals treated with the methanolic extract of *C. diffusa* at a dose of 2000 mg/kg returned to normal thereafter [83]. Meanwhile, by using a brine shrimp lethality assay, Mahmuda Nasrin and colleagues indicated that the dichloromethane–methanolic crude extract of *C. diffusa* had cytotoxic ability to tested animals, with an $LD_{50}$ index of 3.79 μg/mL when compared to the standard drug, vincristine sulfate ($LD_{50}$ = 0.81 μg/mL) [219]. Differences in acute toxicity results for *C. diffusa* across studies may be due to variation in extraction solvents and experimental animal models.

### 4.2.3. Antihyperglycemic effects of CD.EAF on the high-fat diet and streptozotocin-induced type 2 diabetic mice

Streptozotocin (STZ) is a well-established antineoplastic agent, which is commonly used to induce both insulin-dependent (type 1) and non-insulin-dependent (type 2) diabetes mellitus in experimental animal models [220]. The type of diabetes induced mainly depends on the STZ dosage. STZ enters pancreatic β-cells via the GLUT2 glucose transporter due to its structural similarity to glucose, resulting in selective accumulation and cytotoxicity in β-cells [221]. At high doses, STZ causes direct β-cell destruction through DNA alkylation and depletion of nicotinamide adenine

dinucleotide ($NAD^+$) levels. In contrast, low-dose STZ induces β-cell damage indirectly through immune-mediated mechanisms, including the generation of glutamic acid decarboxylase (GAD) autoantigens that trigger T lymphocyte infiltration into pancreatic islets [222].

Hayashi et al. [223] developed a type 2 diabetes mouse model using a single intraperitoneal injection of STZ (100 mg/kg). In the present study, a combination of STZ and a high-fat diet (HFD) was used to induce a type 2-like diabetic condition. It is proven that obesity, caused by HFD, is known to enhance circulating free fatty acid levels, promoting lipid over glucose utilization in muscle tissue. These effects contribute to insulin resistance, which is a key feature of type 2 diabetes [224]. The obtained results demonstrated that mice exhibited a significant increase (79.2%) in body weight compared to the time point "Before studying" after 8 weeks of HFD feeding. Besides, blood glucose levels were markedly elevated in HFD-fed mice compared to those on a normal-fat diet (NFD). Moreover, 72 hours after STZ administration, a significant increase was observed in serum glucose concentrations of HFD-fed mice, which were approximately three times higher than the values at the beginning of treatment. These findings confirmed the successful establishment of a type 2 diabetes phenotype through combined HFD and STZ treatment, providing an appropriate model for evaluating the antihyperglycemic effect of CD.EAF.

Our present study revealed that the repeated daily doses of gliclazide (80 mg/kg/day) and the ethyl acetate fraction of *C. diffusa* (CD.EAF) at both tested doses (100 mg/kg/day and 300 mg/kg/day) via oral administration over 14 days significantly decreased the plasma glucose concentrations in HFD and STZ-induced type 2 diabetic mice compared to the negative control group. The antihyperglycemic property of CD.EAF may be attributed to the interactions between its major phytoconstituents and key metabolic enzymes, including α-amylase and α-glucosidase. Moreover, the reduction in blood glucose following CD.EAF treatment suggests that this fraction contains constituents capable of modulating pathways involved in glucose homeostasis. Recently, Chuong Thi

Ngoc Hieu and her colleagues [225] also found the hypoglycemic effect of *C. diffusa* extracts in STZ-induced diabetic mice using both fasting blood glucose and glucose tolerance tests. In this study, the diabetic animal model was induced via a single intraperitoneal injection of STZ at a dose of 170 mg/kg. Among the extracts tested, the 96% ethanol extract at doses of 0.21 and 0.42 g/kg produced a more typical antihyperglycemic potential than the aqueous extract (0.36 and 0.72 g/kg) and 50% ethanol extract (0.49 and 0.98 g/kg) in both fasting serum glucose and glucose tolerance assays after 7 days of treatment. Furthermore, the effect of 96% ethanol extract on reducing fasting blood glucose was comparable to that of glibenclamide (5 mg/kg), while its efficacy in the glucose tolerance test was slightly lower than that of the reference drug.

In contrast to its remarkable antihyperglycemic effect, CD.EAF did not significantly improve the serum lipid profile at either tested dose. Notably, a previous study has shown that ethanolic extracts of *C. diffusa* leaves at doses of 200 mg/kg and 400 mg/kg significantly reduced TC, TG, and LDL-C concentrations while increasing HDL-C levels in doxorubicin-induced cardiomyopathic rats [226]. The difference from our current findings may be attributed to variations in experimental models, treatment conditions, plant parts used, and the polarity of extraction solvents. Besides, the obtained results suggested that the glucose-lowering effect of CD.EAF may not be accompanied by a similar effect on lipid metabolism under the experimental conditions of this study. The absence of significant changes in lipid parameters may be related to differences in the mechanisms regulating glucose and lipid metabolism, the relatively short treatment period, or the specific composition of the fraction. Therefore, the present findings support the antihyperglycemic potential of CD.EAF but do not provide sufficient evidence to confirm a lipid-lowering effect. Further studies involving longer treatment periods and investigations of the mechanisms underlying lipid metabolism are needed to clarify the potential effects of CD.EAF on lipid homeostasis.

Diabetes mellitus and obesity contribute to liver and kidney degeneration through different pathways, involving oxidative stress, inflammation, and metabolic

dysregulation [227]. In diabetes, chronic hyperglycemia generates oxidative stress by upregulating the production of reactive oxygen species through NADH-oxidase and the formation of advanced glycation end-products, which damage hepatocytes and renal cells. This contributes to non-alcoholic fatty liver disease in the liver, marked by steatosis, inflammation, and progression to non-alcoholic steatohepatitis, and diabetic nephropathy in the kidneys, characterized by glomerulosclerosis and tubular injury. Obesity exacerbates these effects through lipotoxicity, where excessive free fatty acids from adipose tissue overload the liver, promoting lipid accumulation and fibrosis, and inducing podocyte damage [228,229]. In our study, histopathological analysis revealed marked structural improvements in both the liver and pancreas of mice treated with CD.EAF after two weeks of intervention. Hence, these effects may contribute to the observed antihyperglycemic activity of CD.EAF. However, an apparent discrepancy was observed between the histopathological findings and the serum aminotransferase results. Although histopathological observations suggested improvement in liver morphology following CD.EAF treatment, the changes in serum AST and ALT were not statistically significant. This might be due to serum aminotransferases and histopathological examination providing different types of information regarding hepatic status. In particular, serum AST and ALT provide biochemical indicators of hepatocellular injury, whereas histopathology provides direct morphological information at the tissue level. In the present study, AST and ALT levels in the diabetic control group were slightly higher than those of the normal control group, which may partly explain why treatment-related changes in serum AST and ALT were not detected. This finding contrasts with a previous study of *C. diffusa* extract, which reported reductions in AST and ALT together with improvements in hepatic oxidative stress markers [81]. The difference may be related to variations in the plant extract, experimental model, treatment conditions, and duration of treatment. Furthermore, the present histopathological assessment was qualitative rather than quantitatively or semi-quantitatively scored. Therefore, the observed histological improvement was considered supportive rather than definitive evidence of

hepatoprotective activity. Further studies using quantitative histological scoring and additional liver biomarkers are needed to clarify the effects of CD.EAF on hepatic injury.

Besides, a recent study indicated that oral treatment with *C. diffusa* extract could significantly improve the testicular tissue injury induced by diabetes in HFD and STZ-induced male Wistar rats. Specifically, after 21 days of administration, it exhibited antioxidant activity by reducing malondialdehyde concentrations and enhancing levels of glutathione, glutathione peroxidase, superoxide dismutase, total antioxidant capacity, nitric oxide, and catalase, which led to the protection of oxidative damage caused by diabetes and the improvement of antioxidant defense systems. It also decreased all sexual hormone levels, including testosterone, AMH, FSH, and LH, resulting in the maintenance of reproductive hormone balance. Additionally, a substantial increase was observed in the sperm parameters, including motility, count, and viability, in the diabetic animals treated with *C. diffusa* extract [230]. On this basis, *C. diffusa* may offer a promising therapeutic option for both diabetes and its associated complications.

### 4.3. Molecular docking simulation

#### 4.3.1. Docking result analysis of isolated compounds against the targets

Molecular docking was performed to explore the potential interactions between the isolated compounds and the target enzymes, α-amylase and α-glucosidase. The protein structures with PDB IDs 2QV4 (α-amylase) and 3W37 (α-glucosidase) were selected as structural models corresponding to the two carbohydrate-digesting enzymes evaluated in the *in vitro* inhibition assays [103,104]. These structures contained co-crystallized acarbose, enabling evaluation of the docking protocol by redocking the reference ligand. Although the protein structure of α-glucosidase used for docking was obtained from a different biological source than the enzyme used in the *in vitro* experiments, it provided an experimentally determined structural model of the corresponding enzyme target. Therefore, the molecular docking analysis was considered a complementary approach to explore potential compound–enzyme interactions that may

help explain the experimentally observed inhibitory activities, rather than as a direct validation of the *in vitro* results.

To evaluate the docking procedures, acarbose was redocked into the active sites of the target proteins. The similarity between the conformations of the native ligands and the re-docked ligand, as well as the RMSD values for both targets being less than 1.5 Å, indicated the accuracy of the molecular docking simulation method used in this study. In the analysis of the binding interactions between acarbose and the enzymes, it interacted with three key amino acids of 2QV4, including Asp197, Glu233, and Asp300, via conventional hydrogen bonds. These residues take responsibility for the catalytic action of 2QV4. Furthermore, the hydrogen bonds between the sugar hydroxyls of acarbose and the His101 and His201 amino acids of 2QV4 contributed to stabilizing and enhancing the binding specificity [231]. Meanwhile, acarbose also formed conventional hydrogen bonds with the residues Asn237, Arg552, and Asp568 in the active site of 3W37. These interactions played a crucial role in improving the binding affinity and specificity of the complex [232].

The binding affinity between a protein and ligand is defined by binding free energy, which can be accurately predicted using computationally intensive methods, such as free energy perturbation and thermodynamic integration. However, these approaches are not suitable for large-scale virtual screening due to their high cost. Meanwhile, molecular docking provides a faster alternative by employing scoring functions to estimate protein–ligand binding free energy from a single complex structure, enabling efficient screening of large molecular libraries. These scoring functions are widely used in structure-based drug design to rank putative ligand poses, thereby identifying the most favorable conformation with its score assigned for binding affinity [233]. In molecular docking simulation, the binding free energy, which is often expressed in kcal/mol, serves as a computational estimate of the Gibbs free energy change ($\Delta G$) upon formation of a ligand–protein complex. The more negative values of binding energies indicate more favorable and stable binding interactions, as they correspond to

greater predicted binding affinity and enhanced thermodynamic stability of the ligand–protein complex [234].

Our study presented that among the investigated compounds, 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**) and isoschaftoside (**14**) had the highest binding affinity toward both 2QV4 and 3W37 proteins due to their lower docking scores than the positive control, acarbose. Further analysis of possible binding interactions proved that these substances all displayed hydrogen bonds with two main amino acids, Asp197 and Glu233, in the active site of the 2QV4 enzyme. Moreover, they could create conventional hydrogen bonds, Pi-Sigma, Pi-Pi T-shaped, and Pi-Alkyl interactions with other key amino acid residues, such as Thr163, Tyr62, Trp59, Arg195, Lys200, Ile235, His101, His201, and His305, which might contribute to their strongest potency. In the case of 3W37, both substances had hydrogen-bond interactions with the Asp568 residue, which is a catalytic acid amine in the target's active site. Besides, they also displayed hydrophobic bonds with several important acid amines, including Trp432, Phe476, Phe601, Lys506, Asn496, Ala231, Ala234, Ile233, Asp357, Asp469, and Arg552. The molecular docking results provide a possible structural explanation for the experimentally observed enzyme inhibition [232]. The favorable predicted interactions of compounds **10** and **14** with residues within the active sites of α-glucosidase and α-amylase suggest that these compounds may interfere with substrate binding or enzyme catalysis, thereby potentially contributing to their inhibitory effects. Nevertheless, these docking predictions were considered supportive rather than confirmatory evidence, as docking scores and predicted binding poses cannot independently establish binding affinity or biological activity.

### 4.3.2. *In silico* analysis of drug-likeness properties and pharmacokinetic parameters

#### *Drug-likeness properties*

Lipinski's Rule of Five, which was introduced by Christopher A. Lipinski in 1997, is a set of guidelines used in drug discovery to predict the oral bioavailability of

small-molecule drug candidates. According to this rule, a compound is more likely to exhibit poor absorption or permeation when it violates more than one of the following criteria, including a molecular weight less than 500 Da, a calculated octanol-water partition coefficient (log P) less than 5, no more than 5 hydrogen bond donors, and no more than 10 hydrogen bond acceptors. These criteria are based on the physicochemical properties of most orally administered drugs, reflecting their ability to be absorbed and permeate membranes. While adherence to these rules increases the likelihood of a compound being a viable oral drug candidate, satisfying them does not guarantee success in drug development. Several factors, such as toxicity, efficacy, metabolic stability, and target specificity, must also be considered. Notably, certain drug classes, such as antibiotics, antitumor, and antifungals, are effective although they violate one or more rules. Thus, Lipinski's rules are considered a useful initial screening tool in drug discovery but are not definitive predictors of a compound becoming a drug [133,235].

In this study, among fourteen isolated compounds from *C. diffusa*, four substances did not satisfy Lipinski's Rule of Five, including 3*α*-acetyl-20*S*,24*S*-epoxydammarane-25-ol (**2**), 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**), quercitrin (**12**), and isoschaftoside (**14**). Although compounds **10** and **14** did not satisfy the overall Lipinski drug-likeness criteria, their *in vitro* inhibitory activities indicate that violation of the Rule of Five does not necessarily exclude biological activity. Additionally, it is proven that acarbose, a drug therapy for diabetic treatment and management, which is widely used in clinical settings, does not meet the criteria for this rule [236]. Consequently, compounds that violate Lipinski's rules may still possess clinical potential, and promising candidates should not be eliminated solely based on these guidelines. These findings also highlighted that Lipinski's Rule of Five is primarily a guideline for assessing drug-likeness and oral bioavailability rather than a direct predictor of pharmacological activity. Meanwhile, the overall pharmacological potential of a compound should be evaluated together with its biological activity, pharmacokinetic properties, toxicity, and target-related evidence.

***Prediction of pharmacokinetic parameters***

*In silico* studies were performed with the isolated compounds from *C. diffusa* to determine their pharmacokinetic parameters, including absorption, distribution, metabolism, excretion, and toxicity. The absorption properties were evaluated by predicting the Caco-2 cell permeability and human intestinal absorption (HIA). Caco-2 cells, which are derived from human colon adenocarcinoma, can differentiate into polarized monolayers that mimic the morphology and function of intestinal epithelial cells. Thus, this assay provides an estimate of the fraction of an orally administered drug absorbed into systemic circulation via the intestine [236]. The Caco-2 cell permeability of substances with values of log Papp in $10^{-6}$ cm/s below 0 is classified as having low permeability, whereas those between 0 and 1 are classified as moderate permeability, and values exceeding 1 indicate high permeability [237]. The obtained results showed that most tested compounds had moderate and high Caco-2 cell permeability. Meanwhile, HIA refers to the fraction of an administered dose that crosses the intestinal wall and enters the portal circulation following ingestion. Based on established threshold values, drugs are generally classified into three categories as poorly absorbed (HIA ≤ 30%), highly absorbed (HIA ≥ 80%), and moderately absorbed (values between these limits) [236]. In this study, the calculated HIA values for the evaluated compounds indicate favorable oral absorption profiles.

To determine the distribution characteristics of the isolated compounds, the volume of distribution, blood-brain barrier (BBB), and central nervous system (CNS) permeability were predicted. The volume of distribution at steady state (VDss) represents the theoretical volume in which a drug must be uniformly distributed to achieve the observed plasma concentration. A higher VDss indicates greater distribution of the drug into tissues relative to plasma. Typically, log VDss values less than −0.15 are considered poorly distributed, whereas values greater than 0.45 are regarded as well distributed [238]. With the exception of quercitrin (**12**) and isoschaftoside (**14**), the remaining compounds exhibited moderate and weak distribution results. The permeability across

the BBB and penetration into the CNS are key factors in assessing the neurological safety of a drug. In the distribution process, substances with log BB values greater than 0.3 are considered to exhibit good absorption through the BBB, while log PS values greater than -2 reflect good penetration through the CNS [239]. The present study showed that all investigated compounds had a poor ability to penetrate the CNS except for corosolic acid (**1**), 3*α*-acetyl-20*S*,24*S*-epoxydammarane-25-ol (**2**), and stigmasterol (**4**); however, only stigmasterol (**4**) could cross the BBB readily.

Drug metabolism and biotransformation are primarily mediated by cytochrome P450 isoenzymes, such as CYP2D6 and CYP3A4. Thus, understanding the role of the cytochrome P450 system is essential for elucidating drug–drug interactions that can lead to toxicities or diminished therapeutic efficacy [240]. Furthermore, a previous study demonstrated that the interaction of antidiabetic drugs with other medications through CYP3A4 and CYP2D6 inhibition results in an additive effect on the activity of antidiabetic agents [241]. Our present work indicated that none of the tested compounds were found to inhibit CYP2D6, while only *N-trans*-feruloyltyramine (**8**) and 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**) could act as CYP3A4 inhibitors.

In terms of excretion properties, total clearance, expressed as log ml/min/kg, represents the rate at which a drug is removed from the body, reflecting hepatic metabolism, renal excretion, and other elimination processes [242]. In the present work, all isolated compounds from *C. diffusa* were predicted to exhibit a low total clearance, except lyratol F (**5**). Besides, none of the compounds was a substrate of renal organic cation transporter 2 (OCT2). This is a key transporter that facilitates the renal secretion of diverse cationic drugs, thereby playing crucial roles in drug disposition and therapeutic response [243].

Regarding toxicity, AMES mutagenicity, hERG inhibitor, oral rat acute toxicity ($LD_{50}$), hepatotoxicity, and skin sensitization were used to predict the toxicity profile of

the isolated compounds from *C. diffusa*. The AMES toxicity test is a widely used assay for assessing the mutagenic and potential carcinogenic properties of chemical molecules [244]. Among 14 tested compounds, only methyl gallate (**7**) and benzyl-O-β-D-glucopyranoside (**13**) demonstrated AMES toxicity. Otherwise, acquired long QT syndrome is a condition linked to dangerous ventricular arrhythmias, which commonly arises from inhibition of the hERG potassium channel, a key regulator of cardiac electrical activity [245]. The obtained result indicated that all isolated compounds from *C. diffusa* were non-inhibitors of hERG. For hepatotoxicity, a substance is classified as hepatotoxic if it exhibits any pathological or physiological effect that disrupts the normal function of the liver, an essential organ involved in detoxification, metabolism of xenobiotics, and elimination of nitrogenous waste. Similarly, skin sensitization assessment or irritant screening evaluates the possible ability of a molecule to cause allergic reactions upon contact with the skin [240,245]. In our present work, corosolic acid (**1**) and *N-trans*-feruloyltyramine (**8**) were predicted to have hepatotoxicity, while lyratol F (**5**) was predicted to cause skin sensitisation. According to the hazard classification system of the U.S. Environmental Protection Agency (EPA), the toxicity levels of chemicals are classified into four types, including Category I (highly toxic, $LD_{50} \leq 50$ mg/kg), Category II (moderately toxic, $50 < LD_{50} \leq 500$ mg/kg), Category III (slightly toxic, $500 < LD_{50} \leq 5000$ mg/kg), and Category IV (practically non-toxic, $LD_{50} > 5000$ mg/kg) [246]. Based on the calculated $LD_{50}$ values, the isolated compounds from *C. diffusa* were considered non-toxic.

### 4.3.3. Pros and cons of the method

Virtual screening based on docking is a popular technique in drug research and development, allowing simulation of interactions between proteins and ligands or between proteins to predict the protein inhibition potential of compounds [247]. By analyzing binding energies and interactions, such as hydrogen bonds, van der Waals, and electrostatics, docking allows preliminary identification of potential compounds, narrowing the database by eliminating inactive compounds before conducting *in vitro* or

*in vivo* testing, optimizing the guide compound, and saving time and cost compared to traditional chemical synthesis methods [248].

However, this method also has many limitations. The docking results depend on the accuracy of the software and algorithms, which can lead to errors due to the use of multiple tools with different theoretical bases. The scoring function in docking is based on a subjective theory, which cannot evaluate the entire protein-ligand interaction, leading to false negatives or false positives [249]. To overcome this, the Receiver Operating Characteristic (ROC) curve method is used to validate the docking procedure by comparing the docking scores of two groups of standards (inhibitors and non-inhibitors) with experimental data [250]. In addition, determining the exact 3D structure of proteins and ligands is challenging, as the structure can be changed by interactions such as hydrogen bonds or van der Waals forces. The discrepancy between computer models and biological reality in the human body also reduces the reliability of *in silico* results [249]. Therefore, docking scores were interpreted as computational estimates of the relative favorability of ligand–protein interactions rather than as direct evidence of biological activity. A favorable docking score and interactions with residues in the active site may support a potential binding mode and provide a mechanistic hypothesis, but they cannot independently confirm enzyme inhibition, binding affinity, or biological efficacy. In the present study, the docking results were therefore considered complementary to the *in vitro* enzyme inhibition findings rather than as independent evidence of inhibitory activity. Further experimental validation, including enzymatic kinetic studies, direct binding assays, and *in vivo* investigations, is required to confirm the predicted interactions and determine their biological relevance. Overall, the integration of *in silico*, *in vitro*, *in vivo*, and clinical studies provides a more comprehensive approach to evaluating the biological significance and therapeutic potential of candidate compounds.

Similarly, the ADMET predictions were interpreted as preliminary computational assessments rather than experimental evidence of pharmacokinetic behavior or safety.

Although the predicted ADMET profiles may provide useful information for prioritizing compounds for further investigation, these predictions are subject to the limitations of the underlying algorithms and input structures and therefore require experimental validation.

Overall, the experimental design of this study combined complementary phytochemical, *in vitro*, *in vivo*, and molecular approaches to address the research objectives. Phytochemical investigation was conducted to identify the constituents of *C. diffusa* that may contribute to its biological activities, while enzyme inhibition assays were used to provide an initial assessment of the potential of the extracts and isolated compounds to inhibit key carbohydrate-digesting enzymes. Based on these results, the most active fraction was further evaluated in a high-fat diet and streptozotocin-induced hyperglycemic mouse model to determine whether the observed *in vitro* activity was associated with antihyperglycemic effects *in vivo*. Biochemical and histopathological analyses were performed to provide complementary evidence for interpreting treatment-related effects. In addition, molecular docking was employed to explore potential interactions between selected compounds and the target enzymes. Taken together, these findings provide complementary evidence for the antihyperglycemic potential of *C. diffusa.* However, each experimental model provides distinct, limited evidence rather than independent proof of a therapeutic effect. In particular, docking predictions do not establish biological activity or binding affinity, the *in vivo* effects of individual isolated compounds were not evaluated, and the relatively short treatment period and absence of direct investigation of insulin-related and molecular signaling pathways limit the mechanistic interpretation of the observed antihyperglycemic effect. Hence, further studies integrating enzyme kinetics, target-binding assays, molecular mechanistic investigations, pharmacokinetic and toxicity evaluation, and in vivo testing of individual compounds are needed.

# Conclusion & Recommendations

The doctoral thesis focused on the research of chemical constituents and the investigation of bioactive extracts and substances with hypoglycemic activities from *Commelina diffusa* Burm.f. From the detailed results and appropriate discussions, some main conclusions are drawn as follows:

1. Fourteen compounds were extracted, isolated, and structural elucidated from *C. diffusa*, including corosolic acid (**1**), 3*α*-acetyl-20*S*,24*S*-epoxydammarane-25-ol (**2**), 11-oxo-α-amyrin acetate (**3**), stigmasterol (**4**), lyratol F (**5**), 4-hydroxybenzoic acid (**6**), methyl gallate (**7**), *N-trans*-feruloyltyramine (**8**), *N-trans-p*-coumaroyl-3',4'-dihydroxyphenylethylamine (**9**), 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**10**), daucosterol (**11**), quercitrin (**12**), benzyl-O-β-D-glucopyranoside (**13**), and isoschaftoside (**14**). Among these substances, compounds **1-3**, **5**, **8-10**, and **13** were isolated for the first time not only in the *C.diffusa* species but also in the *Commelina* genus. Meanwhile, this is the first report of the separation and purification of compounds **7**, **11**, **12**, and **14** from this plant species. The chemical investigation substantially expanded the known phytochemical profile of *C. diffusa*, while the identification of several newly reported constituents with inhibitory activity against α-glucosidase and α-amylase provides a basis for their further pharmacological investigation.

2. The inhibitory effects of fractions and isolated compounds from *C. diffusa* against α-glucosidase and α-amylase enzymes were evaluated. The obtained results indicated that the ethyl acetate fraction (CD.EAF) exhibited the highest inhibitory activity against both tested enzymes. Thus, CD.EAF was further studied for its acute toxicity and antihyperglycemic potential in an *in vivo* experiment. Regarding isolated compounds, all investigated compounds showed concentration-dependent inhibitory effects, with compounds **9**, **10**, and **14** exhibiting the strongest inhibitory activities against both enzymes. These three compounds therefore represent promising candidates

for further investigation as potential lead molecules for the development of antidiabetic agents.

3. The acute toxicity and the antihyperglycemic effect of CD.EAF on high-fat diet and streptozotocin-induced type 2 diabetic mice were determined. Specifically, the CD.EAF was relatively safe with an $LD_{50}$ value of over 2000 mg/kg. Meanwhile, the repeated daily oral administration of the CD.EAF at both doses of 100 mg/kg/day and 300 mg/kg/day for 14 days exhibited a significant antihyperglycemic effect by reducing blood glucose levels and improving hepatocyte and islet injuries in mice with a high-fat diet and streptozotocin-induced type 2 diabetes. However, the repeated daily administration of the CD.EAF at both tested doses did not affect the serum lipid levels of mice.

4. The inhibitory potential of the isolated compounds against α-glucosidase (PDB: 3W37) and α-amylase (PDB: 2QV4) was further explored using molecular docking. The docking results revealed that compounds **10** and **14** showed favorable predicted interactions with both target enzymes, with docking scores lower than those of acarbose and interactions involving several active-site residues. These findings provided structural hypotheses that may help explain their experimentally observed enzyme inhibitory activities; however, experimental studies are required to confirm the predicted binding interactions and their biological relevance. Additionally, the isolated compounds from *C. diffusa* were analyzed for their physicochemical properties and ADMET parameters through *in silico* studies.

Overall, this study substantially expanded the phytochemical profile of *C. diffusa* and provided new scientific evidence from *in vitro* enzyme inhibition assays, *in vivo* evaluation of the most active fraction, and *in silico* analyses of isolated compounds. The identification of compounds **10** and **14** as promising α-glucosidase and α-amylase inhibitors supports their further investigation as potential lead compounds for the development of antidiabetic agents. On that basis, we propose the following recommendations:

1. Further phytochemical studies of *C. diffusa* should be continued to investigate additional constituents and their biological activities, particularly those that may contribute to the observed antihyperglycemic effects.

2. Further studies should be employed to explore the mechanisms of the antihyperglycemic activity of the CD.EAF, including glucose tolerance, insulin sensitivity, relevant metabolic and molecular pathways, and longer-term treatment effects.

3. Compounds **10** and **14** should be further investigated as potential lead candidates for antidiabetic drug development. These approaches should include detailed structure–activity relationship investigations, confirmation of their enzyme inhibitory mechanisms and kinetics, direct target-binding validation, evaluation of pharmacokinetic and pharmacodynamic properties, comprehensive toxicity and safety assessment, and *in vivo* efficacy studies using appropriate diabetic animal models.

# List of publications

➢ **02 publications in international journals:**

1. Duc Loi Vu, Thi Van Anh Nguyen, Tien Dat Nguyen, Viet Hau Dang, Hong Duong Le, **Xuan Tung Nguyen*** (2023), "Anti-diabetic effect of major compounds from *Commelina diffusa*", *Revista Brasileira de Farmacognosia*, **33**(3), 657-661. (Q2, SCIE, IF = 2.464).

2. **Xuan Tung Nguyen**, Duc Loi Vu*, Thi Van Anh Nguyen, Thi Minh Thu Nguyen, Hong Duong Le (2024), "Acute toxicity and antidiabetic effect of the ethyl acetate fraction of *Commelina diffusa* Burm.f. on the high-fat diet and streptozotocin-induced type 2 diabetic mice", *Pakistan Journal of Pharmaceutical Sciences*, 37(4), 855-861. (Q3, SCIE, IF = 0.7).

➢ **02 publications in national scientific journals (Vietnam):**

3. **Nguyen Xuan Tung**, Vu Duc Loi*, Le Hong Duong, Nguyen Thi Van Anh (2023), "Extraction and isolation of several compounds from *Commelina diffusa* Burm.f.", *Vietnam Journal of Traditional Medicine and Pharmacy*, **2**(49), 21-28.

4. **Nguyen Xuan Tung**, Vu Duc Loi*, Nguyen Thi Van Anh, Le Hong Duong, Nguyen Thi Thao Vy, Nguyen Thi Quynh Trang (2024), "Evaluation of the inhibitory effect on α-glucosidase and α-amylase enzymes of triterpenes isolated from *Commelina diffusa* Burm.f.", *VNU Journal of Science: Medical and Pharmaceutical Sciences*, **40**(2), 74-82.

➢ **01 patent:**

5. Vu Duc Loi*, **Nguyen Xuan Tung**, Le Hong Duong. Patent: "Process for extracting a mixture of active ingredients with the effect of lowering blood glucose from *Commelina diffusa* Burm.f.", No. 3900, granted by the Intellectual Property Office of Vietnam under Decision No. 142452/QĐ-SHTT dated 05/12/2024.

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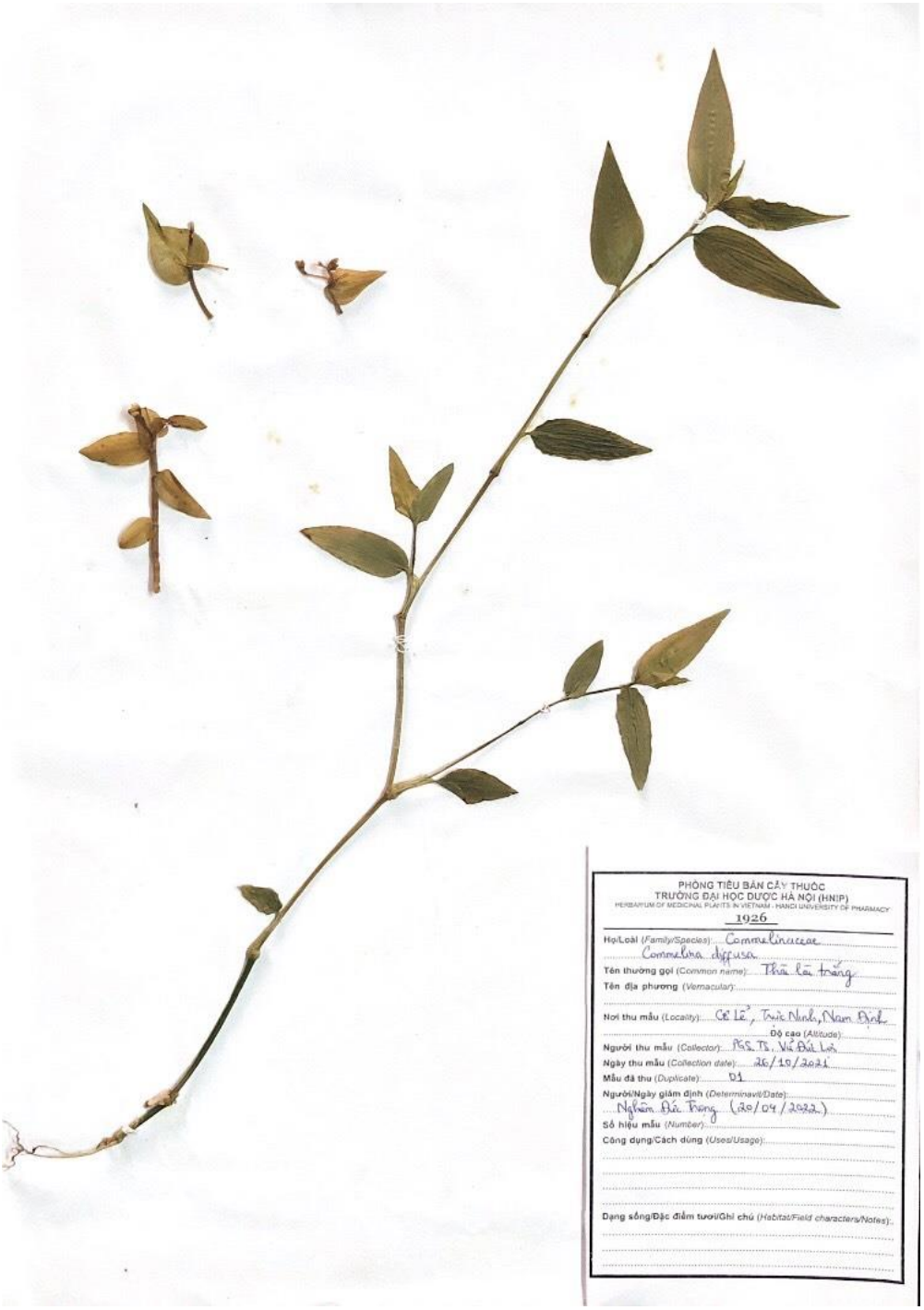
# APPENDIX

**1. The results of the scientific name identification and the voucher specimen of *Commelina diffusa* Burm.f.**



**TRƯỜNG ĐẠI HỌC DƯỢC HÀ NỘI**
BỘ MÔN THỰC VẬT
*****

# PHIẾU GIÁM ĐỊNH TÊN KHOA HỌC

Số: 03/2022

**Người thu mẫu**: PGS. TS. Vũ Đức Lợi
**Người gửi mẫu**: DS. Lê Hồng Dương
**Ngày thu mẫu**: 26/10/2021
**Nơi thu mẫu**: Thị trấn Cổ Lễ, huyện Trực Ninh, tỉnh Nam Định
**Mô tả mẫu**: Cả cây mang hoa

**Kết quả giám định**: *Căn cứ vào các tài liệu thực vật hiện có tại Trường Đại học Dược Hà Nội với các đặc điểm của các bộ phận mẫu cây, đã xác định mẫu trên có:*

- Tên khoa học: *Commelina diffusa* Burm.f.
- Họ: Commelinaceae
- Tên thường gọi: Thài lài trắng, Rau trai

*Hà Nội, ngày 25 tháng 01 năm 2022*

| **Trưởng Bộ môn** | **Người giám định** |
|---|---|
| **TS. Hoàng Quỳnh Hoa** | **ThS. Nghiêm Đức Trọng** |

**Tài liệu tham khảo**


1. Phạm Hoàng Hộ (2000), *Cây cỏ Việt Nam*, NXB Trẻ, Quyển 3.
2. Deyuan Hong, Robert A. DeFilipps (2000), Commelinaceae, in: Wu, Z. Y., P. H. Raven (eds), *Flora of China. Vol. 24 (Flagellariaceae through Marantaceae)*, Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.

TRƯỜNG ĐẠI HỌC DƯỢC HÀ NỘI

*BỘ MÔN THỰC VẬT*

**PHÒNG TIÊU BẢN CÂY THUỐC (HNIP)**

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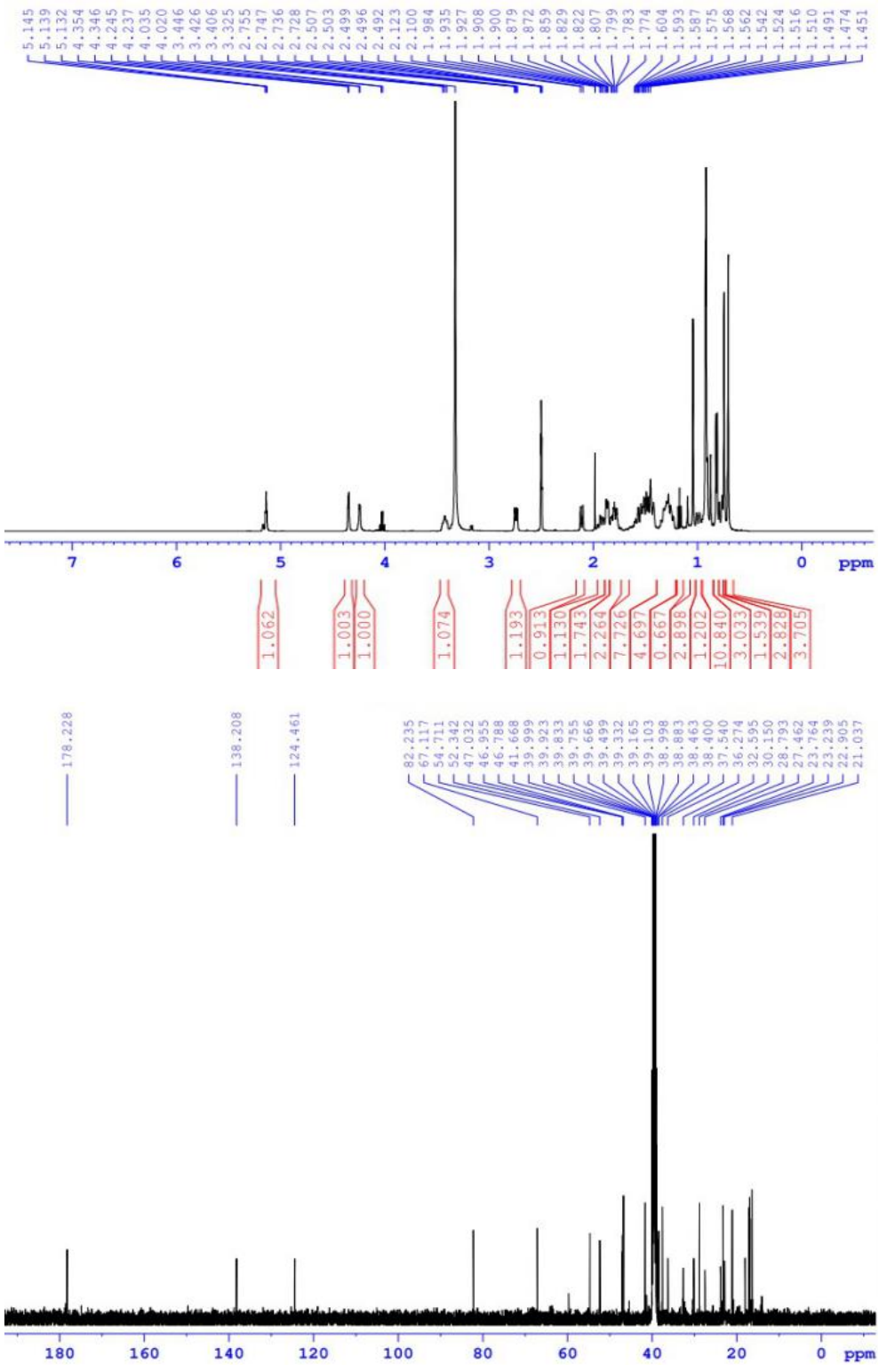
# GIẤY CHỨNG NHẬN MÃ SỐ TIÊU BẢN

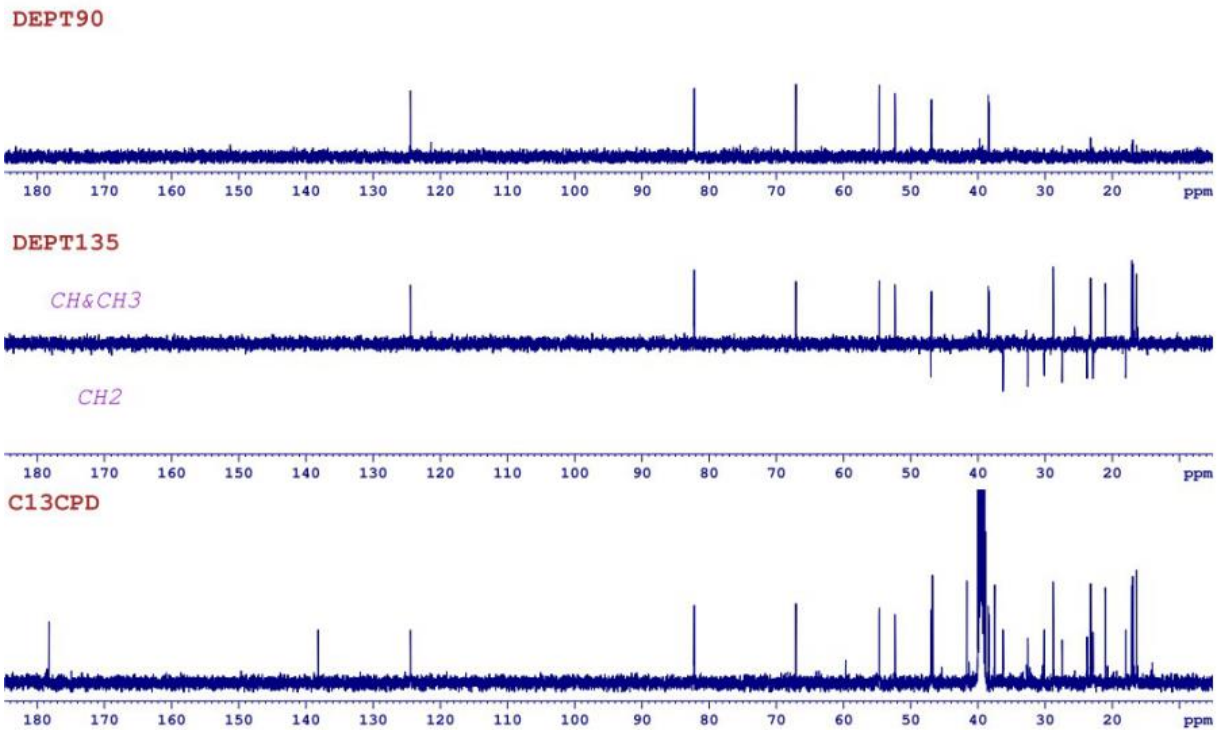
1. **Tên mẫu cây**
   - Tên khoa học: *Commelina diffusa* Burm.f.
   - Tên thường dùng: Thài lài trắng, Rau trai
2. **Nguồn gốc:** Thị trấn Cổ Lễ, huyện Trực Ninh, tỉnh Nam Định
3. **Ngày thu mẫu:** 26/10/2021
4. **Ngày nộp mẫu:** 20/4/2022
5. **Người thu mẫu:** Vũ Đức Lợi
6. **Người nộp mẫu:** Lê Hồng Dương
7. **Số hiệu phòng tiêu bản:** HNIP/18651/22
8. **Người giám định tên khoa học:** Nghiêm Đức Trọng
9. **Số lượng mẫu đã nộp: (Duplicate)** 03

| **Người nộp mẫu** | **Người nhận mẫu** |
| --- | --- |
| **Lê Hồng Dương** | **Nghiêm Đức Trọng** |

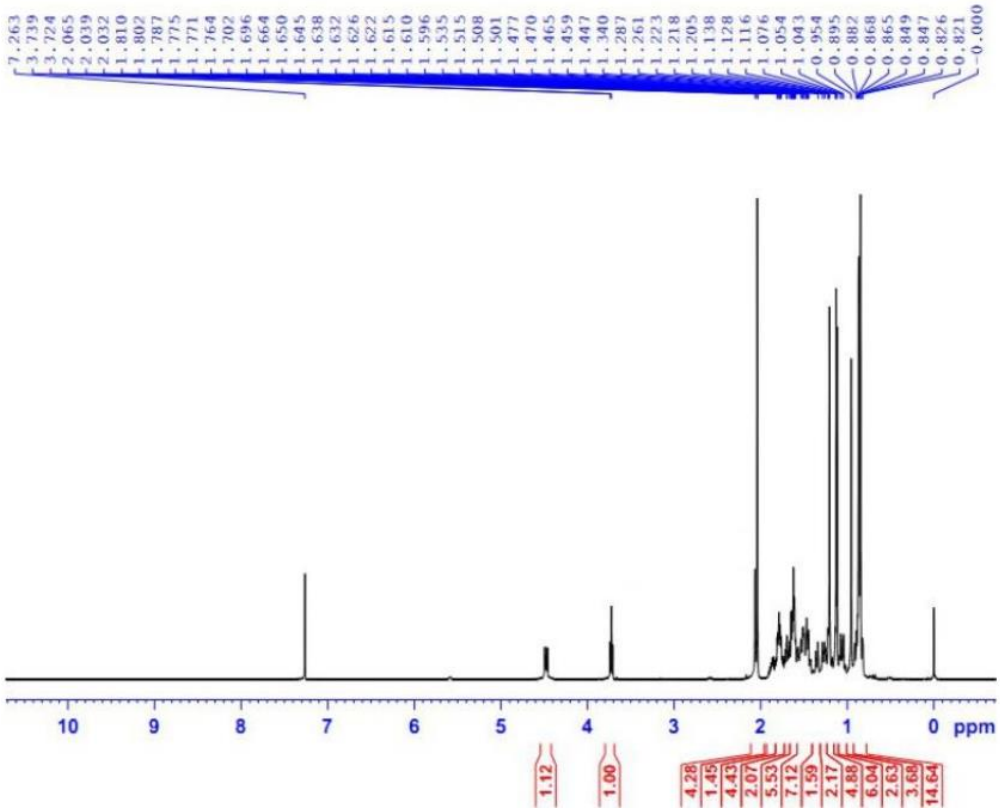
## 2. Spectral data of isolated compounds from *Commelina diffusa* Burm.f.

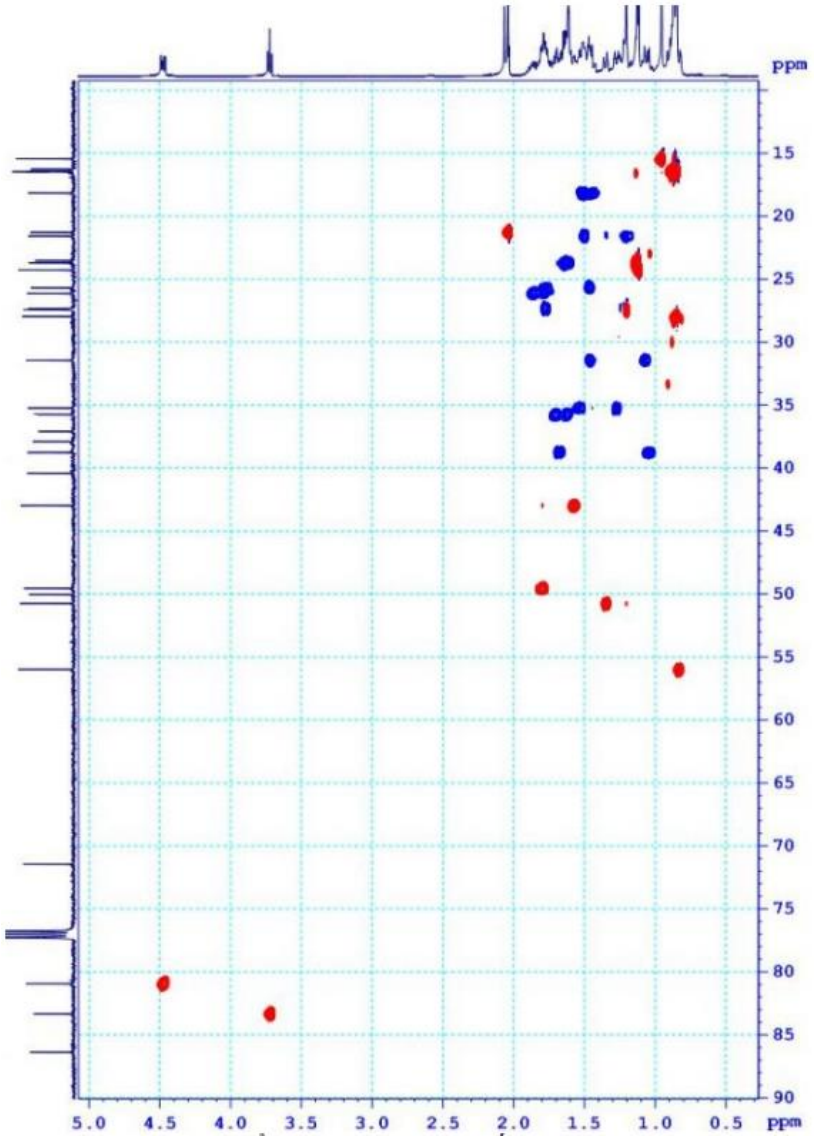
### 2.1. The $^{1}$H-NMR, $^{13}$C-NMR, and DEPT spectra of Corosolic acid (1)






**2.2. The $^{1}$H-NMR, $^{13}$C-NMR, HSQC, and HMBC spectra of 3*α*-acetyl-20*S*,24*S*-epoxydammarane-25-ol (2)**

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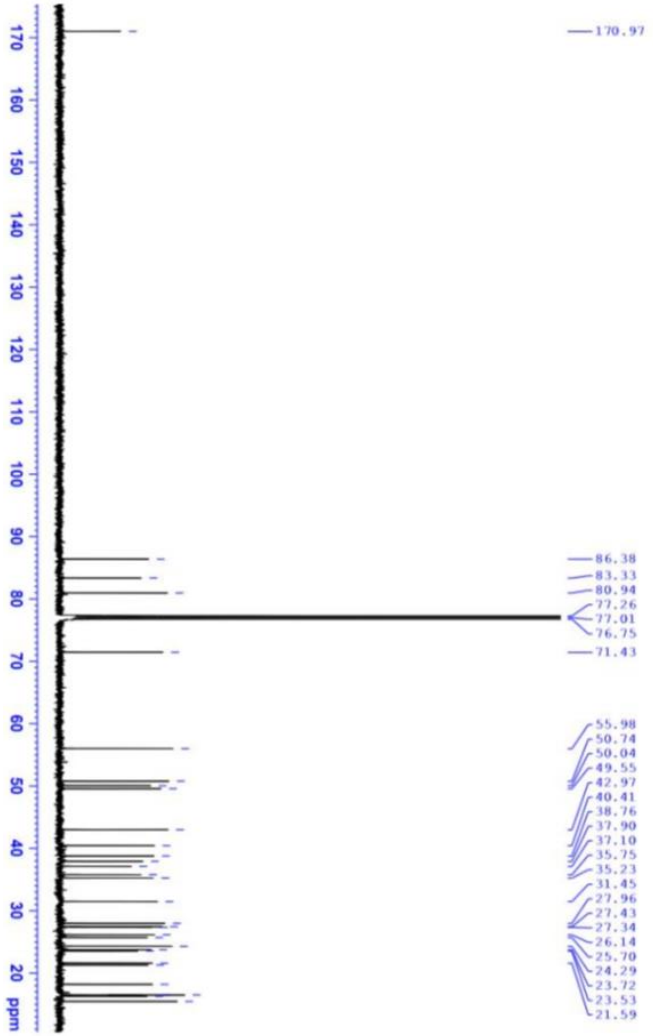
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90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77.01
76.75
71.43
55.98
50.74
50.04
49.55
42.97
40.41
38.76
37.90
37.10
35.75
35.23
31.45
27.96
27.43
27.34
26.14
25.70
24.29
23.72
23.53
21.59
170
160
150
140
130
120
110
100
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80
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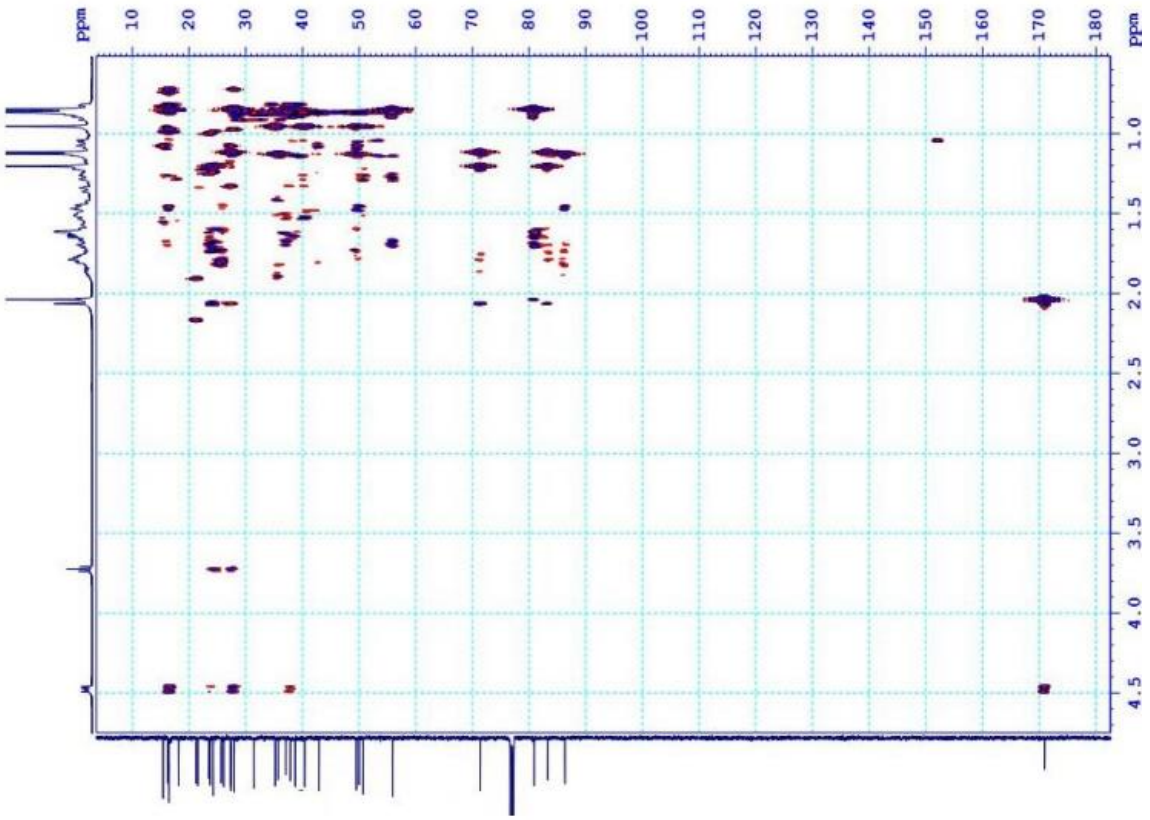
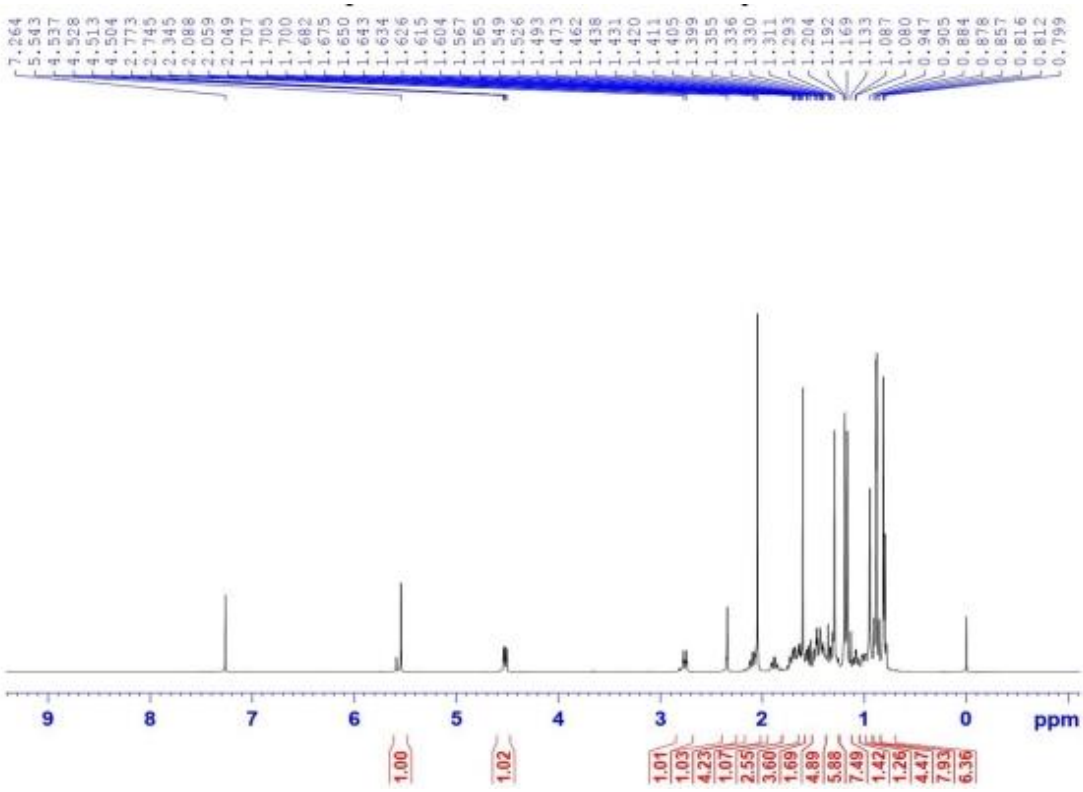
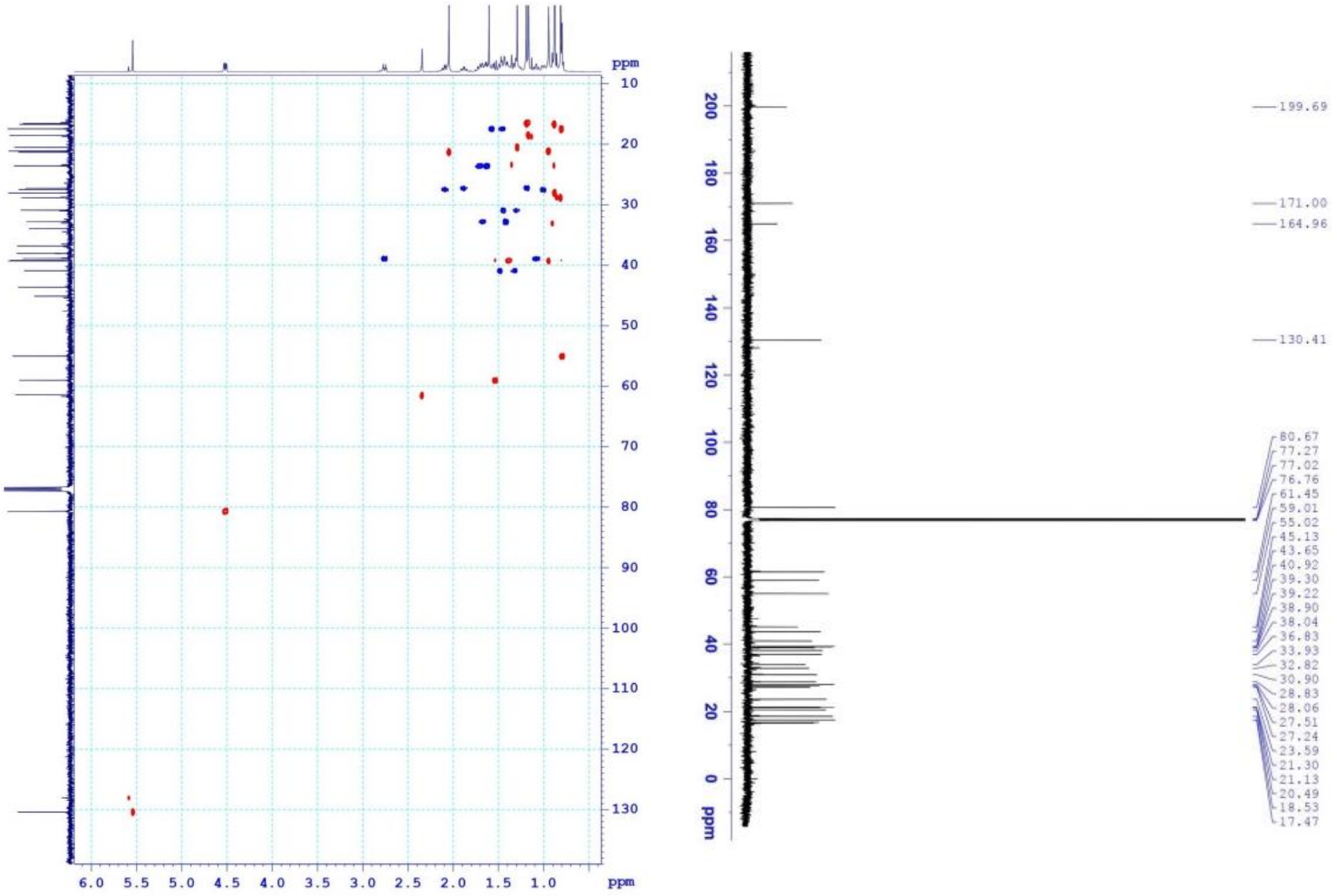
60
50
40
30
20
ppm




**2.3. The $^{1}$H-NMR, $^{13}$C-NMR, HSQC, and HMBC spectra of 11-oxo-α-amyrin acetate (3)**

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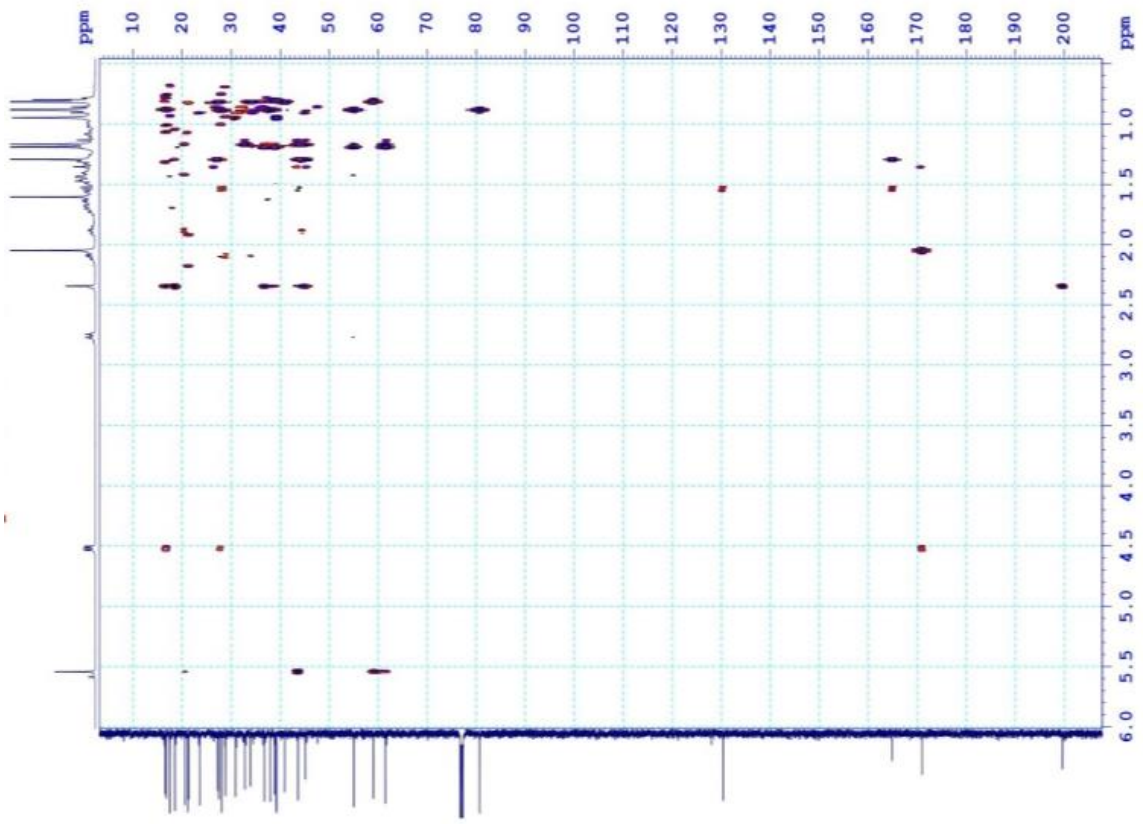
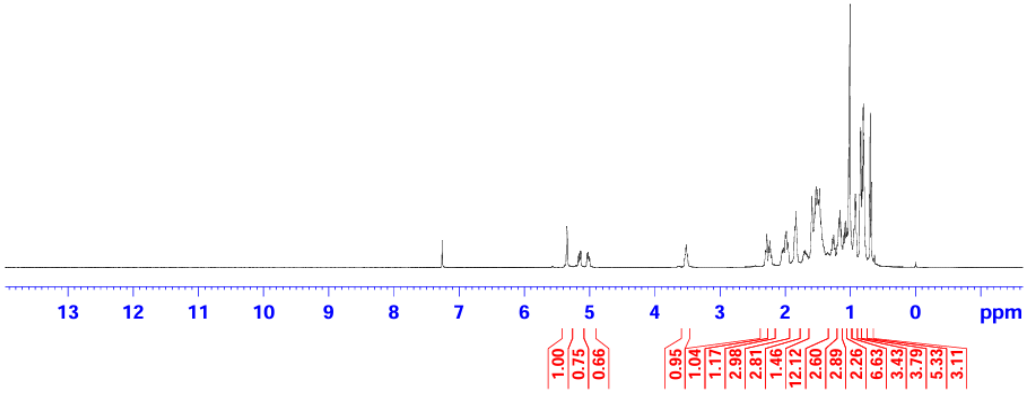
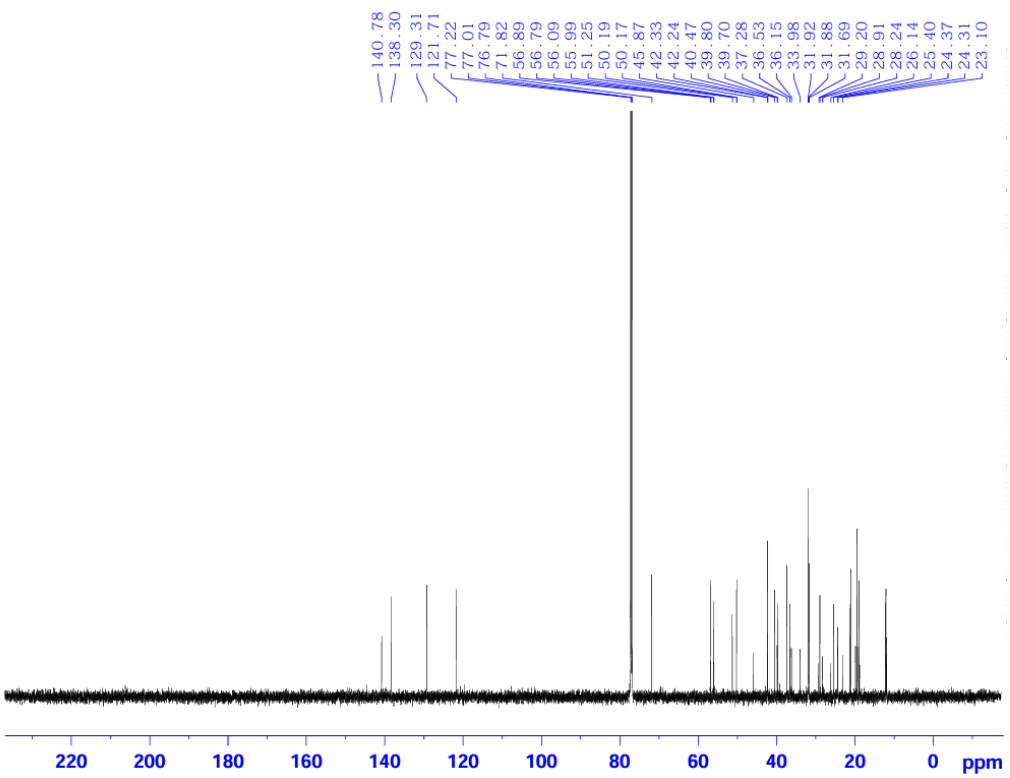
50
60
70
80
90
100
110
120
130
6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
ppm
200 180 160 140 120 100 80 60 40 20 0
ppm
199.69
171.00
164.96
130.41
80.67
77.27
77.02
76.76
61.45
59.01
55.02
45.13
43.65
40.92
39.30
39.22
38.90
38.04
36.83
33.93
32.82
30.90
28.83
28.06
27.51
27.24
23.59
21.30
21.13
20.49
18.53
17.47

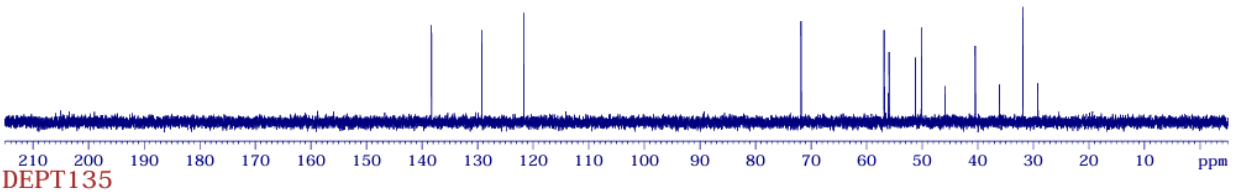



## 2.4. The $^{1}$H-NMR, $^{13}$C-NMR, and DEPT spectra of Stigmasterol (4)

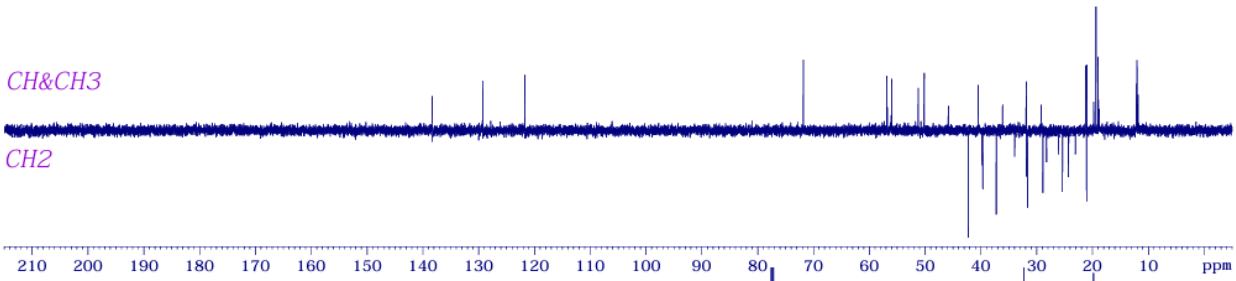





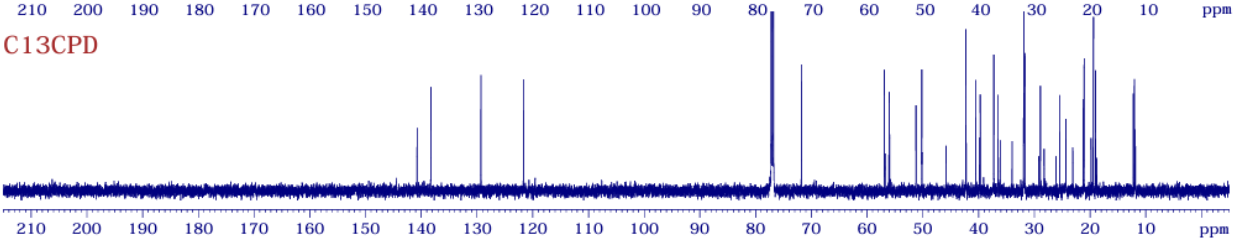

DEPT90



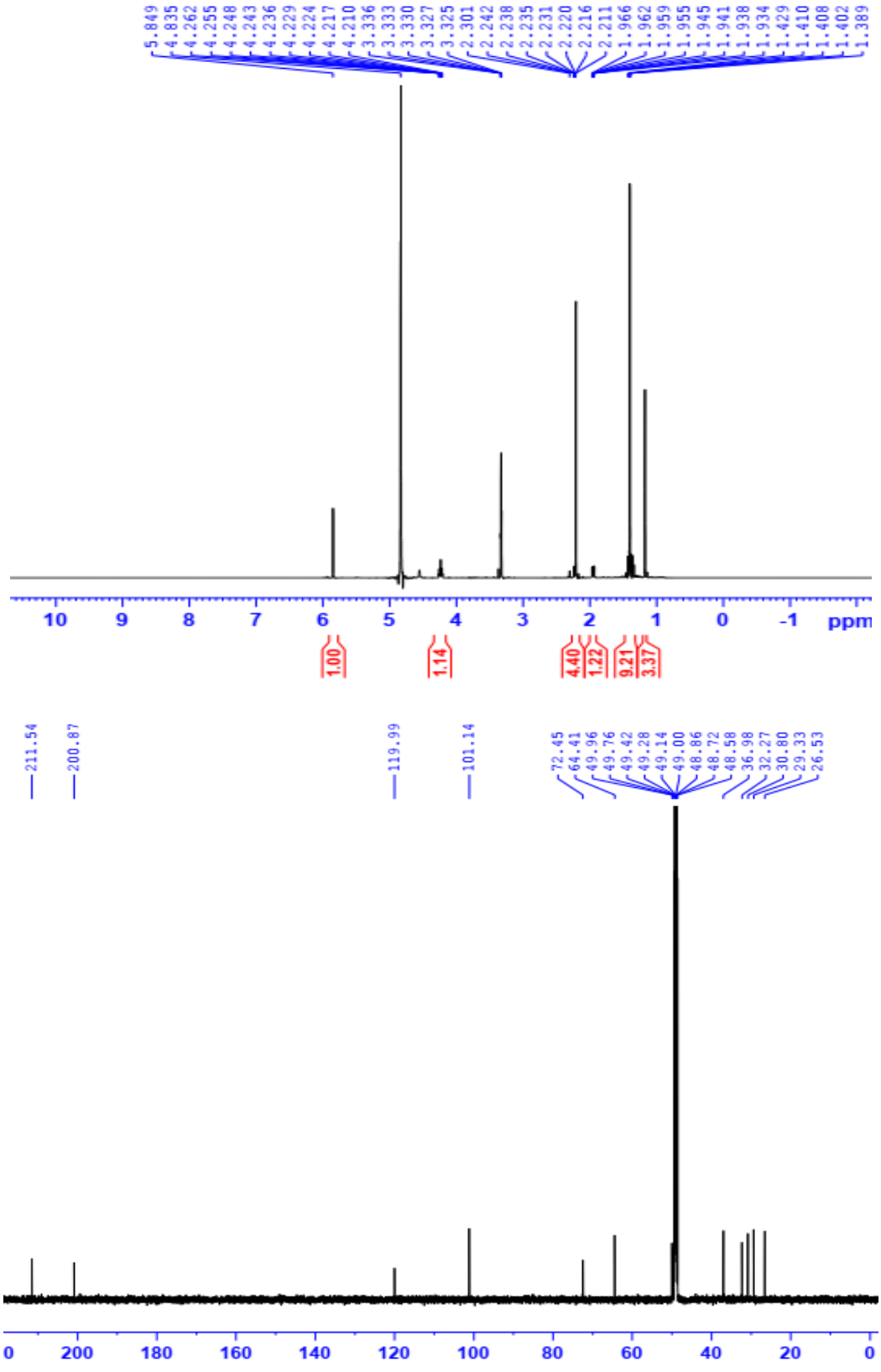

DEPT135

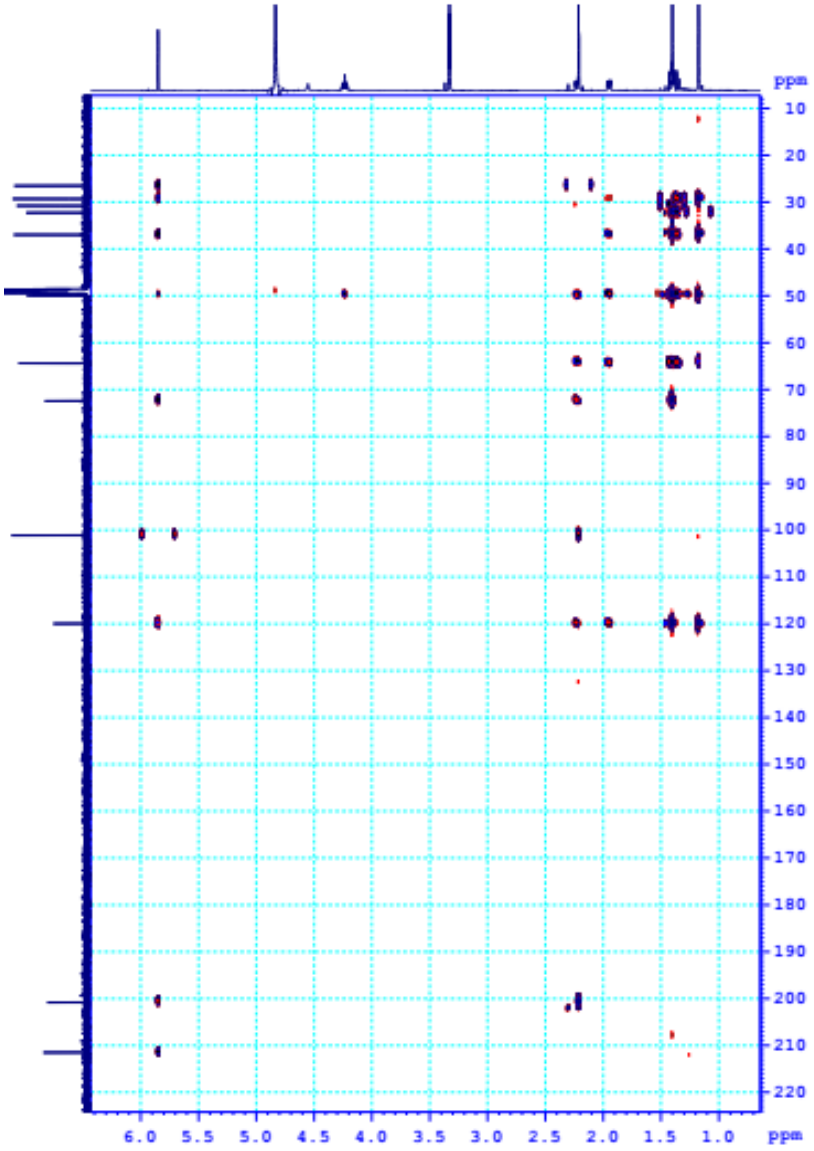



C13CPD



## 2.5. The $^{1}$H-NMR, $^{13}$C-NMR, HSQC, HMBC, and ESI-MS spectra of Lyratol F (5)

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120
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140
150
160
170
180
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200
210
220
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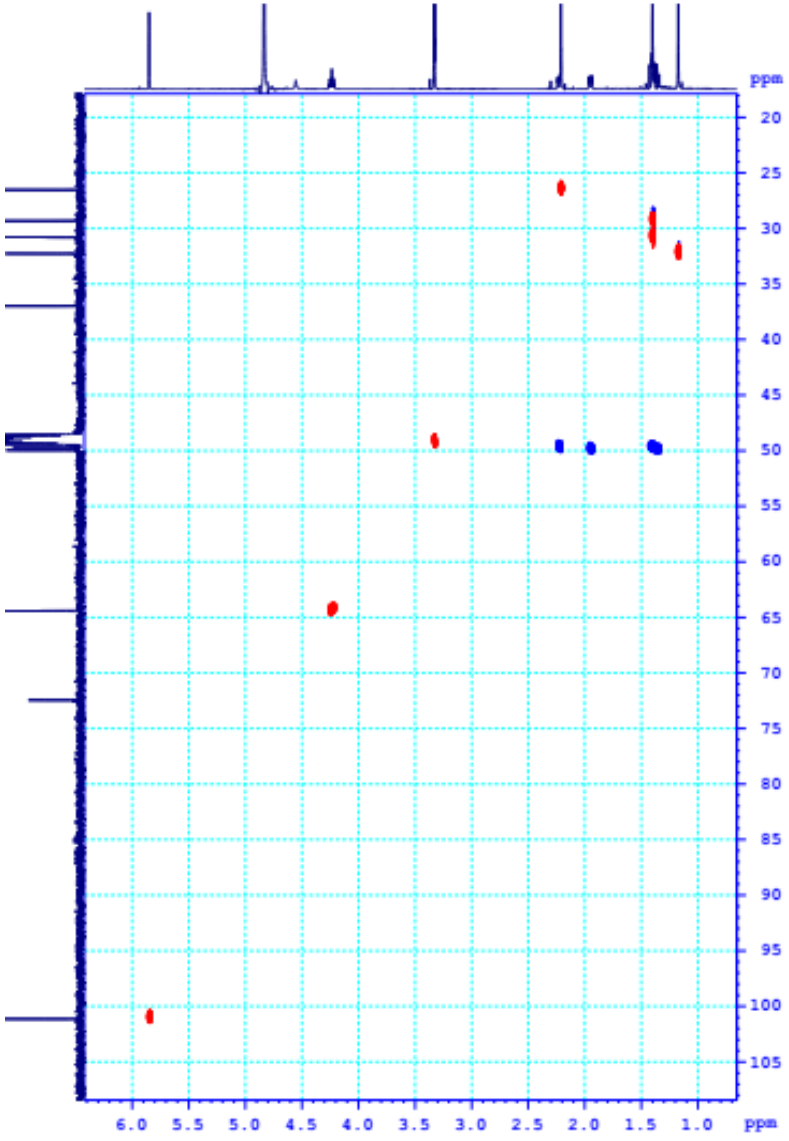
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm


ppm
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55
60
65
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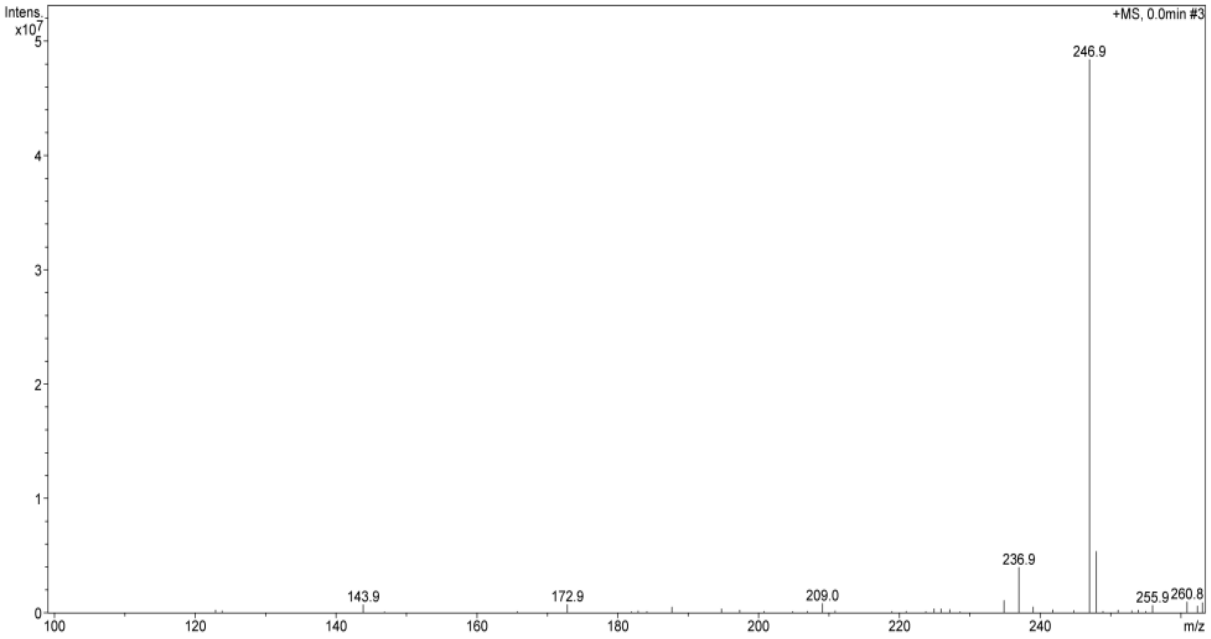
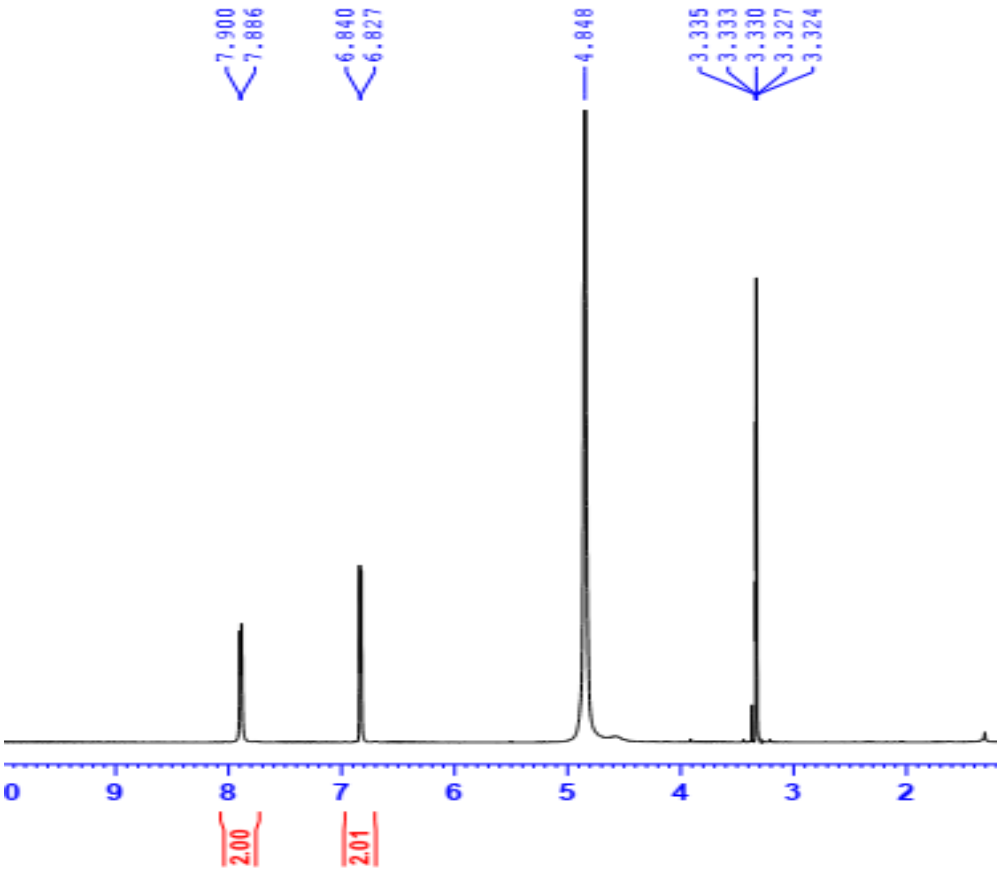
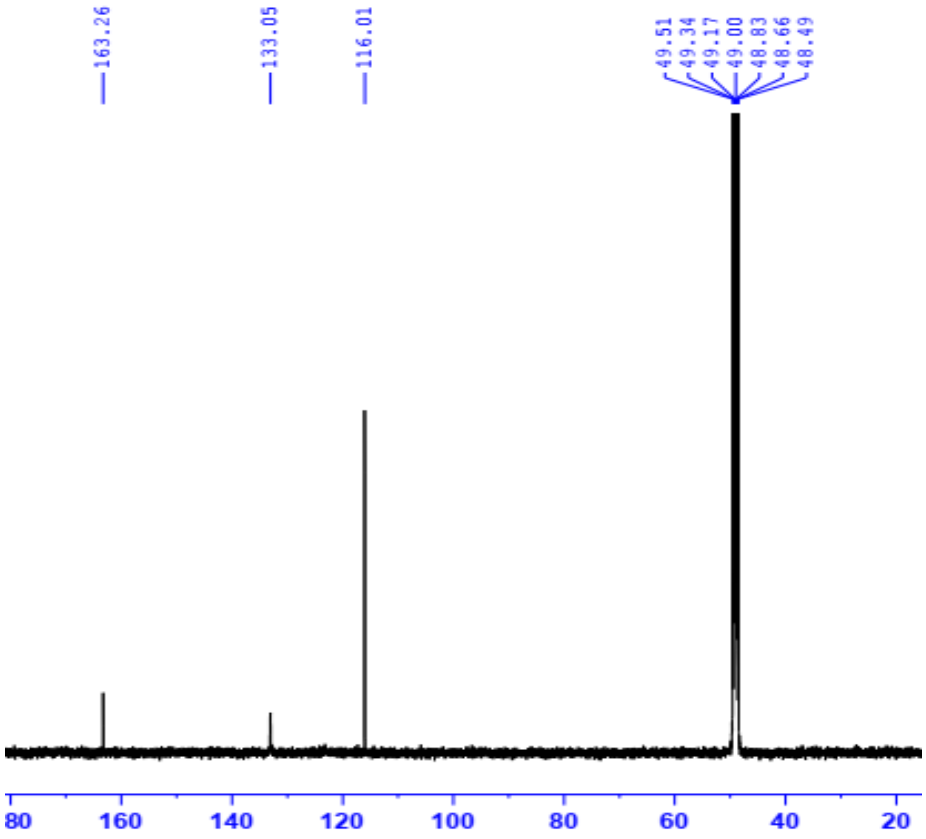
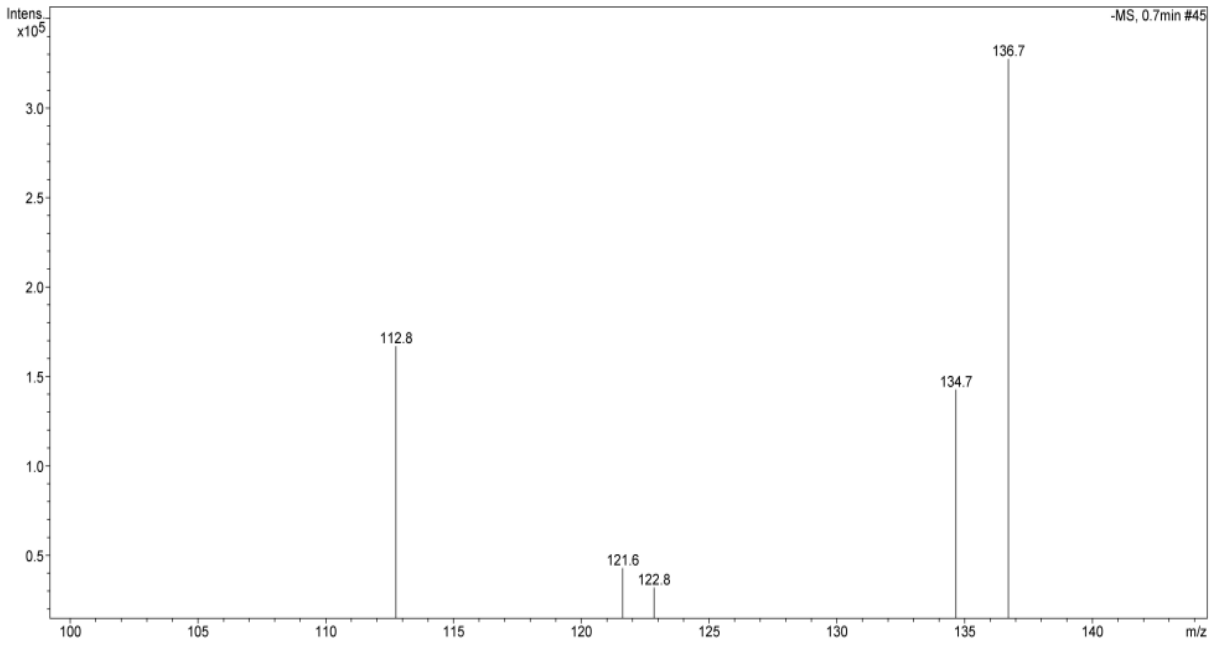
75
80
85
90
95
100
105
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm



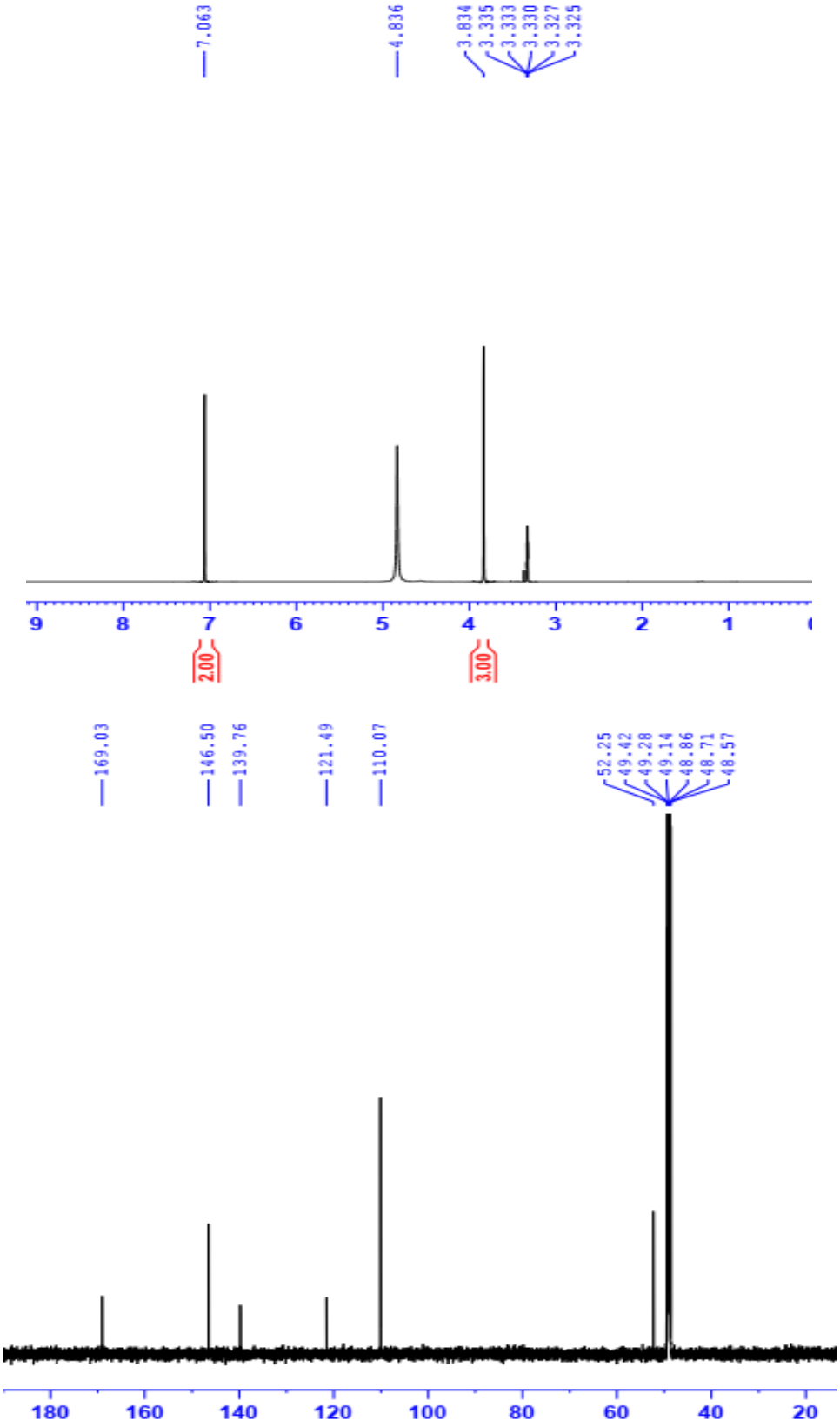

## 2.6. The $^{1}$H-NMR, $^{13}$C-NMR, and ESI-MS spectra of 4-hydroxybenzoic acid (6)



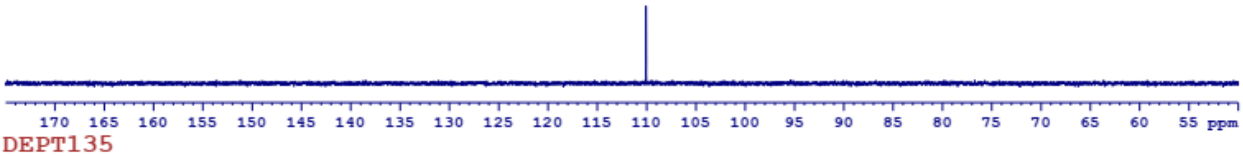

163.26
133.05
116.01
49.51
49.34
49.17
49.00
48.83
48.66
48.49
80
160
140
120
100
80
60
40
20


Intens.
x10^5
-MS, 0.7min #45
136.7
112.8
134.7
121.6
122.8
3.0
2.5
2.0
1.5
1.0
0.5
100
105
110
115
120
125
130
135
140
m/z

## 2.7. The $^{1}$H-NMR, $^{13}$C-NMR, DEPT, and ESI-MS spectra of Methyl gallate (7)



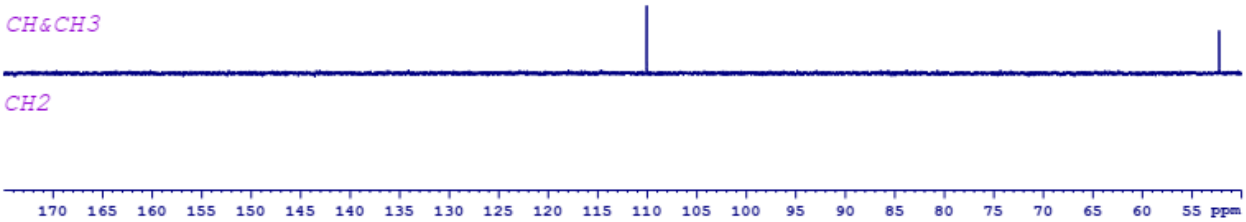
DEPT90



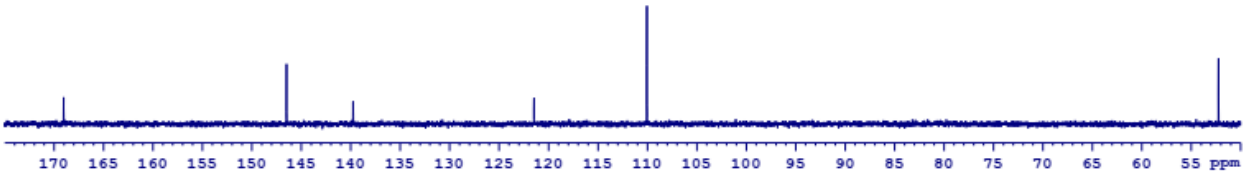

DEPT135

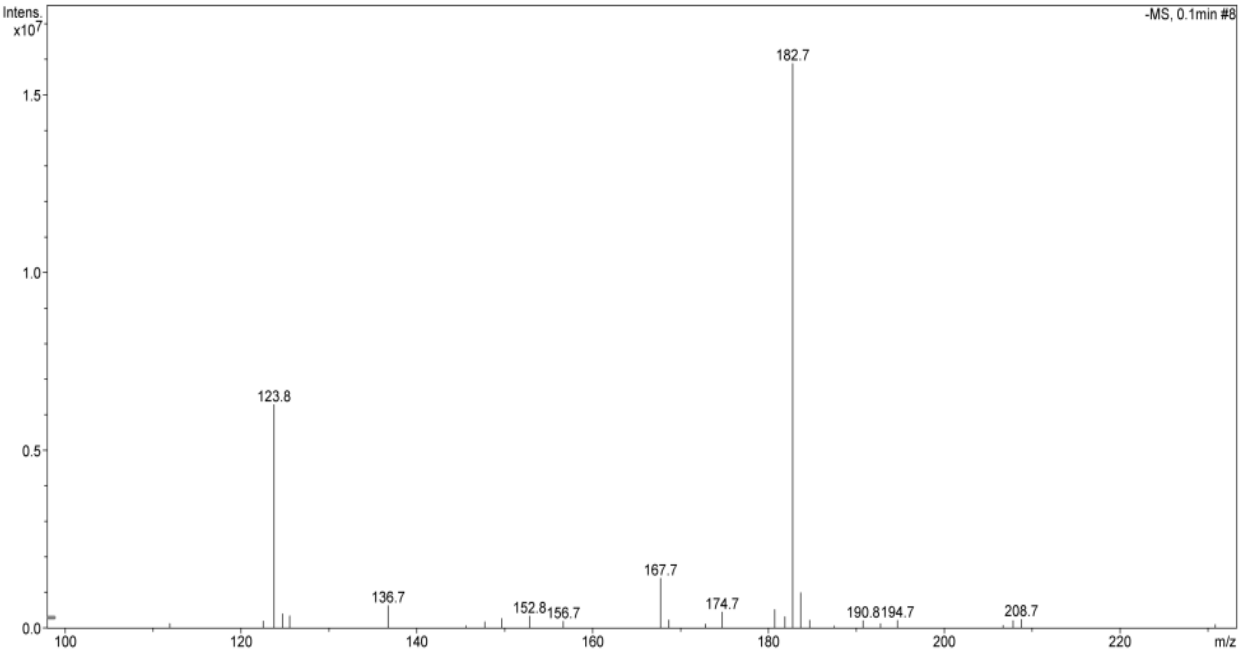
*CH&CH3*

*CH2*

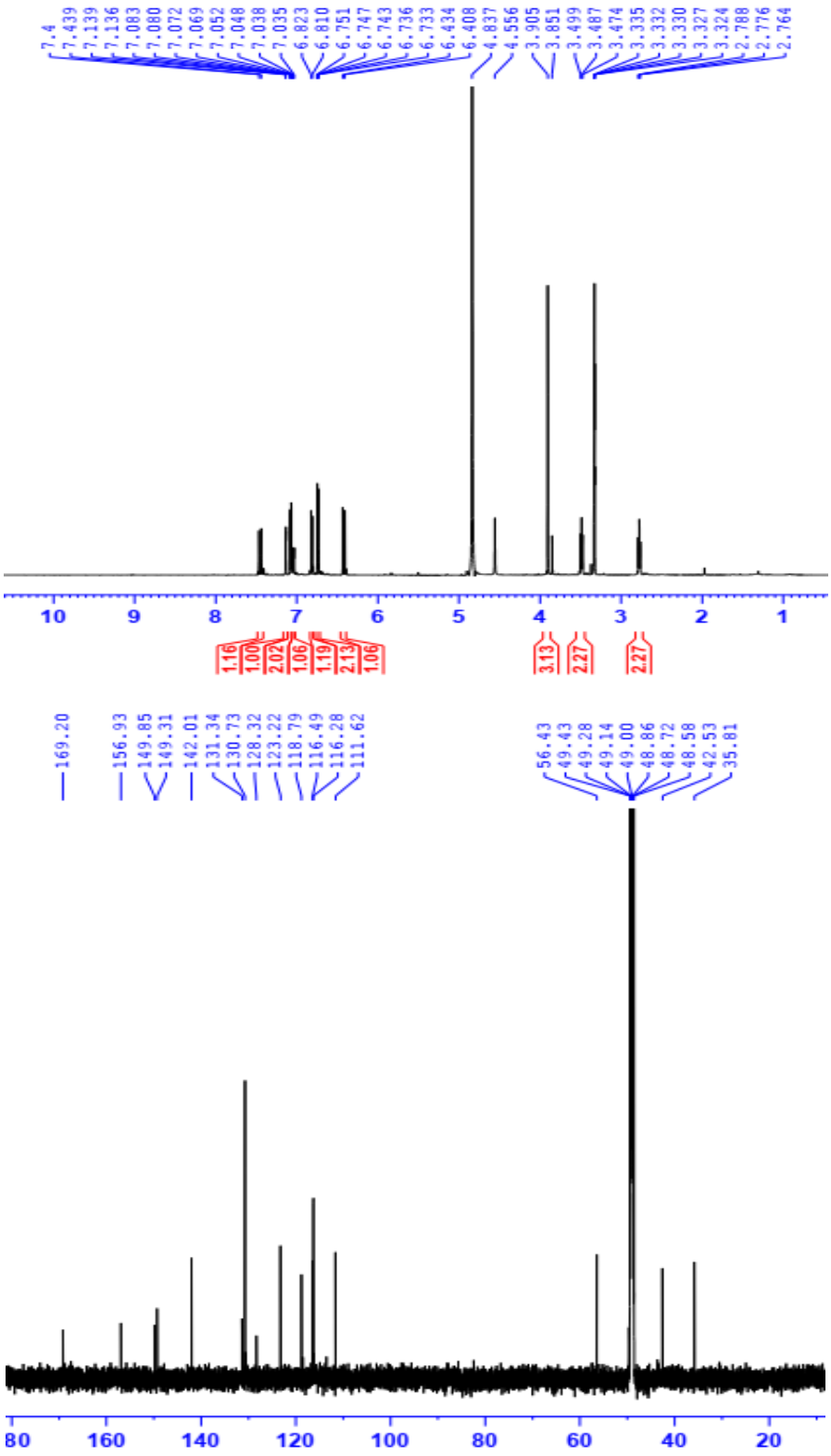


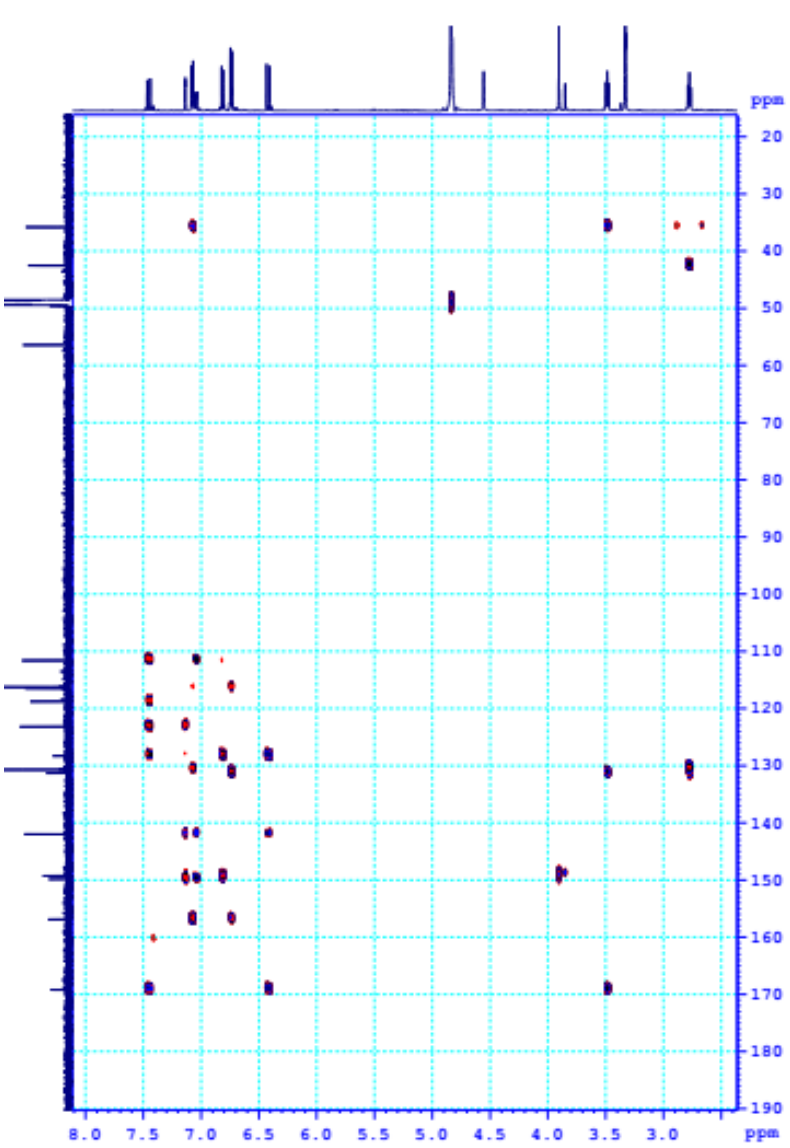

C13CPD



**2.8. The $^{1}$H-NMR, $^{13}$C-NMR, HSQC, HMBC, and ESI-MS spectra of *N-trans*-feruloyltyramine (8)**

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50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
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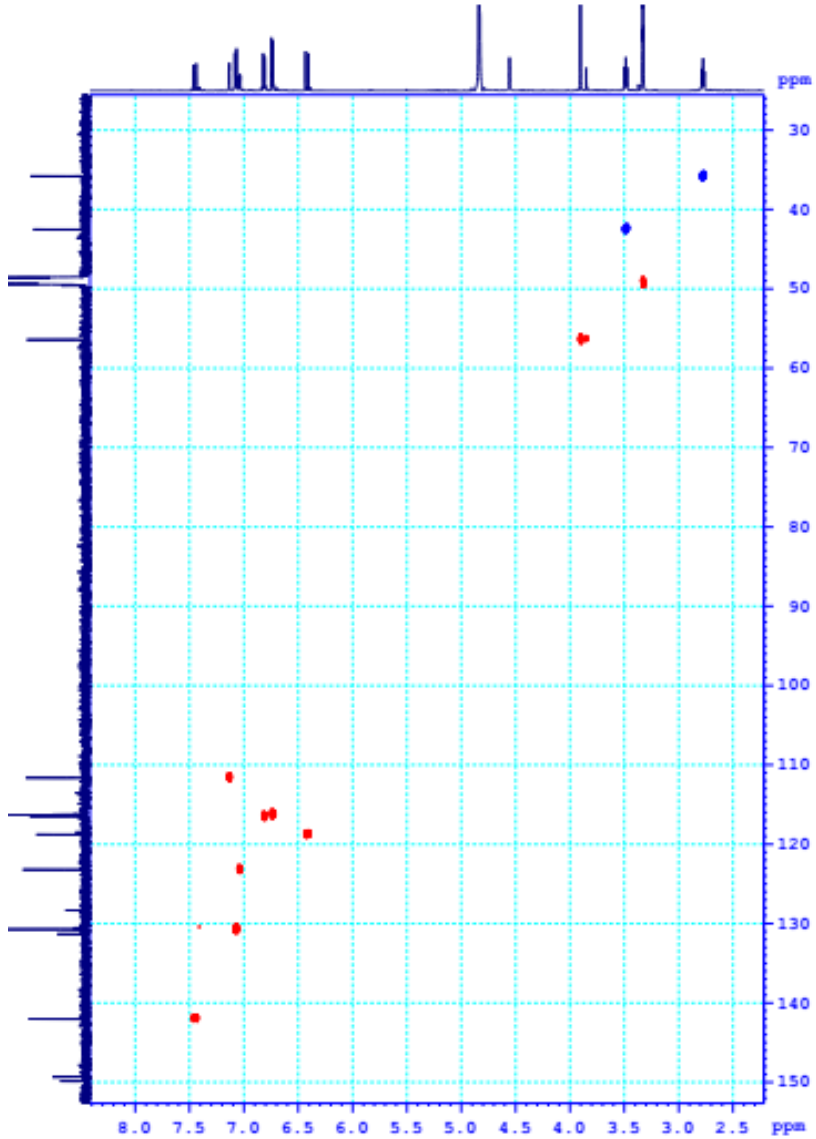
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
ppm


ppm
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60
70
80
90
100
110
120
130
140
150
8.0
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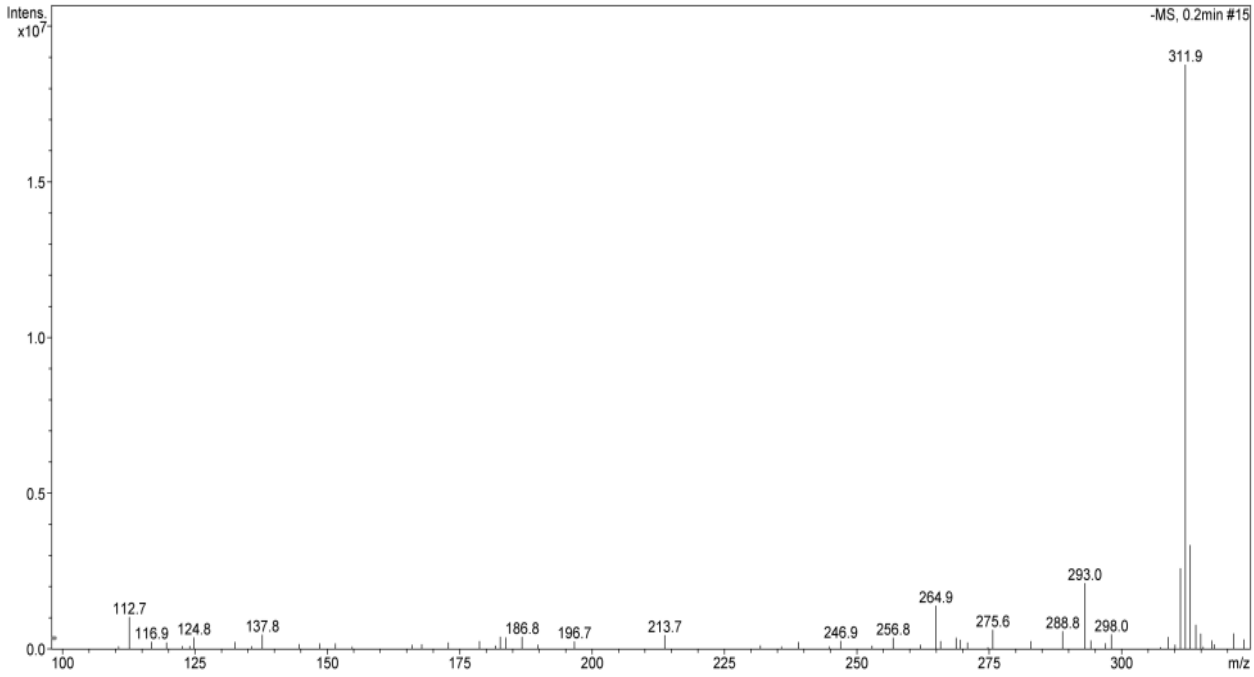
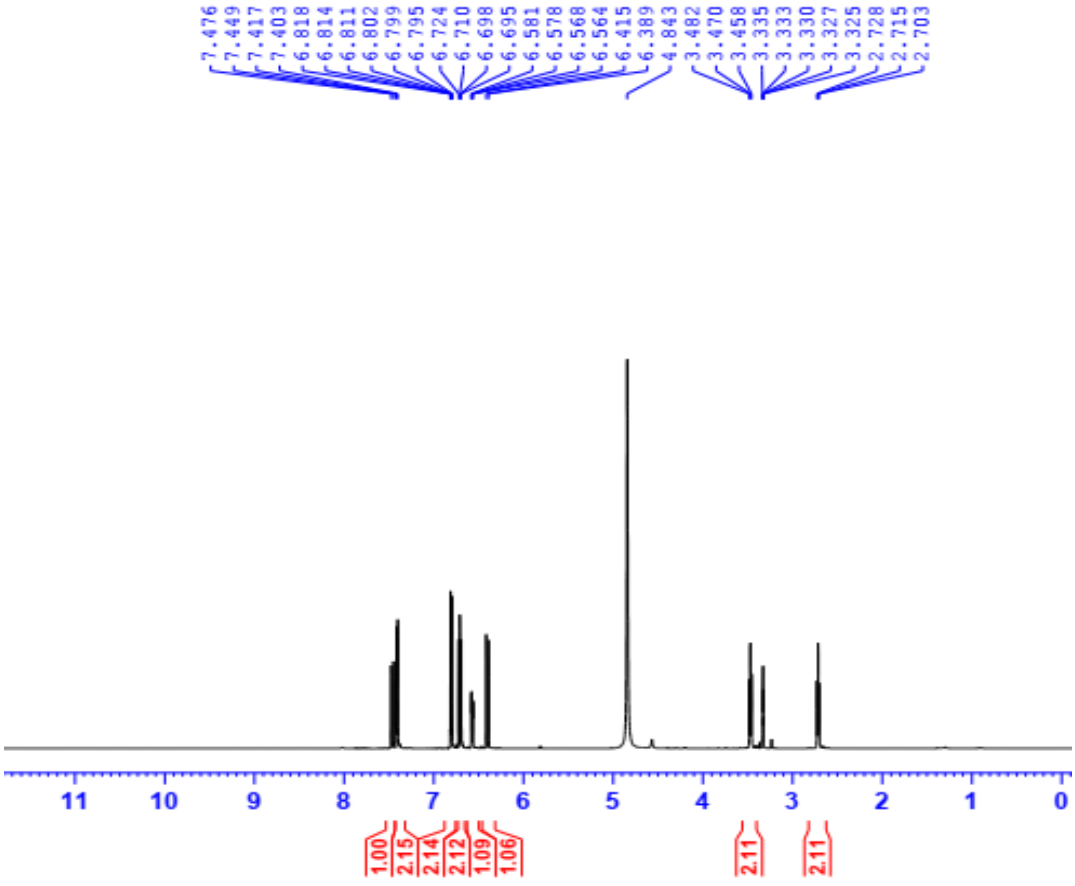
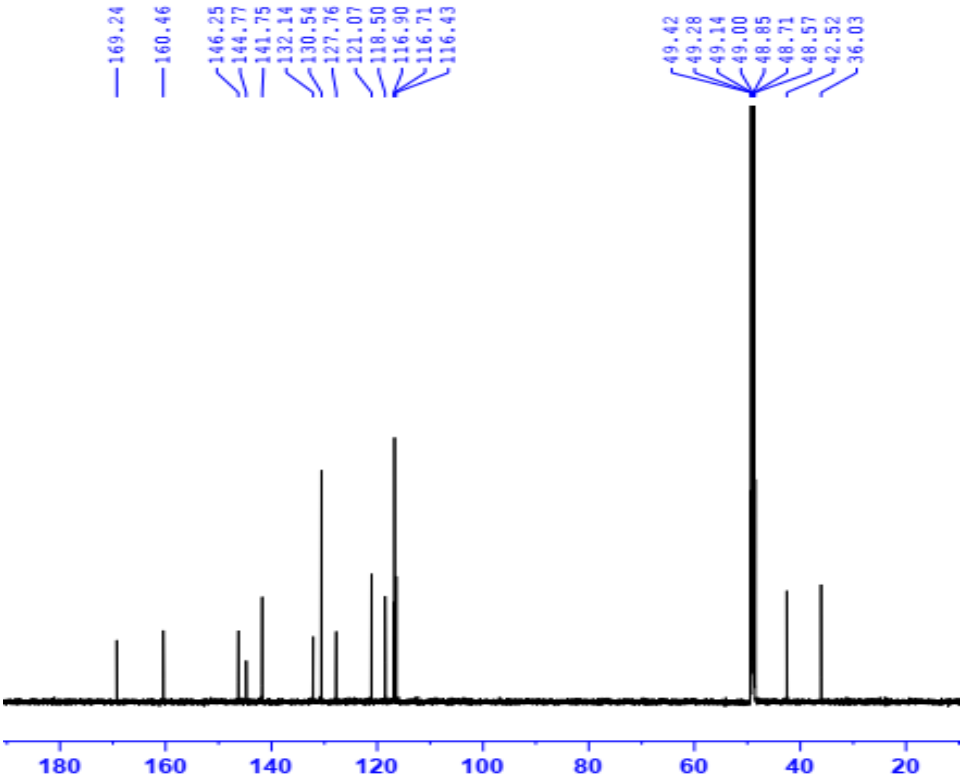
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
ppm




**2.9. The $^{1}$H-NMR, $^{13}$C-NMR, HSQC, HMBC, and ESI-MS spectra of *N-trans-p*-coumaroyl-3',4'-dihydroxyphenylethylamine (9)**




169.24
160.46
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144.77
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132.14
130.54
127.76
121.07
118.50
116.90
116.71
116.43
49.42
49.28
49.14
49.00
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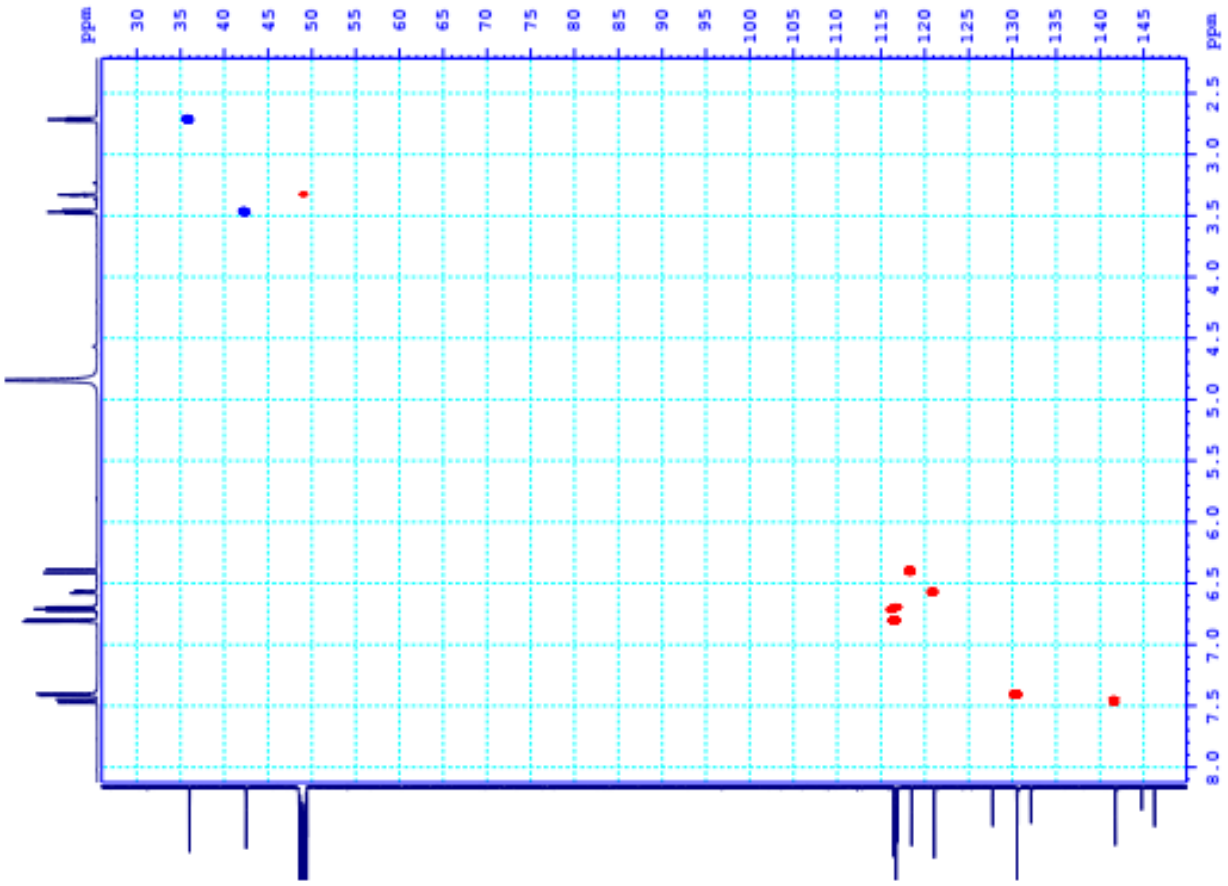
48.71
48.57
42.52
36.03
180
160
140
120
100
80
60
40
20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55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
145
ppm
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3.0
3.5
4.0
4.5
5.0
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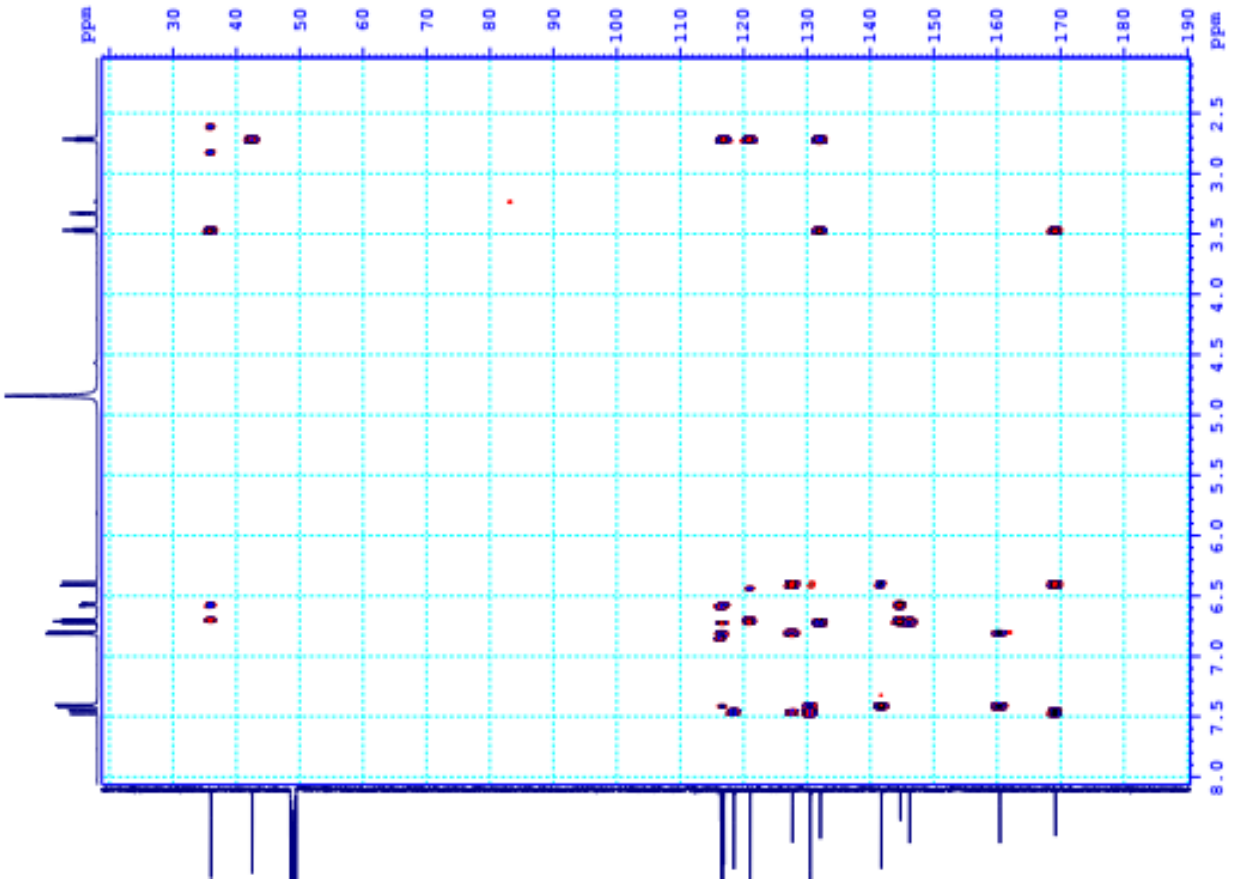
6.0
6.5
7.0
7.5
8.0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80
90
100
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150
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170
180
190
ppm
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4.0
4.5
5.0
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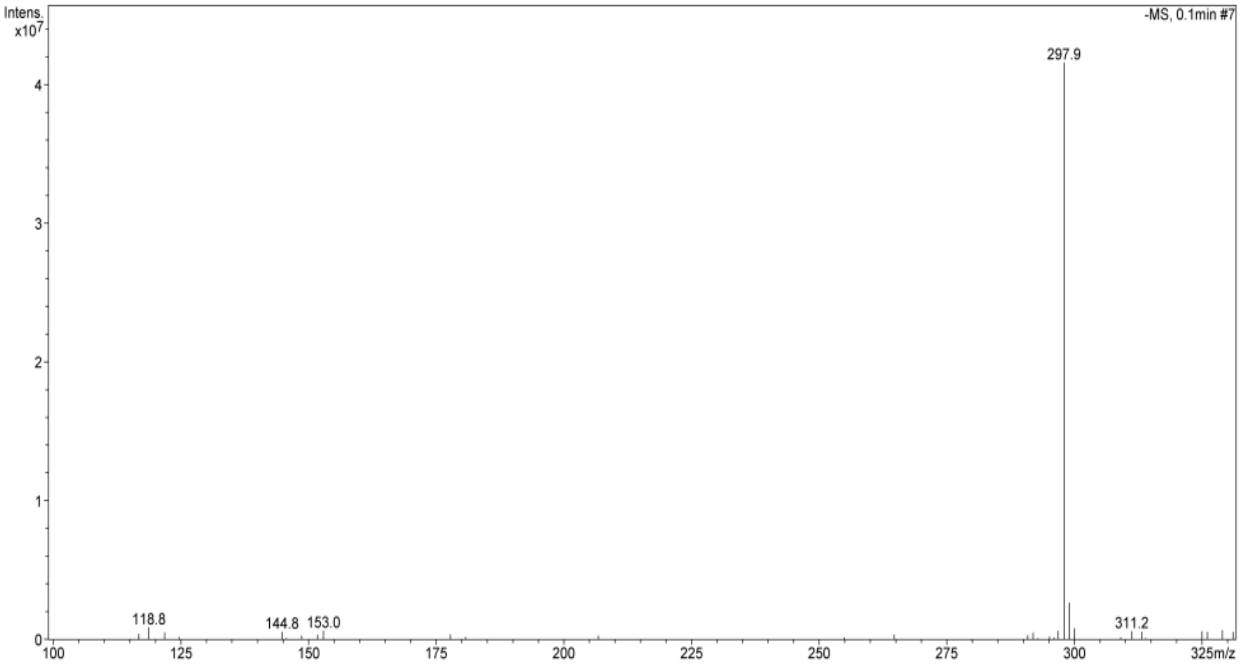
6.0
6.5
7.0
7.5
8.0


Intens.
x10^7
-MS, 0.1min #7
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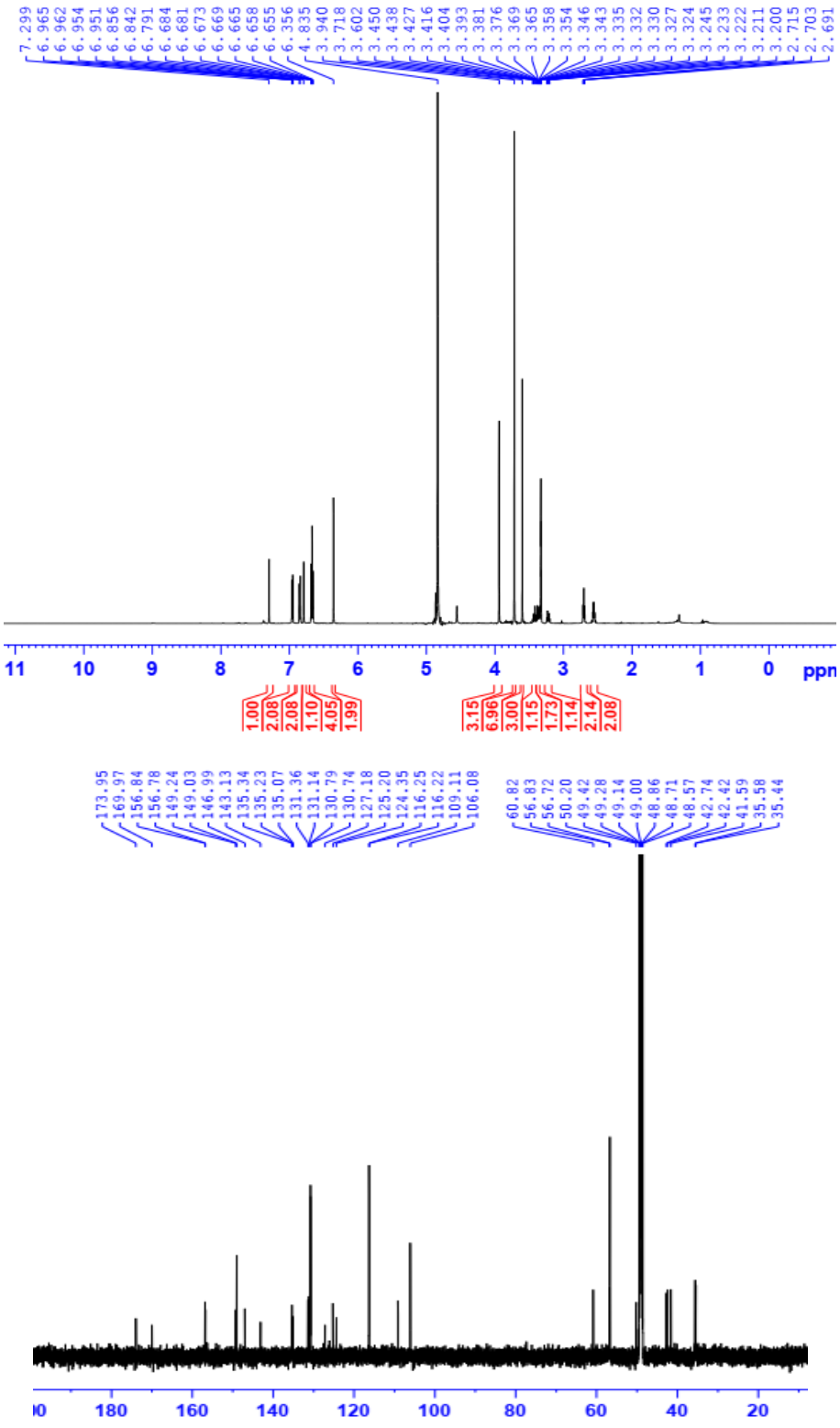
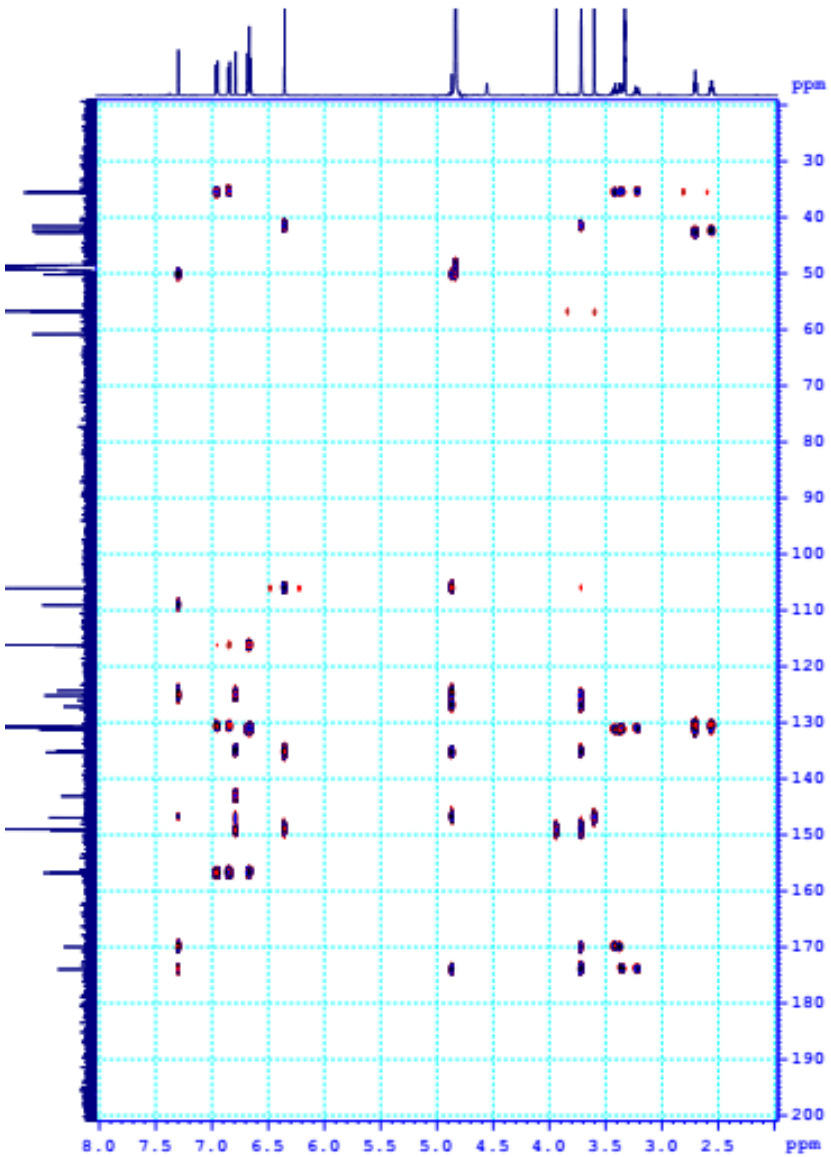
144.8
153.0
311.2
0
1
2
3
4
100
125
150
175
200
225
250
275
300
325m/z

**2.10. The $^{1}$H-NMR, $^{13}$C-NMR, HSQC, HMBC, and ESI-MS spectra of 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-*N*$^{1}$,*N*$^{2}$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (10)**




ppm
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40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
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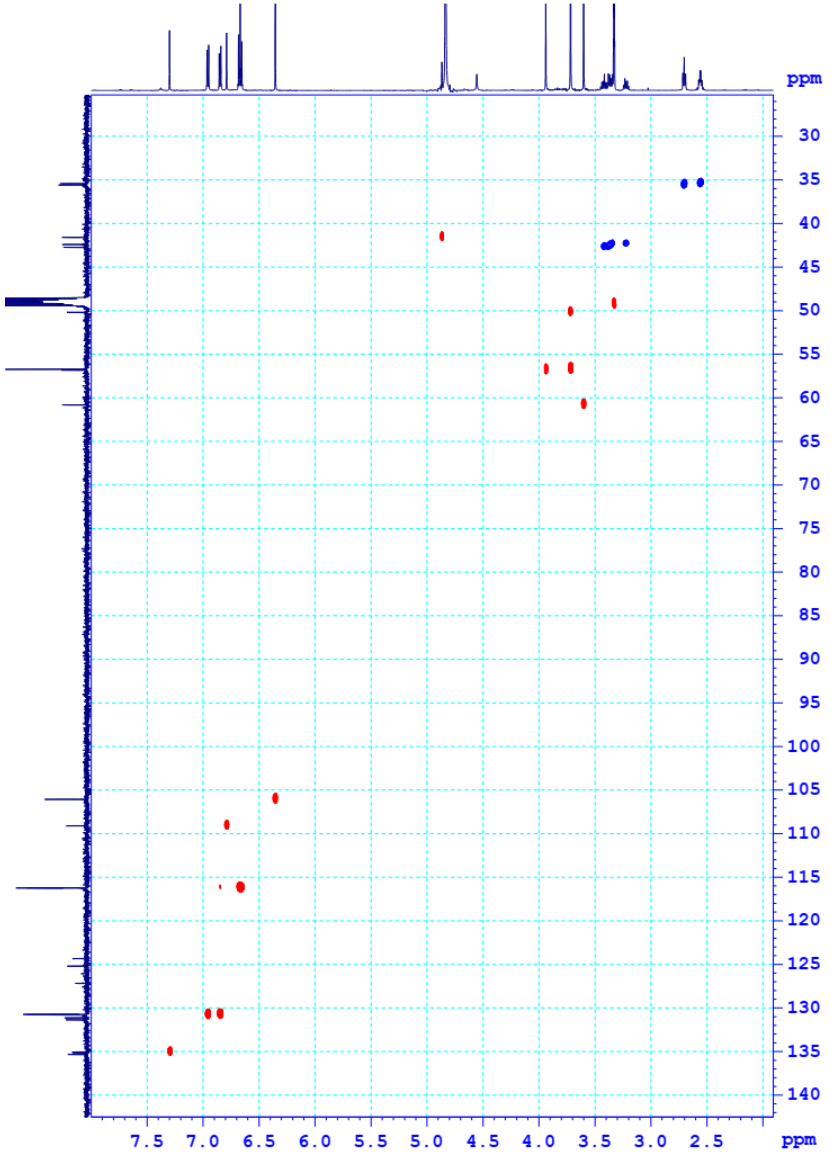
ppm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75
80
85
90
95
100
105
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115
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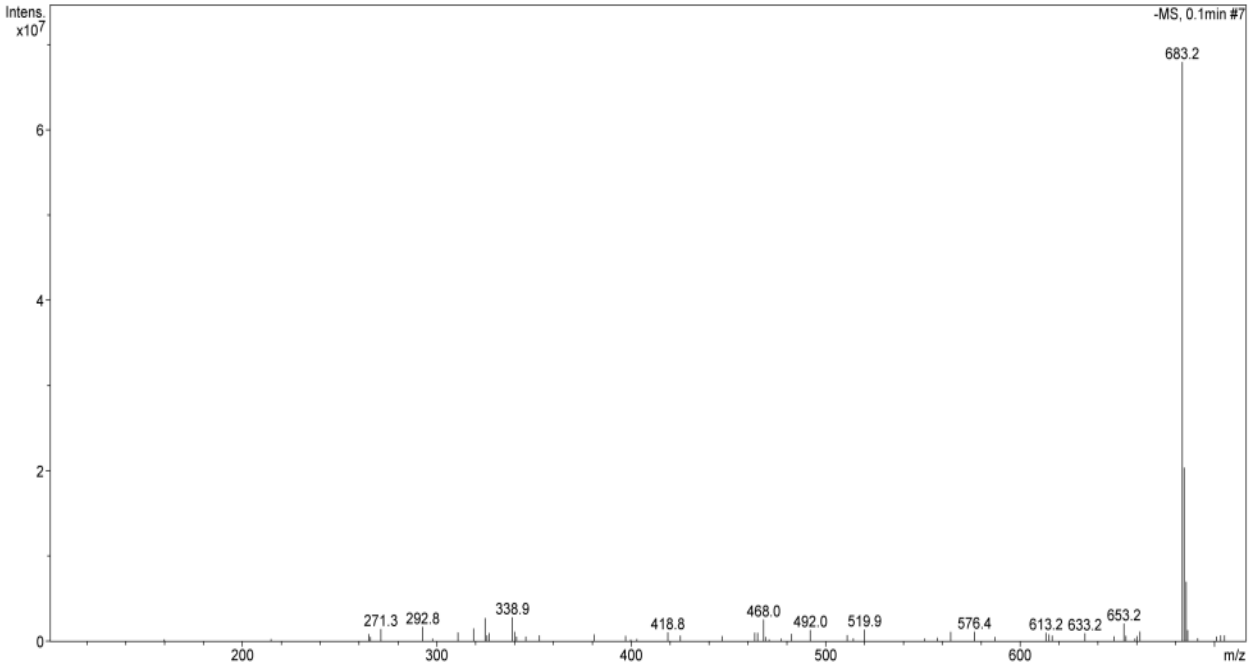
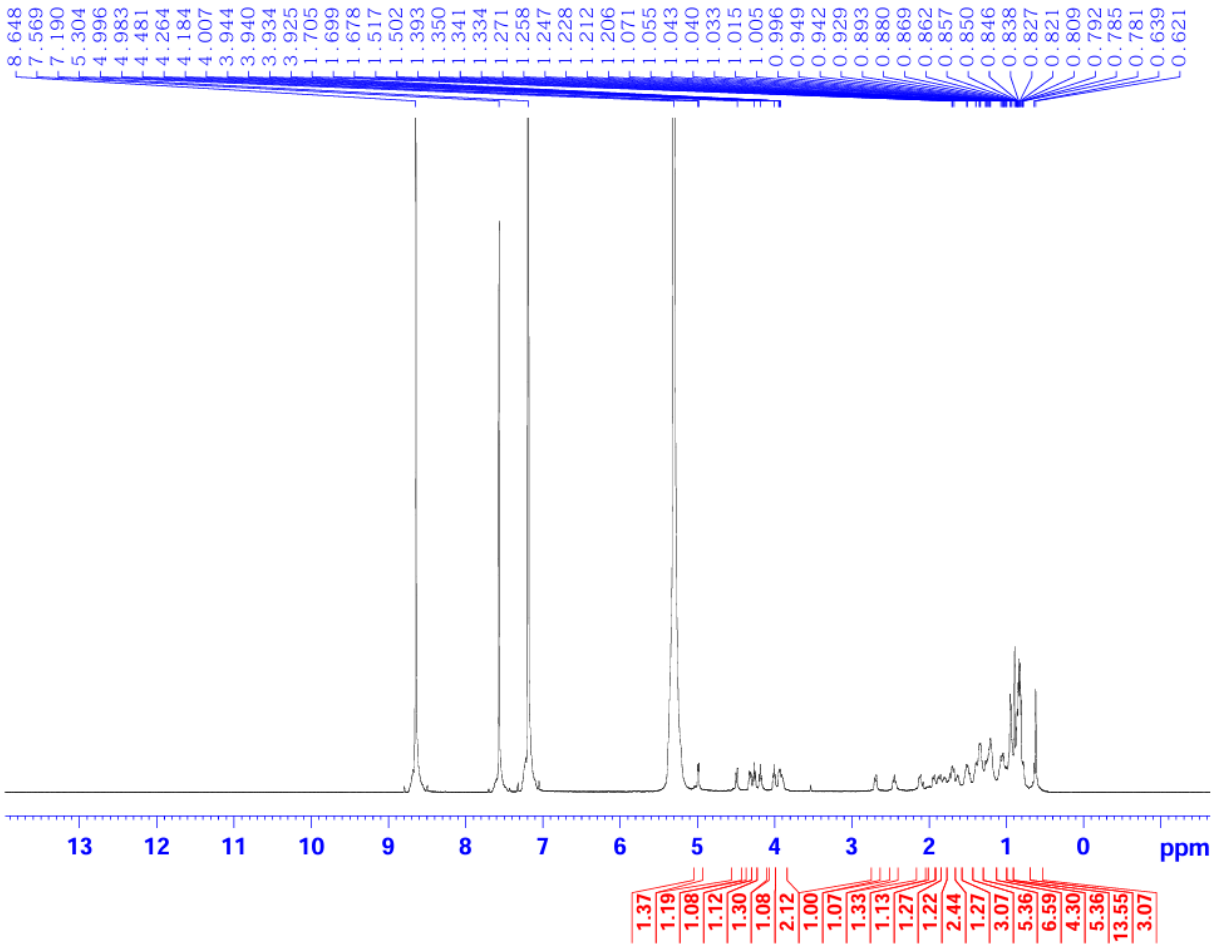
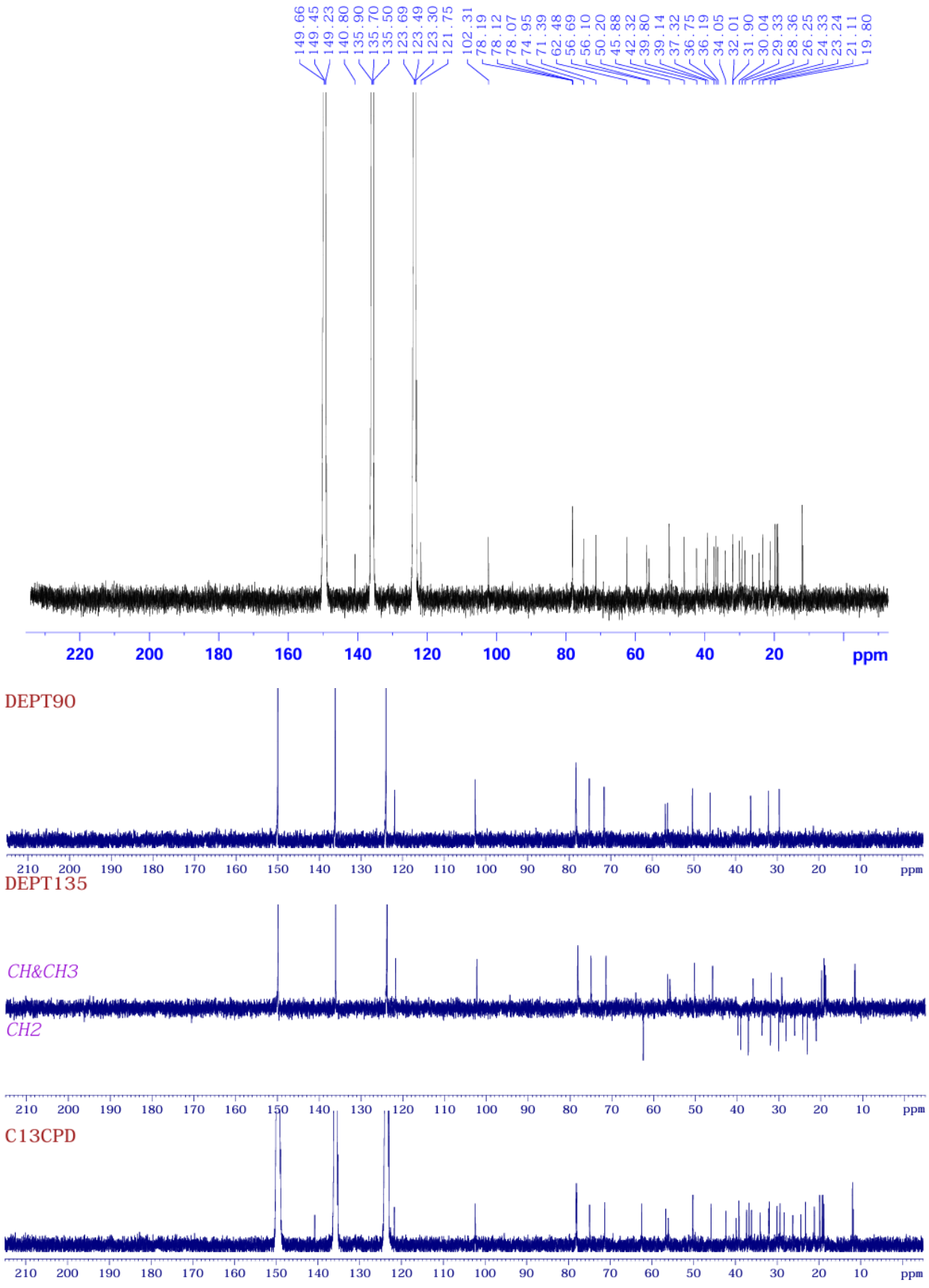
125
130
135
140
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5
ppm

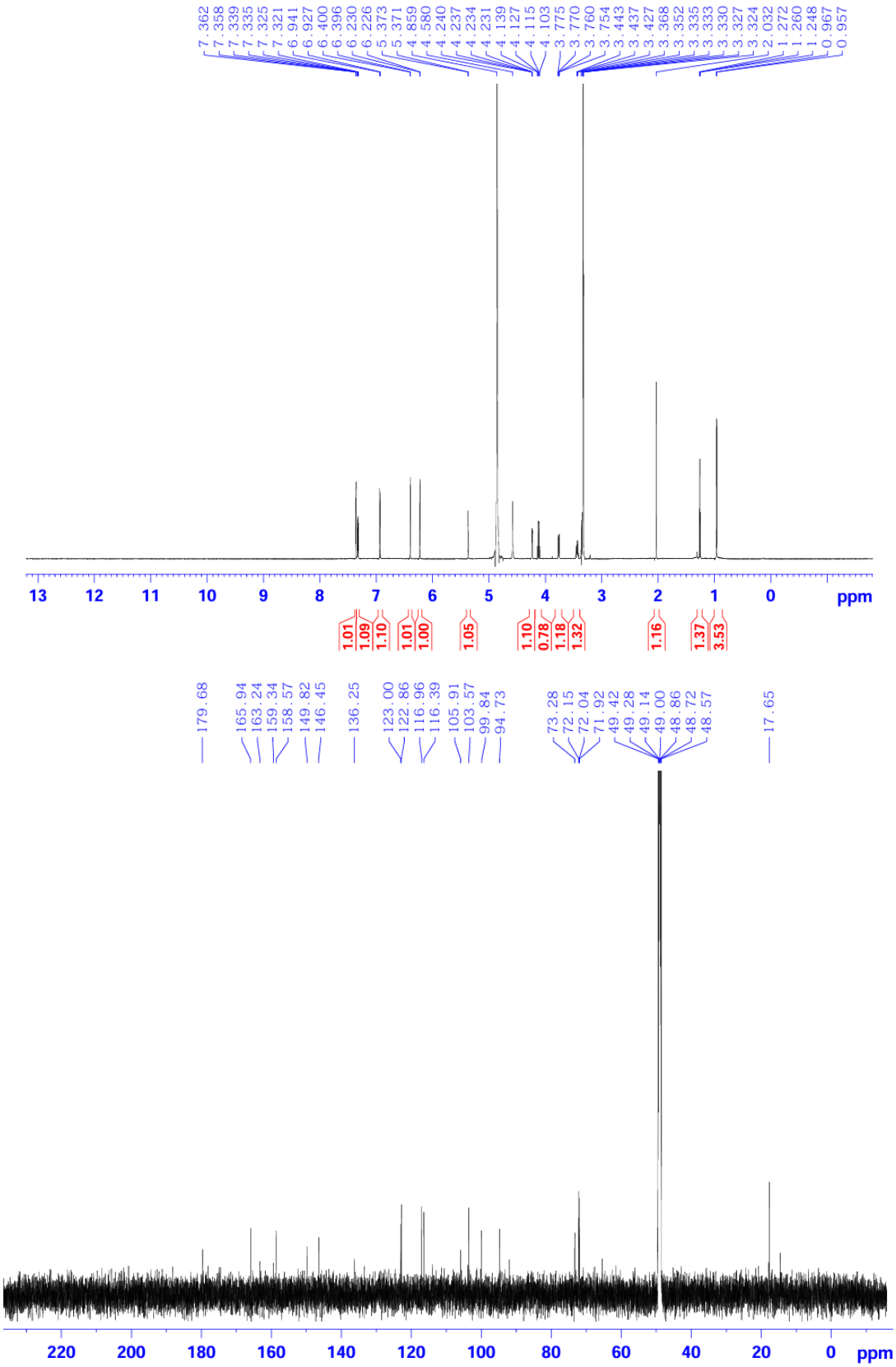



## 2.11. The $^{1}$H-NMR, $^{13}$C-NMR, and DEPT spectra of Daucosterol (11)

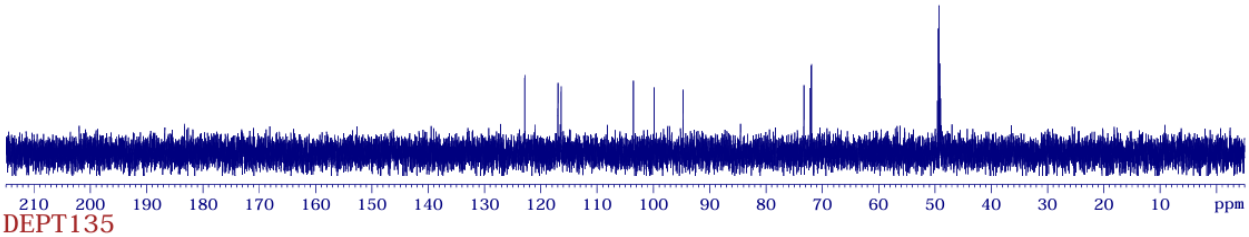



149.66
149.45
149.23
140.80
135.90
135.70
135.50
123.69
123.49
123.30
121.75
102.31
78.19
78.12
78.07
74.95
71.39
62.48
56.69
56.10
50.20
45.88
42.32
39.80
39.14
37.32
36.75
36.19
34.05
32.01
31.90
30.04
29.33
28.36
26.25
24.33
23.24
21.11
19.80
220
200
180
160
140
120
100
80
60
40
20
ppm
DEPT90
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm
DEPT135
CH&CH3
CH2
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm
C13CPD
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

## 2.12. The $^{1}$H-NMR, $^{13}$C-NMR, DEPT, and ESI-MS spectra of Quercitrin (12)



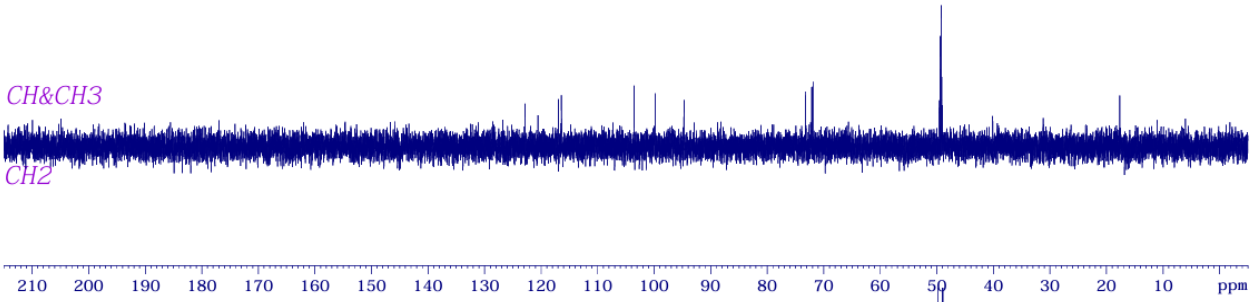
DEPT90



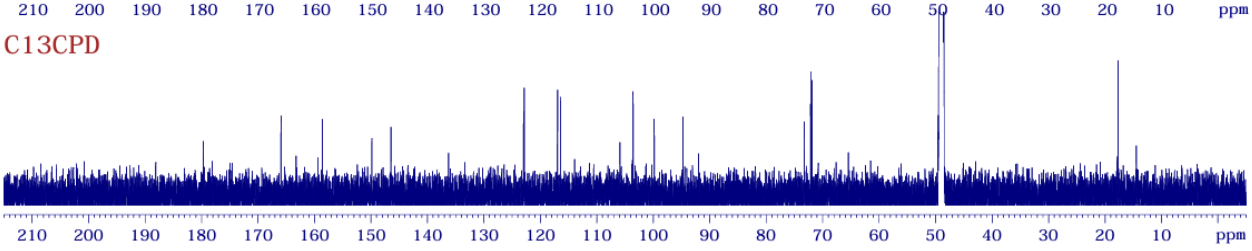

DEPT135

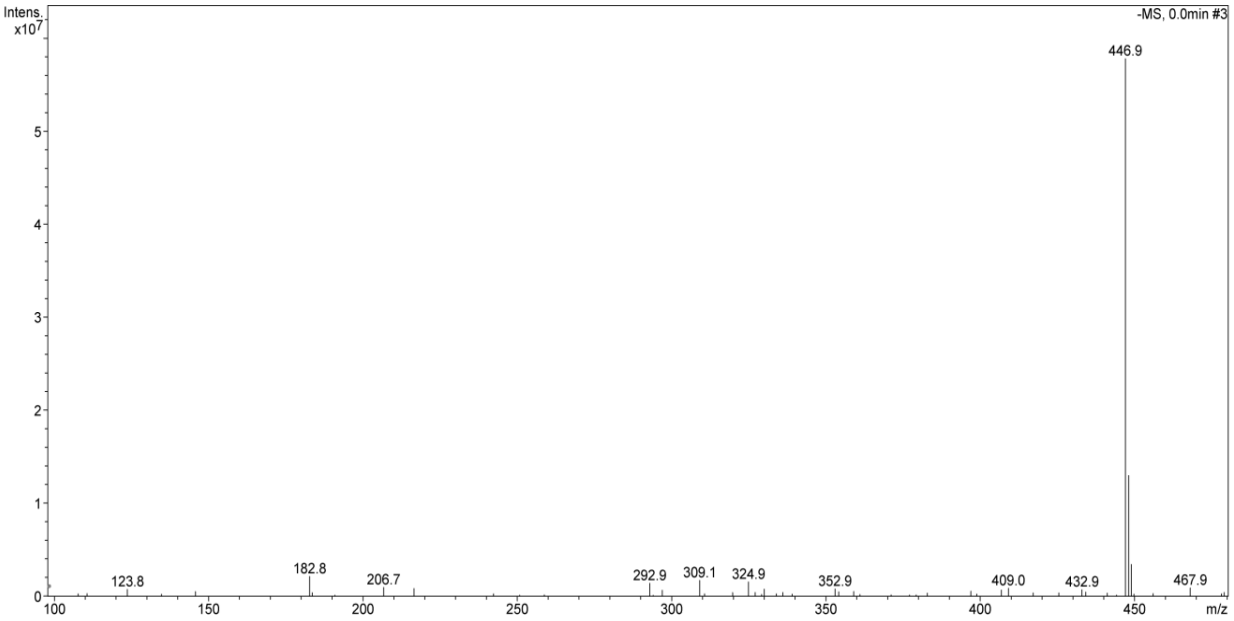
*CH&CH3*

*CH2*

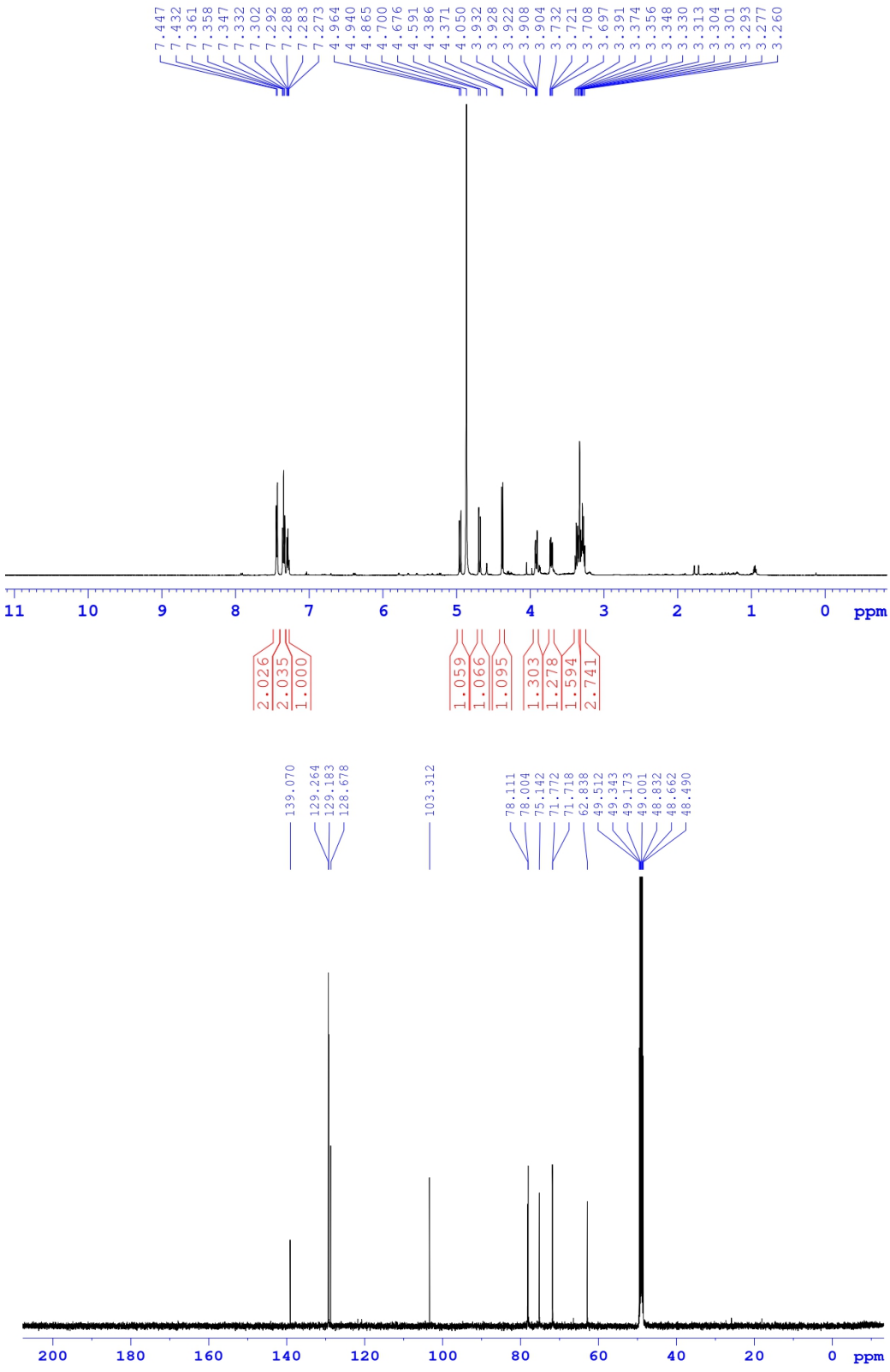


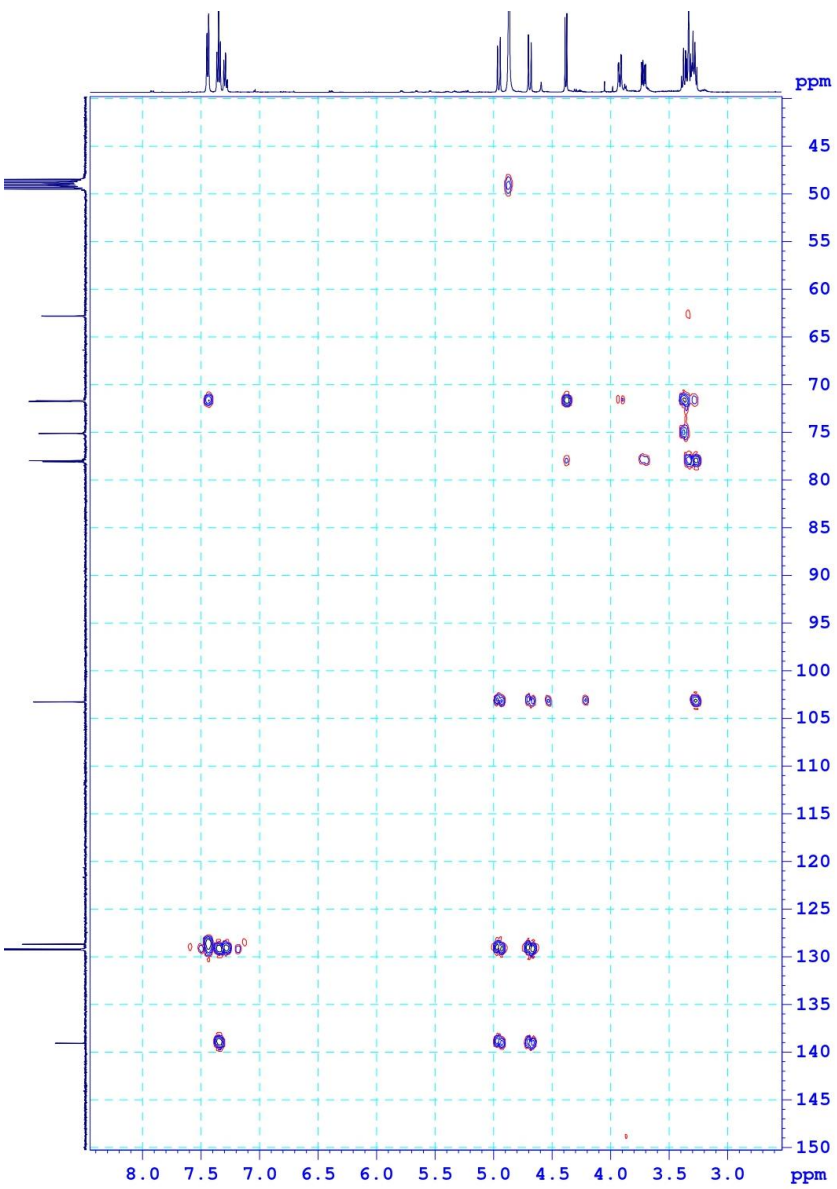

C13CPD

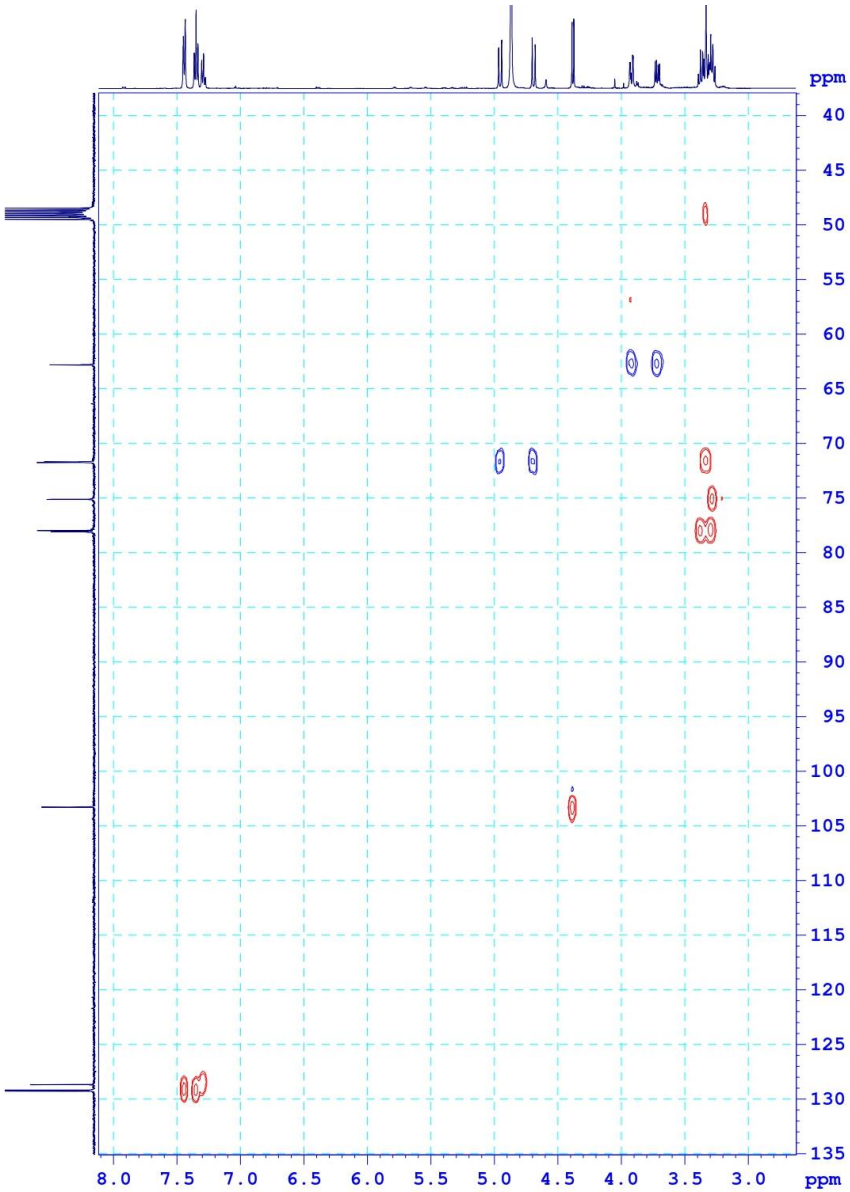


## 2.13. The $^{1}$H-NMR, $^{13}$C-NMR, HSQC, HMBC, DEPT, and ESI-MS spectra of Benzyl-O-*β*-D-glucopyranoside (13)




ppm
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
145
150
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
ppm


ppm
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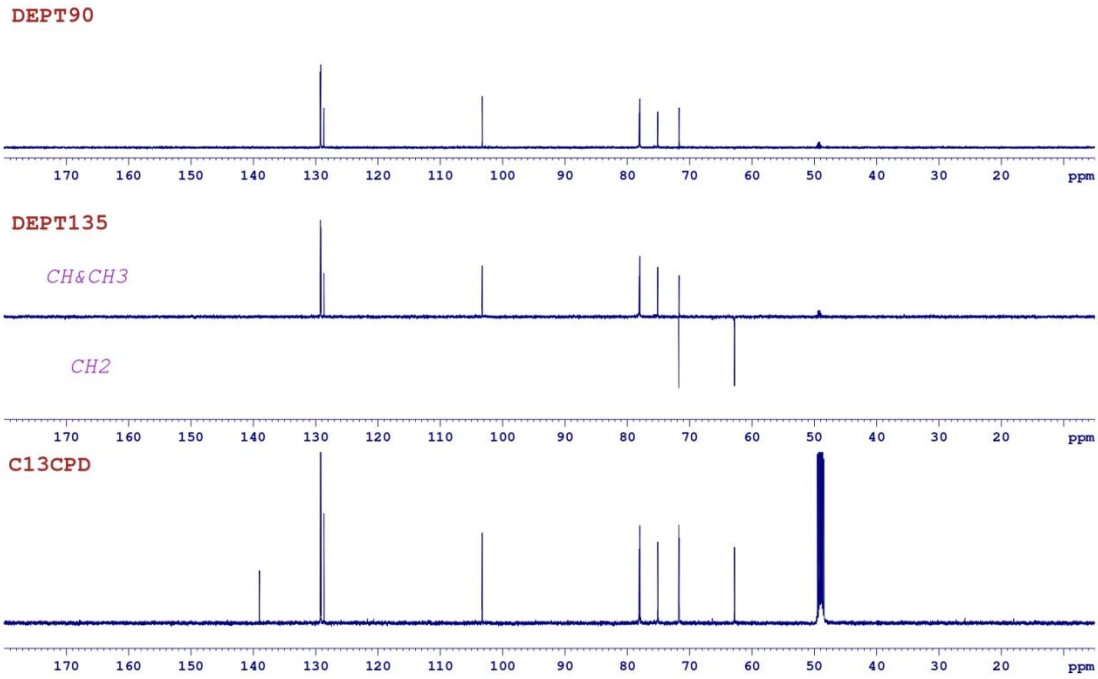
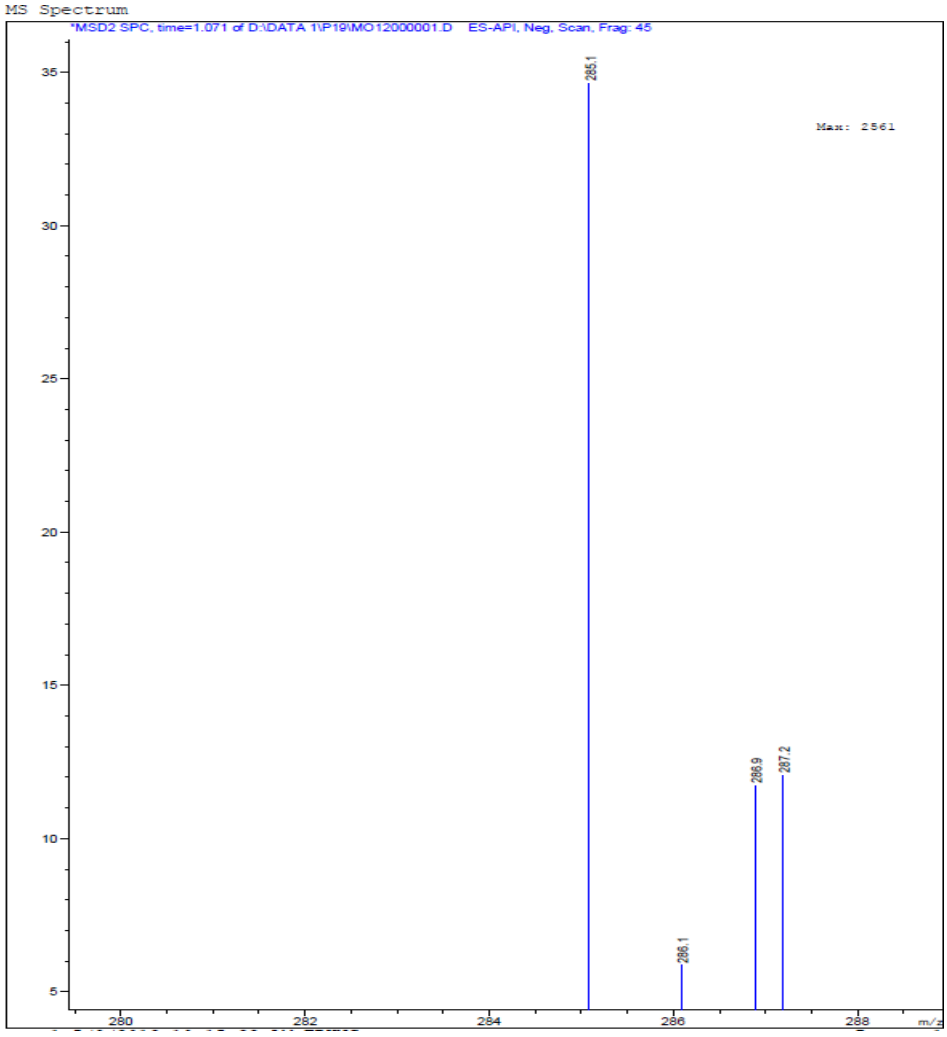
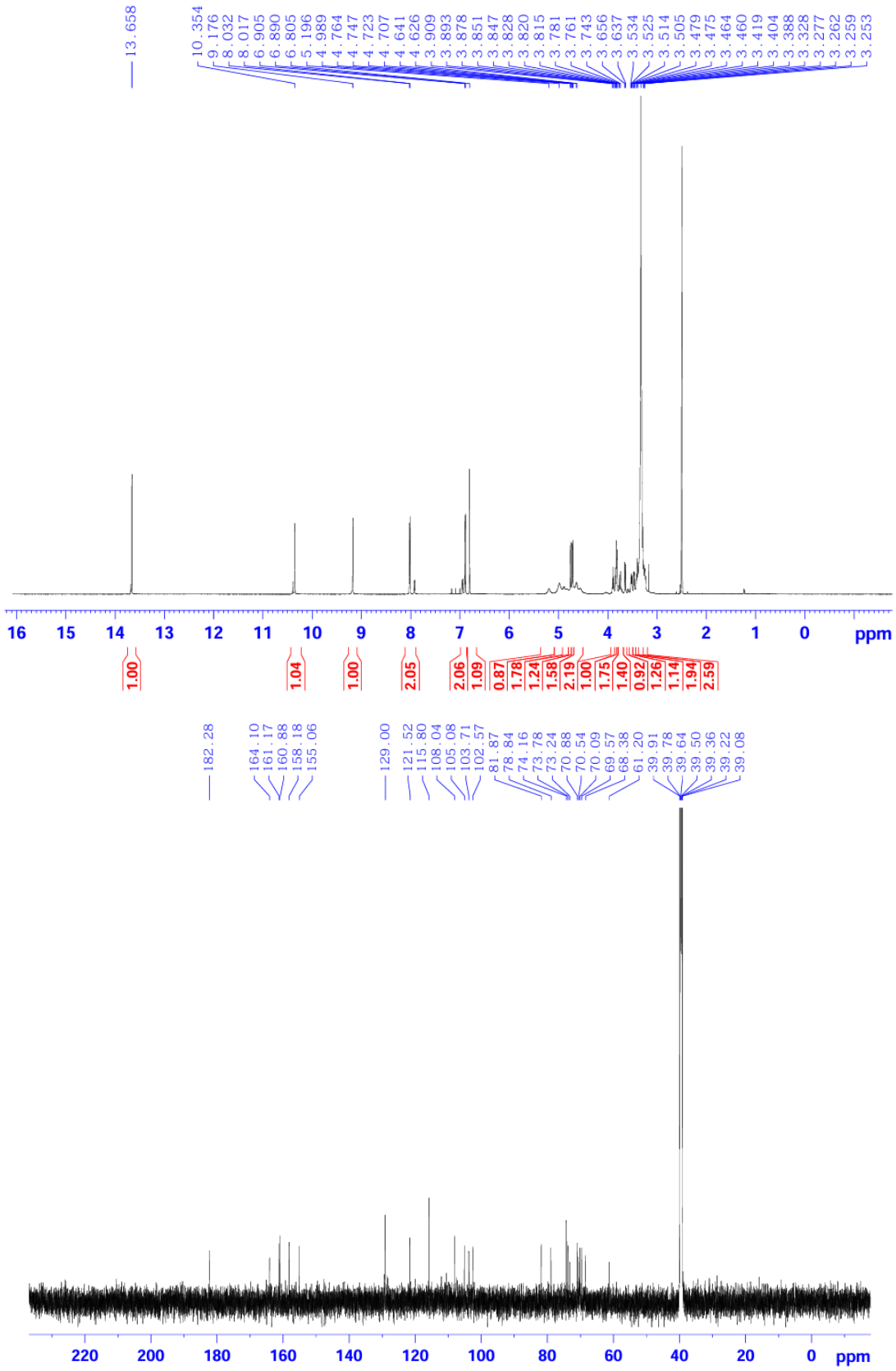
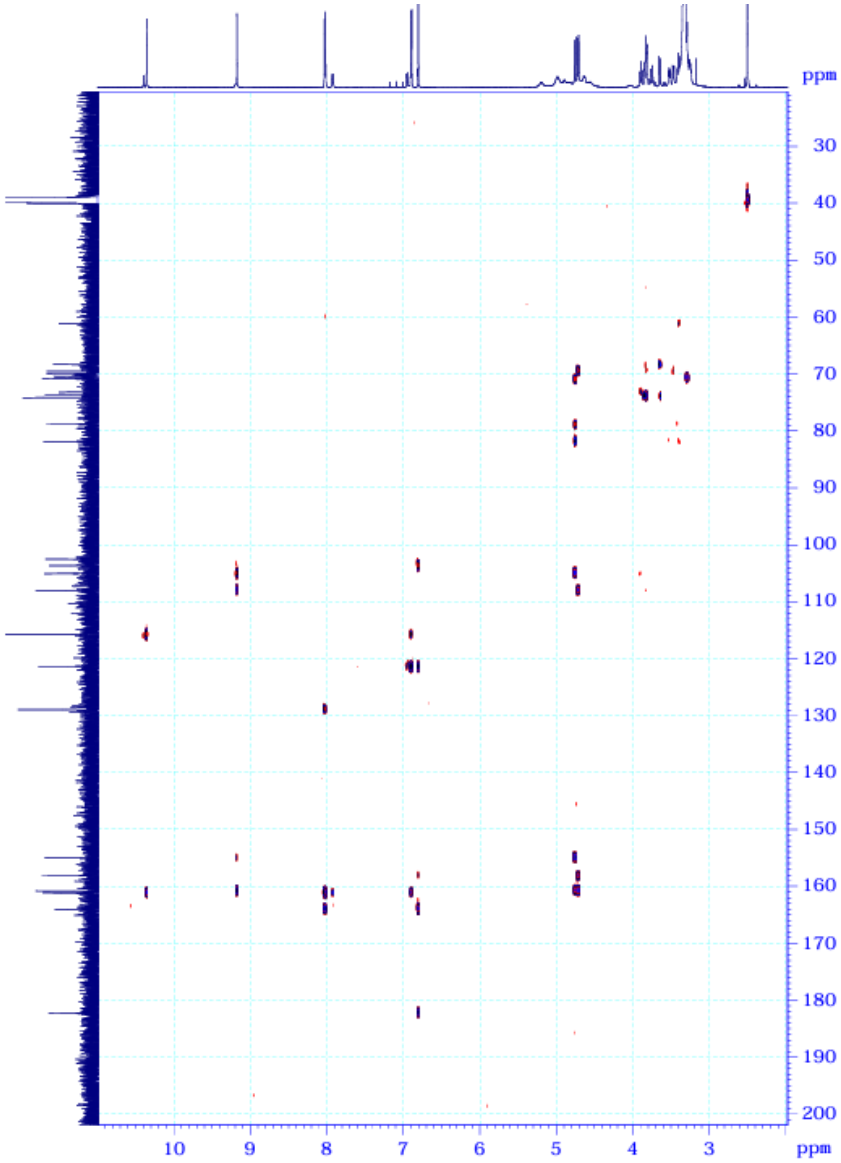
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
ppm

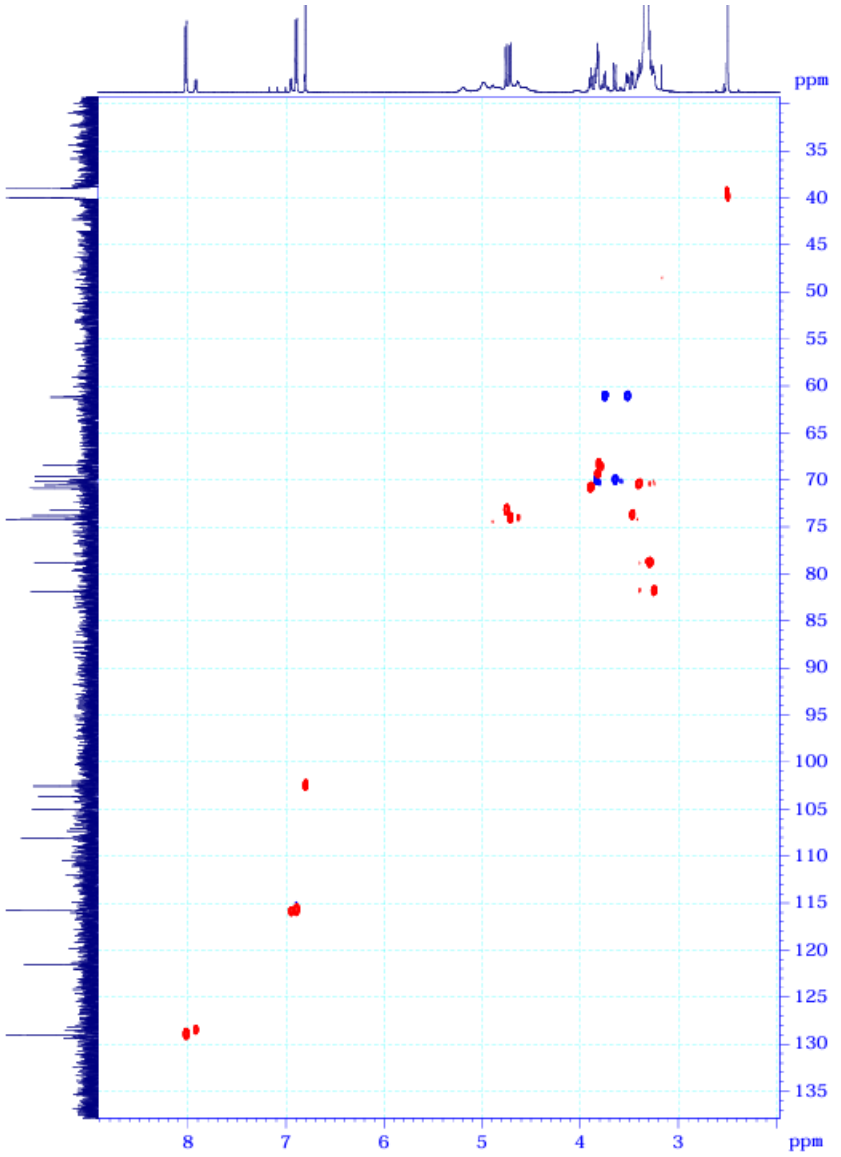

DEPT90
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm
DEPT135
CH&CH3
CH2
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm
C13CPD
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

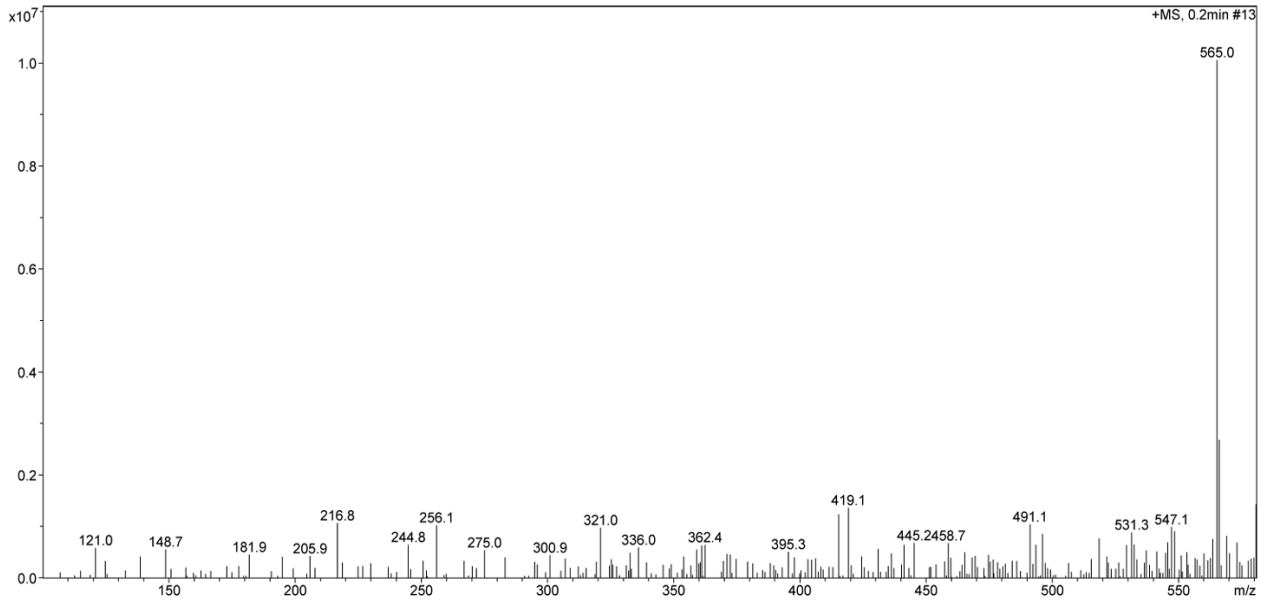

MS Spectrum
*MSD2 SPC, time=1.071 of D:\DATA 1\P19\MO12000001.D ES-API, Neg, Scan, Frag: 45
Max: 2561
285.1
286.9
287.2
286.1
35
30
25
20
15
10
5
280
282
284
286
288
m/z

## 2.14. The $^{1}$H-NMR, $^{13}$C-NMR, HSQC, HMBC, and ESI-MS spectra of Isoschaftoside (14)




ppm
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
10
9
8
7
6
5
4
3
ppm


ppm
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
8
7
6
5
4
3
ppm


x10^7
+MS, 0.2min #13
565.0
1.0
0.8
0.6
0.4
0.2
0.0
121.0
148.7
181.9
205.9
216.8
244.8
256.1
275.0
300.9
321.0
336.0
362.4
395.3
419.1
445.2458.7
491.1
531.3
547.1
150
200
250
300
350
400
450
500
550
m/z

## 3. NMR data of isolated compounds from *C. diffusa* and comparison with those in published literature

**Table S1.** Comparison of NMR data of compound **1** and corosolic acid [117]

| Position | Compound 1 (DMSO-$d_6$) | | Corosolic acid (DMSO-$d_6$) | |
|---|---|---|---|---|
| | $^{13}$C (150 MHz) $\delta_C$ (ppm) | $^1$H (600 MHz), $\delta_H$ (ppm) | $^{13}$C (125 MHz) $\delta_C$ (ppm) | $^1$H (500 MHz), $\delta_H$ (ppm) |
| 1 | 47.1 | - | 46.8 | - |
| 2 | 67.2 | 4.24 (1H, d, $J$ = 4.8 Hz) | 67.1 | 4.22 (1H, d, $J$ = 8.7 Hz) |
| 3 | 82.1 | 4.35 (1H, d, $J$ = 10.8 Hz) | 82.2 | 4.33 (1H, dd, $J$ = 9.0, 11.5 Hz) |
| 4 | 39.0 | - | 38.8 | - |
| 5 | 54.6 | - | 54.7 | - |
| 6 | 18.0 | - | 18.0 | - |
| 7 | 32.5 | - | 32.6 | - |
| 8 | 39.3 | - | 39.0 | - |
| 9 | 46.9 | - | 47.0 | - |
| 10 | 37.6 | - | 37.5 | - |
| 11 | 22.9 | - | 22.9 | - |
| 12 | 124.5 | 5.14 (1H, brs) | 124.4 | 5.17 (1H, t, $J$ = 3.4 Hz) |
| 13 | 138.3 | - | 138.2 | - |
| 14 | 41.7 | - | 41.6 | - |
| 15 | 27.6 | - | 27.4 | - |
| 16 | 23.8 | - | 23.7 | - |
| 17 | 47.0 | - | 46.9 | - |
| 18 | 52.2 | 3.42 (1H, t, $J$ = 11.4 Hz) | 52.3 | 3.42 (1H, t, $J$ = 9.8 Hz) |
| 19 | 38.4 | 2.75 (1H, dd, $J$ = 4.8, 11.4 Hz) | 38.4 | 2.74 (1H, t) |
| 20 | 38.5 | 2.11 (1H, d, $J$ = 13.8 Hz) | 38.4 | 2.11 (1H, t) |
| 21 | 30.2 | - | 30.1 | - |
| 22 | 36.3 | - | 36.2 | - |
| 23 | 28.8 | 1.03 (3H, s) | 28.8 | 1.00 (3H, s) |
| 24 | 17.2 | 0.88 (3H, s) | 17.1 | 0.88 (3H, s) |
| 25 | 16.5 | 0.82 (3H, s) | 16.4 | 0.82 (3H, s) |
| 26 | 16.9 | 0.91 (3H, s) | 16.9 | 0.92 (3H, s) |
| 27 | 23.3 | 0.92 (3H, s) | 23.2 | 0.92 (3H, s) |
| 28 | 178.2 | - | 178.2 | - |
| 29 | 21.1 | 0.71 (3H, s) | 21.0 | 0.71 (3H, s) |
| 30 | 17.1 | 0.75 (3H, s) | 16.9 | 0.75 (3H, s) |

**Table S2.** Comparison of NMR data of compound **2** and 3α-acetyl-20*S*,24*S*-epoxydammarane-25-ol [118]

| Position | Compound 2 ($CDCl_3$) | | 3α-acetyl-20*S*,24*S*-epoxydammarane-25-ol ($CDCl_3$) | |
|---|---|---|---|---|
| | $^{13}C$ (150 MHz) $\delta_C$ (ppm) | $^{1}H$ (600 MHz), $\delta_H$ (ppm) | $^{13}C$ (75 MHz) $\delta_C$ (ppm) | $^{1}H$ (300 MHz), $\delta_H$ (ppm) |
| 1 | 33.4 | - | 33.9 | - |
| 2 | 23.5 | - | 22.5 | - |
| 3 | 80.9 | 4.48 (1H, m) | 77.8 | 4.47 (1H, brs) |
| 4 | 36.9 | - | 36.3 | - |
| 5 | 50.7 | - | 50.3 | - |
| 6 | 18.2 | - | 17.7 | - |
| 7 | 35.8 | - | 34.7 | - |
| 8 | 40.4 | - | 40.1 | - |
| 9 | 50.0 | - | 50.2 | - |
| 10 | 38.7 | - | 36.7 | - |
| 11 | 21.5 | - | 21.2 | - |
| 12 | 27.3 | - | 26.8 | - |
| 13 | 42.9 | - | 42.4 | - |
| 14 | 49.5 | - | 49.7 | - |
| 15 | 31.4 | - | 31.1 | - |
| 16 | 25.7 | - | 25.5 | - |
| 17 | 49.5 | 1.11 (3H, s) | 49.4 | 1.11 (3H, s) |
| 18 | 15.5 | 0.96 (3H, s) | 15.1 | 0.98 (3H, s) |
| 19 | 16.2 | 0.87 (3H, s) | 15.6 | 0.87 (3H, s) |
| 20 | 86.4 | - | 86.0 | - |
| 21 | 27.1 | 1.13 (3H, s) | 26.6 | 1.15 (3H, s) |
| 22 | 35.2 | - | 34.4 | - |
| 23 | 26.1 | - | 26.0 | - |
| 24 | 83.3 | 3.72 (1H, t, $J$ = 9.0 Hz) | 85.9 | 3.65 (1H, dd, $J$ = 9.5, 5.7 Hz) |
| 25 | 71.4 | - | 69.8 | - |
| 26 | 27.5 | 1.20 (3H, s) | 27.2 | 1.19 (3H, s) |
| 27 | 24.3 | - | 23.9 | - |
| 28 | 28.0 | 0.85 (3H, s) | 27.5 | 0.84 (3H, s) |
| 29 | 21.6 | 0.88 (3H, s) | 21.4 | 0.88 (3H, s) |
| 30 | 16.6 | 0.90 (3H, s) | 16.2 | 0.93 (3H, s) |
| 31 | 21.3 | 2.07 (3H, s) | 20.9 | 2.09 (3H, s) |
| 32 | 171.0 | - | 171.1 | - |

**Table S3.** Comparison of NMR data of compound **3** and 11-oxo-α-amyrin acetate [119]

| Position | Compound 3 ($CDCl_3$) | | 11-oxo-α-amyrin acetate ($CDCl_3$) | |
|---|---|---|---|---|
| | $^{13}$C (150 MHz) $\delta_C$ (ppm) | $^{1}$H (600 MHz), $\delta_H$ (ppm) | $^{13}$C (125 MHz) $\delta_C$ (ppm) | $^{1}$H (500 MHz), $\delta_H$ (ppm) |
| 1 | 38.9 | - | 38.9 | - |
| 2 | 23.6 | - | 23.6 | - |
| 3 | 80.7 | 4.52 (1H, dd, $J$ = 5.4, 11.4 Hz) | 80.7 | 4.52 (1H, dd, $J$ = 12.0, 4.5 Hz) |
| 4 | 38.0 | - | 38.0 | - |
| 5 | 55.0 | - | 55.0 | - |
| 6 | 17.5 | - | 17.5 | - |
| 7 | 32.8 | - | 32.8 | - |
| 8 | 45.1 | - | 45.1 | - |
| 9 | 61.5 | 2.35 (1H, brs) | 61.5 | - |
| 10 | 36.8 | - | 36.8 | - |
| 11 | 199.6 | - | 199.7 | - |
| 12 | 130.4 | 5.54 (1H, s) | 130.4 | 5.54 (1H, s) |
| 13 | 165.0 | - | 165.0 | - |
| 14 | 43.7 | - | 43.7 | - |
| 15 | 27.2 | - | 27.2 | - |
| 16 | 40.9 | - | 40.9 | - |
| 17 | 33.9 | - | 33.9 | - |
| 18 | 59.0 | - | 59.0 | - |
| 19 | 39.2 | - | 39.2 | - |
| 20 | 39.3 | - | 39.3 | - |
| 21 | 30.9 | - | 30.9 | - |
| 22 | 27.5 | - | 27.5 | - |
| 23 | 28.1 | 0.88 (3H, s) | 28.1 | 0.88 (3H, s) |
| 24 | 16.7 | 0.89 (3H, s) | 16.7 | 0.89 (3H, s) |
| 25 | 18.5 | 1.19 (3H, s) | 18.5 | 1.19 (3H, s) |
| 26 | 16.6 | 1.17 (3H, s) | 16.6 | 1.17 (3H, s) |
| 27 | 21.1 | 1.29 (3H, s) | 20.5 | 1.29 (3H, s) |
| 28 | 28.8 | 0.82 (3H, s) | 28.8 | 0.82 (3H, s) |
| 29 | 17.5 | 0.81 (3H, d, $J$ = 2.4 Hz) | 17.5 | 0.81 (3H, d, $J$ = 2.0 Hz) |
| 30 | 20.5 | 0.95 (3H, d, $J$ = 7.8 Hz) | 20.5 | 0.95 (3H, d, $J$ = 6.5 Hz) |
| 31 | 171.0 | - | 171.0 | - |
| 32 | 21.3 | 2.05 (3H, s) | 21.1 | 2.05 (3H, s) |

**Table S4.** Comparison of NMR data of compound **4** and stigmasterol [120]

| Position | Compound 4 ($CDCl_3$) | | Stigmasterol ($CDCl_3$) | |
|---|---|---|---|---|
| | $^{13}$C (150 MHz) $\delta_C$ (ppm) | $^{1}$H (600 MHz), $\delta_H$ (ppm) | $^{13}$C (125 MHz) $\delta_C$ (ppm) | $^{1}$H (500 MHz), $\delta_H$ (ppm) |
| 1 | 37.3 | - | 37.3 | - |
| 2 | 31.7 | - | 31.6 | - |
| 3 | 71.8 | 3.51 (1H, m) | 71.8 | 3.52 (1H, m) |
| 4 | 42.2 | - | 42.3 | - |
| 5 | 140.7 | - | 140.7 | - |
| 6 | 121.7 | 5.35 (1H, brs) | 121.7 | 5.36 (1H, brd, $J$ = 3.5 Hz) |
| 7 | 31.9 | - | 31.8 | - |
| 8 | 31.8 | - | 31.8 | - |
| 9 | 50.2 | - | 50.1 | - |
| 10 | 36.5 | - | 36.6 | - |
| 11 | 21.0 | - | 21.1 | - |
| 12 | 39.8 | - | 39.7 | - |
| 13 | 42.3 | - | 42.3 | - |
| 14 | 56.8 | - | 56.8 | - |
| 15 | 24.4 | - | 24.5 | - |
| 16 | 28.2 | - | 28.9 | - |
| 17 | 56.1 | - | 56.1 | - |
| 18 | 12.0 | 0.85 (3H, s) | 12.1 | 0.85 (3H, s) |
| 19 | 19.1 | 1.02 (3H, s) | 19.4 | 1.02 (3H, s) |
| 20 | 40.5 | - | 40.6 | - |
| 21 | 19.8 | 0.92 (3H, d, $J$ = 6.0 Hz) | 21.1 | 0.92 (3H, d, $J$ = 6.5 Hz) |
| 22 | 138.3 | 5.15 (1H, m) | 138.2 | 5.16 (1H, dd, $J$ = 15.0, 8.5 Hz) |
| 23 | 129.3 | 5.03 (1H, m) | 129.3 | 5.03 (1H, dd, $J$ = 15.0, 8.5 Hz) |
| 24 | 51.2 | - | 51.2 | - |
| 25 | 33.9 | - | 31.9 | - |
| 26 | 21.2 | 0.83 (3H, d, $J$ = 7.2 Hz) | 21.2 | 0.83 (3H, t, $J$ = 8.5 Hz) |
| 27 | 18.9 | 0.81 (3H, d, $J$ = 6.8 Hz) | 19.1 | 0.82 (3H, d, $J$ = 6.5 Hz) |
| 28 | 25.4 | - | 25.3 | - |
| 29 | 12.2 | 0.79 (3H, t, $J$ = 9.6 Hz) | 12.2 | 0.79 (3H, d, $J$ = 9.5 Hz) |

**Table S5.** Comparison of NMR data of compound **5** and lyratol F [121]

| Position | Compound 5 ($CD_3OD$) | | Lyratol F ($CD_3OD$) | |
|---|---|---|---|---|
| | $^{13}$C (150 MHz) $\delta_C$ (ppm) | $^{1}$H (600 MHz), $\delta_H$ (ppm) | $^{13}$C (150 MHz) $\delta_C$ (ppm) | $^{1}$H (600 MHz), $\delta_H$ (ppm) |
| 1 | 37.0 | - | - | - |
| 2 | 49.7 | 2.24 (1H, m, H-2a)<br>1.42 (1H, m, H-2b) | - | 2.22 (1H, m, H-2a)<br>1.41 (1H, m, H-2b) |
| 3 | 64.4 | 4.23 (1H, m) | 64.4 | 4.22 (1H, m) |
| 4 | 49.9 | 1.95 (1H, m, H-4a)<br>1.38 (1H, m, H-4b) | - | 1.93 (1H, m, H-4a)<br>1.33 (1H, m, H-4b) |
| 5 | 72.5 | - | 72.4 | - |
| 6 | 120.0 | - | 119.9 | - |
| 7 | 200.9 | - | 200.9 | - |
| 8 | 101.1 | 5.85 (1H, s) | 101.1 | 5.82 (1H, s) |
| 9 | 211.5 | - | 211.6 | - |
| 10 | 26.5 | 2.21 (3H, s) | 26.5 | 2.19 (3H, s) |
| 11 | 32.3 | 1.12 (3H, s) | 32.2 | 1.15 (3H, s) |
| 12 | 29.3 | 1.41 (3H, s) | 29.3 | 1.38 (3H, s) |
| 13 | 30.8 | 1.41 (3H, s) | 30.7 | 1.38 (3H, s) |

**Table S6.** Comparison of NMR data of compound **6** and 4-hydroxybenzoic acid [122]

| Position | Compound 6 ($CD_3OD$) | | 4-hydroxybenzoic acid ($CD_3OD$) | |
|---|---|---|---|---|
| | $^{13}$C (150 MHz) $\delta_C$ (ppm) | $^{1}$H (600 MHz), $\delta_H$ (ppm) | $^{13}$C (125 MHz) $\delta_C$ (ppm) | $^{1}$H (500 MHz), $\delta_H$ (ppm) |
| 1 | - | - | 123.6 | - |
| 2 | 133.0 | 7.89 (1H, d, $J$ = 8.4 Hz) | 133.8 | 7.8 (1H, d, $J$ = 7.5 Hz) |
| 3 | 116.0 | 6.83 (1H, d, $J$ = 8.4 Hz) | 116.8 | 6.8 (1H, d, $J$ = 7.5 Hz) |
| 4 | 163.3 | - | 164.0 | - |
| 5 | 116.0 | 6.83 (1H, d, $J$ = 8.4 Hz) | 116.8 | 6.8 (1H, d, $J$ = 7.5 Hz) |
| 6 | 133.0 | 7.89 (1H, d, $J$ = 8.4 Hz) | 133.8 | 7.8 (1H, d, $J$ = 7.5 Hz) |
| 7 | - | - | 171.0 | - |

**Table S7.** Comparison of NMR data of compound **7** and methyl gallate [123]

| Position | Compound 7 ($CD_3OD$) | | Methyl gallate ($CD_3OD$) | |
|---|---|---|---|---|
| | **$^{13}$C (150 MHz)** $\delta_C$ (ppm) | **$^{1}$H (600 MHz),** $\delta_H$ (ppm) | **$^{13}$C (125 MHz)** $\delta_C$ (ppm) | **$^{1}$H (500 MHz),** $\delta_H$ (ppm) |
| 1 | 121.5 | - | 120.5 | - |
| 2 | 110.0 | 7.06 (1H, s) | 109.0 | 7.05 (1H, s) |
| 3 | 146.5 | - | 145.3 | - |
| 4 | 139.8 | - | 139.0 | - |
| 5 | 146.5 | - | 145.3 | - |
| 6 | 110.0 | 7.06 (1H, s) | 109.0 | 7.05 (1H, s) |
| 7 | 169.0 | - | 168.0 | - |
| -$OCH_3$ | 52.3 | 3.83 (3H, s) | 52.0 | 3.80 (3H, s) |

**Table S8.** Comparison of NMR data of compound **8** and *N-trans*-feruloyltyramine [124]

| Position | Compound 8 ($CD_3OD$) | | *N-trans*-feruloyltyramine ($CD_3OD$) | |
|---|---|---|---|---|
| | **$^{13}$C (150 MHz)** $\delta_C$ (ppm) | **$^{1}$H (600 MHz),** $\delta_H$ (ppm) | **$^{13}$C (62.5 MHz)** $\delta_C$ (ppm) | **$^{1}$H (250 MHz),** $\delta_H$ (ppm) |
| 1 | 131.3 | - | 131.30 | - |
| 2 | 111.6 | 7.14 (1H, d, $J$ = 1.8 Hz) | 111.55 | 7.12 (1H, d, $J$ = 1.9 Hz) |
| 3 | 149.3 | - | 149.29 | - |
| 4 | 149.9 | - | 149.85 | - |
| 5 | 116.5 | 6.82 (1H, d, $J$ = 8.4 Hz) | 116.47 | 6.79 (1H, d, $J$ = 8.4 Hz) |
| 6 | 123.2 | 7.05 (1H, dd, $J$ = 2.4, 8.4 Hz) | 123.21 | 7.00 (1H, dd, $J$ = 8.4, 1.9 Hz) |
| 7 | 142.0 | 7.46 (1H, d, $J$ = 15.6 Hz) | 142.01 | 7.43 (1H, d, $J$ = 15.7 Hz) |
| 8 | 118.8 | 6.43 (1H, d, $J$ = 15.6 Hz) | 118.75 | 6.40 (1H, d, $J$ = 15.7 Hz) |
| 9 | 169.2 | - | 169.17 | - |
| -$OCH_3$ | 56.4 | 3.90 (3H, s) | 56.39 | 3.88 (3H, s) |
| 1' | 128.3 | - | 128.28 | - |
| 2' | 130.7 | 7.08 (1H, d, $J$ = 8.4 Hz) | 130.72 | 7.06 (1H, d, $J$ = 8.5 Hz) |
| 3' | 116.3 | 6.75 (1H, d, $J$ = 8.4 Hz) | 116.26 | 6.72 (1H, d, $J$ = 8.5 Hz) |
| 4' | 156.9 | - | 156.94 | - |
| 5' | 116.3 | 6.75 (1H, d, $J$ = 8.4 Hz) | 116.26 | 6.72 (1H, d, $J$ = 8.5 Hz) |
| 6' | 130.7 | 7.08 (1H, d, $J$ = 8.4 Hz) | 130.72 | 7.06 (1H, d, $J$ = 8.5 Hz) |
| 7' | 42.5 | 2.77 (2H, t, $J$ = 7.2 Hz) | 42.54 | 2.75 (2H, t, $J$ = 7.4 Hz) |
| 8' | 35.8 | 3.48 (2H, t, $J$ = 7.2 Hz) | 35.81 | 3.47 (2H, t, $J$ = 7.4 Hz) |

**Table S9.** Comparison of NMR data of compound **9** and *N-trans-p*-coumaroyl-3',4'-dihydroxyphenylethylamine [125]

| Position | Compound 9 ($CD_3OD$) | | *N-trans-p*-coumaroyl-3',4'-dihydroxyphenylethylamine ($CD_3OD$) | |
|---|---|---|---|---|
| | $^{13}C$ (150 MHz) $\delta_C$ (ppm) | $^{1}H$ (600 MHz), $\delta_H$ (ppm) | $^{13}C$ (100 MHz) $\delta_C$ (ppm) | $^{1}H$ (400 MHz), $\delta_H$ (ppm) |
| 1 | 127.8 | - | 127.65 | - |
| 2 | 130.5 | 7.41 (1H, d, $J$ = 8.4 Hz) | 130.65 | 7.37 (1H, d, $J$ = 8.6 Hz) |
| 3 | 116.7 | 6.81 (1H, d, $J$ = 8.4 Hz) | 116.77 | 6.77 (1H, d, $J$ = 8.6 Hz) |
| 4 | 160.5 | - | 160.46 | - |
| 5 | 116.7 | 6.81 (1H, d, $J$ = 8.4 Hz) | 116.77 | 6.77 (1H, d, $J$ = 8.6 Hz) |
| 6 | 130.5 | 7.41 (1H, d, $J$ = 8.4 Hz) | 130.56 | 7.37 (1H, d, $J$ = 8.6 Hz) |
| 7 | 141.8 | 7.47 (1H, d, $J$ = 15.6 Hz) | 141.77 | 7.44 (1H, d, $J$ = 15.7 Hz) |
| 8 | 118.5 | 6.41 (1H, d, $J$ = 15.6 Hz) | 118.63 | 6.36 (1H, d, $J$ = 15.7 Hz) |
| 9 | 169.2 | - | 169.28 | - |
| 1' | 132.1 | - | 132.23 | - |
| 2' | 116.9 | 6.69 (1H, d, $J$ = 1.8 Hz) | 116.96 | 6.68 (1H, d, $J$ = 2.0 Hz) |
| 3' | 146.3 | - | 146.29 | - |
| 4' | 144.8 | - | 144.81 | - |
| 5' | 116.4 | 6.72 (1H, d, $J$ = 8.4 Hz) | 116.51 | 6.67 (1H, d, $J$ = 8.0 Hz) |
| 6' | 121.0 | 6.58 (1H, dd, $J$ = 2.4, 8.4 Hz) | 121.13 | 6.53 (1H, dd, $J$ = 8.0, 2.0 Hz) |
| 7' | 36.0 | 2.72 (2H, t, $J$ = 7.2 Hz) | 36.07 | 2.67 (2H, t, $J$ = 7.3 Hz) |
| 8' | 42.5 | 3.48 (2H, t, $J$ = 7.2 Hz) | 42.52 | 3.43 (2H, t, $J$ = 7.3 Hz) |

**Table S10.** Comparison of NMR data of compound **10** and 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide [126]

| Position | Compound 10 ($CD_3OD$) | | 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide ($CD_3OD$) | |
|---|---|---|---|---|
| | $^{13}C$ (150 MHz) $\delta_C$ (ppm) | $^{1}H$ (600 MHz), $\delta_H$ (ppm) | $^{13}C$ (50.3 MHz) $\delta_C$ (ppm) | $^{1}H$ (200 MHz), $\delta_H$ (ppm) |
| 1 | 41.6 | 4.97 (1H, brs) | 41.6 | 5.02 (1H, brs) |
| 2 | 50.2 | 3.72 (1H, overlapped) | 49.2 | 3.70 (1H, s) |
| 2a | 173.9 | - | 174.0 | - |
| 3 | 127.2 | - | 127.1 | - |
| 3a | 170.0 | - | 170.0 | - |

| | | | | |
|---|---|---|---|---|
| 4 | 135.0 | 7.30 (1H, s) | 135.1 | 7.20 (1H, s) |
| 4a | 124.3 | - | 124.3 | - |
| 5 | 109.1 | 6.79 (1H, s) | 109.1 | 6.68 (1H, s) |
| 6 | 149.2 | - | 149.2 | - |
| 7 | 143.1 | - | 143.1 | - |
| 8 | 146.9 | - | 146.9 | - |
| 8a | 125.2 | - | 125.2 | - |
| 1' | 135.2 | - | 135.3 | - |
| 2' | 106.0 | 6.35 (1H, s) | 106.0 | 6.24 (1H, s) |
| 3' | 149.0 | - | 149.0 | - |
| 4' | 135.3 | - | 135.3 | - |
| 5' | 149.0 | - | 149.0 | - |
| 6' | 106.0 | 6.35 (1H, s) | 106.0 | 6.24 (1H, s) |
| 1" | 131.1 | - | 131.1 | - |
| 2" | 130.7 | 6.85 (1H, d, *J* = 8.4 Hz) | 130.7 | 6.73 (1H, d, *J* = 8.5 Hz) |
| 3" | 116.2 | 6.65 (1H, d, *J* = 8.4 Hz) | 116.2 | 6.54 (1H, d, *J* = 8.5 Hz) |
| 4" | 156.8 | - | 156.8 | - |
| 5" | 116.2 | 6.65 (1H, d, *J* = 8.4 Hz) | 116.2 | 6.54 (1H, d, *J* = 8.5 Hz) |
| 6" | 130.7 | 6.85 (1H, d, *J* = 8.4 Hz) | 130.8 | 6.73 (1H, d, *J* = 8.5 Hz) |
| 7" | 35.4 | 2.70 (2H, t, *J* = 7.2 Hz) | 35.4 | 2.58 (2H, t, *J* = 7.1 Hz) |
| 8" | 42.4 | 3.43 (1H, m, H-8"α)<br>3.35 (1H, m, H-8"β) | 42.4 | 3.38 (2H, m) |
| 1"' | 131.3 | - | 131.3 | - |
| 2"' | 130.8 | 6.96 (1H, d, *J* = 8.4 Hz) | 130.8 | 6.84 (1H, d, *J* = 8.5 Hz) |
| 3"' | 116.2 | 6.68 (1H, d, *J* = 8.4 Hz) | 116.2 | 6.56 (1H, d, *J* = 8.5 Hz) |
| 4"' | 156.8 | - | 156.8 | - |
| 5"' | 116.2 | 6.68 (1H, d, *J* = 8.4 Hz) | 116.2 | 6.56 (1H, d, *J* = 8.5 Hz) |
| 6"' | 130.8 | 6.96 (1H, d, *J* = 8.4 Hz) | 130.8 | 6.84 (1H, d, *J* = 8.5 Hz) |
| 7"' | 35.6 | 2.56 (2H, m) | 35.6 | 2.44 (2H, t, *J* = 6.7 Hz) |
| 8"' | 42.7 | 3.35 (1H, m, H-8"'α)<br>3.23 (1H, m, H-8"'β) | 42.8 | 3.26 (2H, m) |
| $OCH_3$-8 | 60.8 | 3.60 (3H, s) | 60.8 | 3.48 (3H, s) |
| $OCH_3$-6 | 56.8 | 3.94 (3H, s) | 56.8 | 3.82 (3H, s) |
| $OCH_3$-3' | 56.7 | 3.72 (3H, overlapped) | 56.7 | 3.60 (3H, s) |
| $OCH_3$-5' | 56.7 | 3.72 (3H, s) | 56.7 | 3.60 (3H, s) |

**Table S11.** Comparison of NMR data of compound **11** and daucosterol [127]

| Position | Compound 11 ($C_5D_5N$) | | Daucosterol ($C_5D_5N$) | |
|---|---|---|---|---|
| | $^{13}$C (150 MHz) $\delta_C$ (ppm) | $^{1}$H (600 MHz), $\delta_H$ (ppm) | $^{13}$C (125 MHz) $\delta_C$ (ppm) | $^{1}$H (500 MHz), $\delta_H$ (ppm) |
| 1 | 37.3 | - | 37.4 | - |
| 2 | 30.0 | - | 30.2 | - |
| 3 | 78.1 | 4.25 (1H, m) | 78.6 | 4.31 (1H, m) |
| 4 | 39.8 | - | 39.3 | - |
| 5 | 140.8 | - | 140.8 | - |
| 6 | 121.8 | 5.30 (1H, overlap) | 121.9 | 5.32 (1H) |
| 7 | 32.0 | - | 32.0 | - |
| 8 | 31.9 | - | 32.1 | - |
| 9 | 50.2 | - | 50.3 | - |
| 10 | 36.8 | - | 36.9 | - |
| 11 | 21.1 | - | 21.2 | - |
| 12 | 39.1 | - | 39.9 | - |
| 13 | 42.3 | - | 42.5 | - |
| 14 | 56.7 | - | 56.8 | - |
| 15 | 24.3 | - | 24.5 | - |
| 16 | 28.4 | - | 28.5 | - |
| 17 | 56.1 | - | 56.2 | - |
| 18 | 12.0 | 0.62 (3H, s) | 11.9 | 0.64 (3H, s) |
| 19 | 19.3 | 0.92 (3H, s) | 19.1 | 0.92 (3H, s) |
| 20 | 36.2 | - | 36.4 | - |
| 21 | 18.9 | 0.95 (3H, m) | 19.0 | 0.97 (3H, d, $J$ = 6.5 Hz) |
| 22 | 34.1 | - | 34.2 | - |
| 23 | 26.3 | - | 26.3 | - |
| 24 | 45.9 | - | 46.0 | - |
| 25 | 29.3 | - | 29.4 | - |
| 26 | 19.8 | 0.89 (3H, s) | 19.9 | 0.89 (3H, d, $J$ = 5.6 Hz) |
| 27 | 19.1 | 0.84 (3H, m) | 19.2 | 0.84 (3H, d, $J$ = 6.5 Hz) |
| 28 | 23.2 | - | 23.3 | - |
| 29 | 11.8 | 0.88 (3H, m) | 12.1 | 0.88 (3H, d, $J$ = 3.1 Hz) |
| 1' | 102.3 | 4.99 (1H, d, $J$ = 7.8 Hz) | 102.5 | 4.99 (1H, d, $J$ = 7.7 Hz) |
| 2' | 75.0 | 3.91 (1H, m) | 75.3 | 3.93 (1H, m) |
| 3' | 78.2 | 4.02 (1H, t, $J$ = 9.0 Hz) | 78.5 | 4.05 (1H, m) |
| 4' | 71.4 | 4.20 (1H, m) | 71.6 | 4.26 (1H, m) |
| 5' | 78.1 | 3.94 (1H, m) | 78.0 | 3.97 (1H, m) |
| 6' | 62.5 | 4.33 (1H, m, H-6'β)<br>4.50 (1H, d, $J$ = 12.0 Hz, H-6'α) | 62.8 | 4.41 (1H, dd, $J$ = 5.1, 11.7 Hz, H-6'β)<br>4.56 (1H, dd, $J$ = 2.1, 11.6 Hz, H-6'α) |

**Table S12.** Comparison of NMR data of compound **12** and quercitrin [128]

| Position | Compound 12 ($CD_3OD$) | | Quercitrin ($CD_3OD$) | |
|---|---|---|---|---|
| | $^{13}C$ (150 MHz) $\delta_C$ (ppm) | $^{1}H$ (600 MHz), $\delta_H$ (ppm) | $^{13}C$ (125 MHz) $\delta_C$ (ppm) | $^{1}H$ (500 MHz), $\delta_H$ (ppm) |
| 2 | 159.3 | - | 159.3 | - |
| 3 | 136.2 | - | 136.2 | - |
| 4 | 179.7 | - | 179.6 | - |
| 5 | 163.2 | - | 163.2 | - |
| 6 | 99.8 | 6.23 (1H, d, $J$ = 2.4 Hz) | 99.8 | 6.20 |
| 7 | 165.9 | - | 165.8 | - |
| 8 | 94.7 | 6.40 (1H, d, $J$ = 2.4 Hz) | 94.7 | 6.37 |
| 9 | 158.6 | - | 158.5 | - |
| 10 | 105.9 | - | 105.9 | - |
| 1' | 122.9 | - | 122.9 | - |
| 2' | 117.0 | 7.36 (1H, d, $J$ = 2.4 Hz) | 116.9 | 7.32 |
| 3' | 146.4 | - | 146.4 | - |
| 4' | 149.8 | - | 149.8 | - |
| 5' | 116.4 | 6.94 (1H, d, $J$ = 8.4 Hz) | 116.4 | 6.91 |
| 6' | 123.0 | 7.33 (1H, dd, $J$ = 2.4, 8.4 Hz) | 122.9 | 7.30 |
| 1" | 103.6 | 5.37 (1H, d, $J$ = 1.2 Hz) | 103.5 | 5.35 |
| 2" | 71.9 | 4.23 (1H, dd; $J$ = 1.8, 3.6 Hz) | 71.9 | 4.22 |
| 3" | 72.1 | 3.77 (1H, dd; $J$ = 3.6, 9.6 Hz) | 72.0 | 3.75 |
| 4" | 73.3 | 3.35 (1H, m) | 73.2 | 3.34 |
| 5" | 72.1 | 3.37 (1H, m) | 72.1 | 3.36 |
| 6" | 17.6 | 0.96 (3H, d; $J$ = 6.0 Hz) | 17.6 | 0.94 |

**Table S13.** Comparison of NMR data of compound **13** and Benzyl-O-*β*-D-glucopyranoside [129]

| Position | Compound 13 ($CD_3OD$) | | Benzyl-O-*β*-D-glucopyranoside ($CD_3OD$) | |
|---|---|---|---|---|
| | $^{13}C$ (150 MHz) $\delta_C$ (ppm) | $^{1}H$ (600 MHz), $\delta_H$ (ppm) | $^{13}C$ (100 MHz) $\delta_C$ (ppm) | $^{1}H$ (400 MHz), $\delta_H$ (ppm) |
| 1 | 139.1 | - | 139.0 | - |
| 2 | 129.2 | 7.44 (1H, t, *J* = 9.0 Hz) | 129.1 | 7.41 (1H, brd, *J* = 8.4 Hz) |
| 3 | 129.3 | 7.35 (1H, t, *J* = 9.0 Hz) | 129.2 | 7.32 (1H, dd, *J* = 8.4, 8.4 Hz) |
| 4 | 128.7 | 7.29 (1H, t, *J* = 8.4 Hz) | 128.6 | 7.26 (1H, m) |
| 5 | 129.3 | 7.35 (1H, t, *J* = 9.0 Hz) | 129.2 | 7.32 (1H, dd, *J* = 8.4, 8.4 Hz) |
| 6 | 129.2 | 7.44 (1H, t, *J* = 9.0 Hz) | 129.1 | 7.41 (1H, brd, *J* = 8.4 Hz) |
| 7 | 71.8 | 4.69 (1H, d, *J* = 14.4 Hz, H-7α)<br>4.95 (1H, d, *J* = 14.4 Hz, H-7β) | 71.7 | 4.66 (1H, d, *J* = 11.6 Hz, H-7α)<br>4.92 (1H, d, *J* = 11.6 Hz, H-7β) |
| 1' | 103.3 | 4.38 (1H, d, *J* = 9.6 Hz) | 103.2 | 4.34 (1H, d, *J* = 7.6 Hz) |
| 2' | 75.1 | 3.27 (1H, m) | 75.1 | 3.23 (1H, overlapped) |
| 3' | 78.0 | 3.38 (1H, m) | 78.0 | 3.35 (1H, overlapped) |
| 4' | 71.7 | 3.30 (1H, m) | 71.6 | 3.27 (1H, overlapped) |
| 5' | 78.1 | 3.34 (1H, m) | 78.0 | 3.31 (1H, overlapped) |
| 6' | 62.8 | 3.91 (1H, dd, *J* = 6.0, 13.2 Hz, H-6'β)<br>3.71 (1H, dd, *J* = 6.0, 13.2 Hz, H-6'α) | 62.8 | 3.88 (1H, dd, *J* = 12.0, 2.0 Hz, H-6'β)<br>3.66 (1H, dd, *J* = 12.0, 5.6 Hz, H-6'α) |

**Table S14.** Comparison of NMR data of compound **14** and isoschaftoside [130]

| Position | Compound 14 ($CD_3OD$) | | Isoschaftoside ($CD_3OD$) | |
|---|---|---|---|---|
| | $^{13}$C (150 MHz) $\delta_C$ (ppm) | $^{1}$H (600 MHz), $\delta_H$ (ppm) | $^{13}$C (125 MHz) $\delta_C$ (ppm) | $^{1}$H (500 MHz), $\delta_H$ (ppm) |
| 2 | 161.2 | - | 161.20 | - |
| 3 | 103.7 | 6.80 (1H, s) | 103.71 | 6.82 (1H, s) |
| 4 | 182.2 | - | 182.31 | - |
| 5 | 158.2 | - | 158.17 | - |
| 6 | 108.0 | - | 108.08 | - |
| 7 | 164.1 | - | 164.10 | - |
| 8 | 105.1 | - | 105.10 | - |
| 9 | 155.0 | - | 155.06 | - |
| 10 | 102.6 | - | 102.59 | - |
| 1' | 121.5 | - | 121.52 | - |
| 2' | 129.0 | 8.03 (1H, d, $J$ = 9.0 Hz) | 129.04 | 8.03 (1H, d, $J$ = 8.8 Hz) |
| 3' | 115.8 | 6.90 (1H, d, $J$ = 9.0 Hz) | 115.80 | 6.89 (1H, d, $J$ = 8.8 Hz) |
| 4' | 160.8 | - | 160.89 | - |
| 5' | 115.8 | 6.90 (1H, d, $J$ = 9.0 Hz) | 115.80 | 6.89 (1H, d, $J$ = 8.8 Hz) |
| 6' | 129.0 | 8.03 (1H, d, $J$ = 9.0 Hz) | 129.04 | 8.03 (1H, d, $J$ = 8.8 Hz) |
| 1" | 74.2 | 4.72 (1H, d, $J$ = 9.6 Hz) | 74.14 | 4.71 (1H, d, $J$ = 9.5 Hz) |
| 2" | 69.6 | 3.83 (1H, m) | 69.58 | 3.83 (1H, m) |
| 3" | 73.8 | 3.46 (1H, m) | 73.79 | 3.46 (1H, m) |
| 4" | 68.4 | 3.80 (1H, m) | 68.39 | 3.80 (1H, m) |
| 5" | 70.0 | 3.82 (1H, m, H-5"α)<br>3.64 (1H, m, H-5"β) | 70.09 | 3.82 (1H, m, H-5"α)<br>3.64 (1H, m, H-5"β) |
| 1"' | 73.2 | 4.76 (1H, d, $J$ = 10.2 Hz) | 73.24 | 4.75 (1H, d, $J$ = 10.4 Hz) |
| 2"' | 70.9 | 3.90 (1H, m) | 70.86 | 3.88 (1H, m) |
| 3"' | 78.8 | 3.27 (1H, m) | 78.85 | 3.28 (1H, m) |
| 4"' | 70.5 | 3.39 (1H, m) | 70.51 | 3.39 (1H, m) |
| 5"' | 81.9 | 3.23 (1H, m) | 81.90 | 3.23 (1H, m) |
| 6"' | 61.2 | 3.74 (1H, m, H-6"'α)<br>3.51 (1H, m, H-6"'β) | 61.17 | 3.74 (1H, m, H-6"'α)<br>3.51 (1H, m, H-6"'β) |

# PUBLICATIONS

# List of publications

➢ **02 publications in international journals:**

1. Duc Loi Vu, Thi Van Anh Nguyen, Tien Dat Nguyen, Viet Hau Dang, Hong Duong Le, **Xuan Tung Nguyen*** (2023), "Anti-diabetic effect of major compounds from *Commelina diffusa*", *Revista Brasileira de Farmacognosia*, **33**(3), 657-661. (Q2, SCIE, IF = 2.464).

2. **Xuan Tung Nguyen**, Duc Loi Vu*, Thi Van Anh Nguyen, Thi Minh Thu Nguyen, Hong Duong Le (2024), "Acute toxicity and antidiabetic effect of the ethyl acetate fraction of *Commelina diffusa* Burm.f. on the high-fat diet and streptozotocin-induced type 2 diabetic mice", *Pakistan Journal of Pharmaceutical Sciences*, 37(4), 855-861. (Q3, SCIE, IF = 0.7).

➢ **02 publications in national scientific journals (Vietnam):**

3. **Nguyen Xuan Tung**, Vu Duc Loi*, Le Hong Duong, Nguyen Thi Van Anh (2023), "Extraction and isolation of several compounds from *Commelina diffusa* Burm.f.", *Vietnam Journal of Traditional Medicine and Pharmacy*, **2**(49), 21-28.

4. **Nguyen Xuan Tung**, Vu Duc Loi*, Nguyen Thi Van Anh, Le Hong Duong, Nguyen Thi Thao Vy, Nguyen Thi Quynh Trang (2024), "Evaluation of the inhibitory effect on α-glucosidase and α-amylase enzymes of triterpenes isolated from *Commelina diffusa* Burm.f.", *VNU Journal of Science: Medical and Pharmaceutical Sciences*, **40**(2), 74-82.

➢ **01 patent:**

5. Vu Duc Loi*, **Nguyen Xuan Tung**, Le Hong Duong. Patent: "Process for extracting a mixture of active ingredients with the effect of lowering blood glucose from *Commelina diffusa* Burm.f.", No. 3900, granted by the Intellectual Property Office of Vietnam under Decision No. 142452/QĐ-SHTT dated 05/12/2024.

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SHORT COMMUNICATION

# Anti-diabetic Effect of Major Compounds from *Commelina diffusa*

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## Abstract

Although *Commelina diffusa* Burm.f., Commelinaceae, has been proven to exhibit many pharmacological activities, the scientific evidence for its antihyperglycemic activities and active substances is still limited. This present study aims to evaluate the *in vitro* antidiabetic ability of *C. diffusa* using α-glucosidase and α-amylase inhibitory assays and isolate its pure compounds with enzymatic inhibitory effects. The ethyl acetate fraction, with the most potent hypoglycemic activity, was chosen to separate and purify six known compounds: 4-hydroxybenzoic acid, methyl gallate, lyratol F, *N-trans*-feruloyltyramine, *N-trans-p*-coumaroyl-3′,4′-dihydroxyphenylethylamine, and 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**6**). Furthermore, the antihyperglycemic effect of the isolated compounds was also evaluated *in vitro* on α-glucosidase and α-amylase enzymes. Compound **6** exhibited the most potent inhibitory activity against both tested enzymes with values of 61.37 ± 0.83 µM in the α-glucosidase assay and 38.23 ± 1.04 µM in the α-amylase assay. These values were much higher than those of positive control (acarbose $IC_{50}$ 210.43 ± 2.78 and 129.19 ± 3.13 µM, respectively). This is the first report on the antidiabetic capacity of *C. diffusa* and its hypoglycemic pure compounds. Our findings suggested that *C. diffusa* might be a promising source of antidiabetic agents.

**Keywords** Chemical constituents · Spectral analysis · Phenolics · Hypoglycemia · α-Glucosidase inhibition · α-Amylase inhibition

## Introduction

Diabetes mellitus is one of the most common non-communicable diseases globally. It causes many dangerous complications and is the leading cause of cardiovascular disease, blindness, kidney failure, and lower-limb amputation (Mandal et al. 2021). Type 2 diabetes, which was previously known as non-insulin-dependent diabetes or adult-onset diabetes, takes responsibility for over 90% of all diabetes cases (Patel et al. 2012). Diabetes mellitus cannot be completely cured. The main goal of hyperglycemia management is maintaining the glycemic levels as close to the non-diabetic range as possible, which can be achieved by proper diet, exercise, and pharmacological approaches. In general, medication therapies are mainly used to lower blood glucose or provide temporary insulin replacement for diabetic patients. However, these existing agents are expensive and expose side effects such as jaundice, diarrhea, headache, liver toxicity, or itchy skin (Nathan et al. 2006; Scheen 2007; Chiabchalard and Nooron 2015). Thus, it is very essential to research and develops antihyperglycemic drugs derived from natural sources, especially medicinal plants that have been widely used in tradition.

*Commelina diffusa* Burm.f., Commelinaceae, is widely distributed in both tropical and subtropical regions of Africa, America, Southeast Asia, and the Pacific Islands (Boyette et al. 2015). In Vietnam, *C. diffusa* can be commonly found in airy and moist areas including fields, gardens, roadsides, ditches, wastelands, and riverbanks. It is proven that the chemical constituents of *C. diffusa* contain

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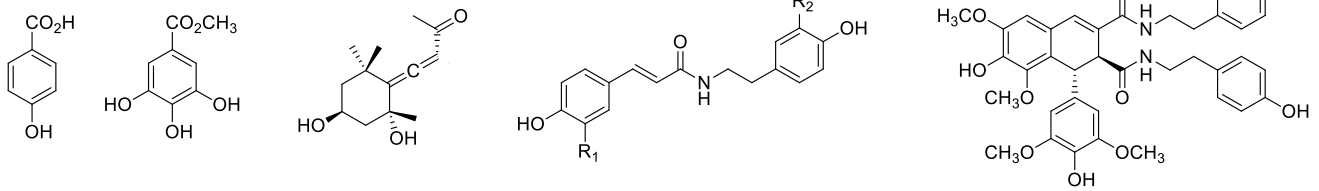
phenolic compounds, flavonoids, alkaloids, tannins, steroids, and vitamins (Khan et al. 2011; Sultana et al. 2018; Kamble 2019). In traditional Vietnamese medicine, *C. diffusa* has long been used for the treatment of respiratory diseases, acute appendicitis, high blood pressure, dysentery, gonorrhea, kidney stones, and genitourinary-urinary tract infections (Ho 2003). In addition, according to modern pharmacological studies, *C. diffusa* exposes several valuable activities such as anti-inflammatory, antioxidant, antibacterial, antifungal, nephroprotective, hepatoprotective, diuretic, and central nervous system depressant (Islam et al. 2021). However, the scientific evidence for the hypoglycemic activities of *C. diffusa* is still very limited. Therefore, this study was designed to determine the *in vitro* antihyperglycemic activity of *C. diffusa* and isolate its pure compounds from the most active fraction using α-glucosidase and α-amylase inhibitory assays. To our best knowledge, this is the first report on the antidiabetic potential of *C. diffusa* and its hypoglycemic pure compounds.

## Materials and Methods

*Commelina diffusa* Burm.f., Commelinaceae, was collected in October 2021 in Nam Dinh province, Vietnam, and identified by Nghiem Duc Trong, Hanoi University of Pharmacy. A voucher specimen (accession no. HNIP/18651/22) is deposited at the Department of Medicinal Materials — Traditional Medicine, University of Medicine and Pharmacy, Vietnam National University. The collected plants were washed, air-dried for 7 days, and ground into a coarse powder (5 kg), which was extracted with MeOH by maceration at room temperature within 24 h and repeated the extraction three times. The combined solutions were filtered and concentrated under reduced pressure at 50 °C. The total MeOH extract (520 g) was consecutively fractionated with hexane, EtOAc, and distilled $H_2O$. The hexane (142.1 g), EtOAc (23.4 g), and aqueous extract (285.5 g) were obtained, evaporated the solvents, and stored at 4–6 °C for further use.

The EtOAc-soluble residue (23.4 g), which exhibited the highest enzymatic inhibitory effects, was subjected to silica gel column chromatography (CC), eluting with a gradient solvent system ($CH_2Cl_2$–MeOH, 1:0 to 0:1) to obtain ten fractions (E1–E10). Chromatography of fraction E4 (1.36 g) over silica gel and elution with a gradient system of hexane–EtOAc (2:1 to 1:4) obtained four subfractions (E4.1–E4.4). Subfraction E4.3 (383.1 mg) was further separated by silica gel CC with hexane–EtOAc (1:1 to 1:2) to provide eight subfractions (E4.3.1–E4.3.8). Subfractions E4.3.2 (18.6 mg), E4.3.3 (24.5 mg), and E4.3.5 (14.8 mg) were purified by Sephadex LH-20 CC with MeOH to afford compounds **1** (3.4 mg), **2** (7.3 mg), and **3** (2.1 mg), respectively. Purification of subfraction E4.3.7 (14.2 mg) over Sephadex LH-20 CC with MeOH-$H_2O$ (9:1) afforded compound **4** (2.4 mg). Fraction E5 (0.94 g) was eluted by silica gel CC with a mixture of $CH_2Cl_2$-MeOH (9:1) to give three subfractions (E5.1–E5.3). Subfraction E5.1 (226.9 mg) was subjected to silica gel CC, eluting with hexane–EtOAc (1:4), and further purified by Sephadex LH-20 CC with MeOH to afford compounds **5** (5 mg) and **6** (7.2 mg).




**1** **2** **3** **4** $R_1$=$OCH_3$; $R_2$=H; **5** $R_1$=H; $R_2$=OH **6**

The antidiabetic capacity of extracts and pure compounds from *C. diffusa* were examined by evaluating their inhibitory effects on α-glucosidase and α-amylase enzymes. The enzymatic inhibition assays were performed in triplicate according to the previously described methods with some modifications (Moradi-Afrapoli et al. 2012; Saltos et al. 2015). The detailed protocols of these experiments are included (Supporting Information, S1). In both tests, acarbose was used as the positive control. The percentage of inhibition of samples was calculated as $\%_{\text{Inhibition}} = \left(1 - \frac{\text{Abs sample}}{\text{Abs control}}\right) \times 100\%$. Then, curves were constructed by plotting the percentage of inhibition against concentration in micrograms per milliliter. The equation of this curve allowed calculating the $IC_{50}$ corresponding to the sample concentrations that inhibited 50% of enzyme activity. The experimental data were expressed as mean ± standard deviation (SD). The SPSS software using

one-way analysis of variance (ANOVA) and independent sample *t*-test was used to analyze the significant differences between results from experimental groups.

## Results and Discussion

*Commelina diffusa* extracts were screened for their antidiabetic capacity by evaluation of the inhibitory effects on α-glucosidase and α-amylase enzymes. The α-amylase enzyme, which is the main secretory product of the salivary and pancreas, activates the initial step of starch and glycogen digestion by hydrolyzing the polysaccharides into oligosaccharides (Lordan et al. 2013). After that, the α-glucosidase enzyme, which is localized in the epithelium of the small intestine, is responsible for the hydrolysis of α-D-(1,4) glycosidic linkages in carbohydrates to generate monosaccharide glucose for intestinal absorption (Bhatia et al. 2019). Hence, inhibiting the activity of these two digestive enzymes can delay the digestion of carbohydrates, and suppress the rate of glucose uptake, thereby reducing blood sugar levels (Alqahtani et al. 2019).

Previous studies have revealed the antidiabetic activities of *Commelina communis* L. and *C. benghalensis* L. (Youn et al. 2004; Shibano et al. 2008; Gurjar et al. 2016). This study indicated that *C. diffusa* also is capable of inducing hypoglycemia. Based on the results included in Table 1, in both assays, the potential antihyperglycemic effects of *C. diffusa* extracts were in the same ascending order as follows: hexane < $H_2O$ < MeOH < EtOAc. In general, the MeOH extracted displayed enzymatic inhibitory activities with $IC_{50}$ values of 36.23 ± 0.46 µg/ml against α-glucosidase and 29.20 ± 1.95 µg/ml against α-amylase, which were significantly higher than those of positive control, acarbose, with $IC_{50}$ values of 135.73 ± 1.79 µg/ml for the former assay and 83.33 ± 2.02 µg/ml for the latter assay, respectively. Among fractions, the EtOAc was the most active fraction with the lowest $IC_{50}$ values of 21.88 ± 2.47 µg/ml in the α-glucosidase method and 25.46 ± 1.32 µg/ml in the α-amylase method, accordingly.

**Table 1** $IC_{50}$ values of enzymatic inhibition assays of *Commelina diffusa* extracts and isolated compounds

| Samples | | $IC_{50}$ (µg/ml) | |
|---|---|---|---|
| | | α-Glucosidase assay | α-Amylase assay |
| Extracts | MeOH | 36.23 ± 0.46 | 29.20 ± 1.95 |
| | Hexane | 152.19 ± 1.39 | 138.25 ± 1.15 |
| | EtOAc | 21.88 ± 2.47 | 25.46 ± 1.32 |
| | $H_2O$ | 93.59 ± 1.13 | 52.81 ± 2.66 |
| Compounds | **1** | > 500 | > 500 |
| | **2** | 121.16 ± 1.28 | 165.42 ± 2.31 |
| | **3** | 119.22 ± 1.09 | 86.10 ± 1.24 |
| | **4** | 32.71 ± 0.35 | > 500 |
| | **5** | 19.63 ± 0.94 | 38.24 ± 0.62 |
| | **6** | 41.98 ± 0.57 | 26.15 ± 0.71 |
| | Acarbose | 135.73 ± 1.79 | 83.33 ± 2.02 |

Data are represented as mean ± SD. **1**, 4-hydroxybenzoic acid; **2**, methyl gallate; **3**, lyratol F; **4**, *N-trans*-feruloyltyramine; **5**, *N-trans-p*-coumaroyl-3′,4′-dihydroxyphenylethylamine; **6**, 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide

The repeated column chromatography of the EtOAc fraction produced six pure compounds **1**–**6**. Their structures were elucidated based on the physicochemical properties, spectroscopic data (Supporting Information, S2 and Fig. S1-S27), and comparison with the published literature. This is the first isolation report of compounds **3**–**6** from the *Commelina* genus in general and the *C. diffusa* species in particular. Similarly, compound **2** was also separated and purified for the first time from this plant species. In contrast, Chumroenphat and Saensouk (2021) discovered, by the use of HPLC, the presence of compound **1** in *C. diffusa* with a content of 46.65 ± 0.53 µg/g.

Among six isolated compounds, compound **6** possessed the highest inhibitory capacity for both enzymatic assays (Table 1). In particular, the $IC_{50}$ values against α-glucosidase and α-amylase of **6** were 61.37 ± 0.83 µM and 38.23 ± 1.04 µM, respectively. These values were about three times higher than those of standard compound acarbose ($IC_{50}$ 210.43 ± 2.78 µM and 129.19 ± 3.13 µM, accordingly). Similarly, compound **5** also performed better inhibitory activity in both tested enzymes when compared to acarbose. Currently, this is the first study of the antihyperglycemic effect of both compounds **5** and **6**. On the other hand, compound **1** exhibited no inhibitory effect on both tested enzymes. However, it is proven that compound **1** was capable of dose-dependent reduction of the serum glucose level in streptozotocin-treated diabetic mice by increasing peripheral glucose consumption (Peungvicha et al. 1998). Though compound **4** strongly suppressed the α-glucosidase ($IC_{50}$ of 104.51 ± 1.12 µM), it was an inefficient inhibitor of α-amylase. Also, this study indicated that compounds **2** and **3** showed moderate inhibitory effects against both tested enzymes. Besides, pharmacological investigations have revealed the ability of anticancer, anti-tumor, antioxidant, antimicrobial, anti-melanogenesis, and anti-inflammatory activities of these compounds (Yue et al. 2012; Jiang et al. 2015; Anzoise et al. 2018).

In summary, this study demonstrated, for the first time, the antidiabetic effects of *C. diffusa* and its pure compounds by measuring the ability of α-glucosidase and α-amylase enzymatic inhibition. The chromatographic methods revealed that the EtOAc soluble fraction, with the greatest hypoglycemia potential, contained six compounds

including 4-hydroxybenzoic acid (**1**), methyl gallate (**2**), lyratol F (**3**), *N-trans*-feruloyltyramine (**4**), *N-trans-p*-coumaroyl-3′,4′-dihydroxyphenylethylamine (**5**), and 1,2-dihydro-6,8-dimethoxy-7-hydroxy-1-(3,5-dimethoxy-4-hydroxyphenyl)-$N^1$,$N^2$-bis-[2-(4-hydroxyphenyl)ethyl]-2,3-naphthalene dicarboxamide (**6**). Among the isolated compounds, compound **6** exposed the highest inhibitory potential against both α-glucosidase and α-amylase enzymes. In addition, compounds **4** and **5** also had good antihyperglycemic activity when compared to the reference drug (acarbose). Polyphenols (active redox agents) are widely spread in nature and fully recognized as antioxidants or scavengers. The pharmacological potential of the analyzed plant material is probably due to the capability of polyphenols to interact with important cellular processes in which key enzymes such as α-glucosidase and α-amylase enzymes, as well as lipoxygenases, phospholipase $A_2$, NADH-oxidase, or glutathione reductase, are involved. Therefore, *C. diffusa* is a potential source of active substances for diabetes treatment and prevention.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s43450-023-00394-7.

**Author Contribution** DLV, TVAN, and TDN conceived and designed the experiments. XTN, VHD, and HDL carried out the plant collection, extraction, and pure compound isolation. TDN and VHD elucidated the compound structures. XTN and TVAN conducted biological activity studies and analyzed the data. XTN prepared the manuscript in consultation with all authors. DLV supervised the project. All authors have read and approved the final submitted version.

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**Data Availability** The data from this study are available from the corresponding author upon reasonable request.

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# Acute toxicity and antidiabetic effect of the ethyl acetate fraction of *Commelina diffusa* Burm.f. on the high-fat diet and streptozotocin-induced type 2 diabetic mice

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**Abstract**: Pharmacological studies proved that *Commelina diffusa* Burm.f. performs various biological activities. Nevertheless, the scientific evidence supporting the hypoglycemic activities of this medicinal plant is insufficient. Thus, this study aims to assess the acute toxicity and the antidiabetic activity of the ethyl acetate fraction of *Commelina diffusa* (CD.EAF) in type 2 diabetic mice model induced by a high-fat diet and streptozotocin injection. The oral acute toxicity assessment was conducted following Lorke's method. The *in vivo* study was conducted by feeding Swiss male mice with a high-fat diet for 8 weeks and giving them a single intraperitoneal injection of streptozotocin at 100mg/kg. When the experimental mice model was successfully induced, the CD.EAF at 100mg/kg/day and 300mg/kg/day doses were orally administered to animals for 14 days. After the treatment period, the repeated daily administration of the CD.EAF at both tested doses exposed significant antihyperglycemic activities in comparison with the untreated diabetic group ($p<0.05$). However, it did not affect the serum lipid levels of mice. Besides, there were significant ameliorations in the histopathological images of the liver and pancreas in mice treated with the CD.EAF. Our findings suggested that the CD.EAF might be a potential agent for drug development to prevent and treat type 2 diabetes.

**Keywords**: *Commelina diffusa*, acute toxicity, antidiabetic, high-fat diet, streptozotocin.

## INTRODUCTION

Type 2 diabetes mellitus is responsible for over 90% of all diabetic patients, becoming a serious public health problem (Alam *et al.*, 2021). The pathophysiology of this disease is primarily described by insulin resistance and a progressive decline in insulin-secreting β-cell function of the pancreas. Due to insulin resistance, in the early stages, β-cells compensate and increase insulin secretion in the blood. If insulin resistance persists or worsens, β-cells will not secrete enough insulin and clinical type 2 diabetes will appear (Eizirik *et al.*, 2020). Currently, there are different groups of oral antihyperglycemic medications, including biguanides, insulin secretagogues, thiazolidinediones, dipeptidyl peptidase-4, sodium-glucose co-transporter-2 and α-glucosidase inhibitors (Blahova *et al.*, 2021). However, severe adverse effects, low efficacy, deficiency of target specificity, poor solubility and low permeability are the main drawbacks of these conventional agents (Padhi *et al.*, 2020). Therefore, developing hypoglycemic drugs derived from medicinal plants with fewer side effects is a top priority.

*Commelina*, which is by far the largest genus of the *Commelinaceae* family, contains about 205-215 species with a worldwide distribution (Pellegrini and Forzza, 2017). It is proven that some species in this genus including *Commelina communis* and *Commelina benghalensis* demonstrate insulin-mimetic and antihyperglycemic activities (Shibano *et al.*, 2008; Dagne *et al.*, 2024). These results suggest that *Commelina diffusa* (*C. diffusa*), which is one of the plant species in the *Commelina* genus, may also have a hypoglycemic property. However, the scientific research to support the antidiabetic effect of this plant is scarce. According to our preliminary investigations, among other solvent fractions, ethyl acetate fraction of *C. diffusa* (CD.EAF) showed the most potent α-glucosidase and α-amylase inhibition activities with $IC_{50}$ values of 21.88±2.47µg/mL and 25.46±1.32µg/mL, respectively when compared to standard agent ($IC_{50}$ values of 135.73±1.79µg/mL and 83.33±2.02µg/mL, accordingly). α-glucosidase and α-amylase involve the hydrolysis process of converting carbohydrates into smaller glucose molecules; as a result, inhibiting these two enzymes will slow down glucose formation and reduce glucose absorption into the blood (Vu *et al.*, 2023). On that basis, the present study aimed to assess the antihyperglycemic activity of the CD.EAF against mice with type 2 diabetes induced by a high-fat diet and streptozotocin.

## MATERIALS AND METHODS

### *Plant preparation*

The whole plant of *C. diffusa* was obtained from Nam Dinh province, the Northeast of Vietnam and was

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authenticated by Hanoi University of Pharmacy, Hanoi, Vietnam. The voucher specimen is kept at the University of Medicine and Pharmacy, Vietnam National University. The raw plants were air-dried and minced to afford a rough powder. The dry powder of plant material (5kg) was macerated three times in 15L of methanol at room temperature within 24 hours and then filtered. The solvent was evaporated under a vacuum. The total methanol extract (520g) was consecutively fractionated with *n*-hexane, ethyl acetate, and water. The ethyl acetate extract was concentrated using a rotary evaporator to obtain 23,4g residue.

***Experimental animals***

Healthy Swiss male mice with an average weight of 25± 2g were obtained from the National Institute of Hygiene and Epidemiology of Vietnam. All mice were kept individually in polycarbonate cages under a light-controlled environment (12h light/dark cycles) at room temperature and were offered pelleted food and water *ad libitum*. The animals were sustained for a quarantine period of seven days to acclimate to the laboratory conditions before the experiment. All experimental procedures were approved by the Ethics Committee of the University of Medicine and Pharmacy, Vietnam National University with a permission number of UMP/062022, and complied with the international regulations for animal research.

***Acute toxicity assay***

The oral acute toxicity of the CD.EAF was examined according to the method described by Lorke (1983). Briefly, the mice were randomly divided into five groups, with 10 mice in each group and fasted overnight. Each group was orally administered with testing samples at ascending doses of 125, 250, 500, 1000 and 2000mg/kg. The general condition, behavior signs of harmfulness, and mortality in each batch were strictly monitored within 72 hours to determine the median lethal dose ($LD_{50}$) values. Animals that survived for 72 hours were further observed for seven days for any signs of delayed toxicity.

***In vivo experimental procedure***

The *in vivo* study was conducted on an experimental mice model with diabetes produced by a high-fat diet and streptozotocin and divided into two stages with some modifications to the previously reported methods (Rivera-Ramírez *et al.*, 2011; Yang *et al.*, 2022). In the first stage, after the acclimation period, mice were randomly assigned into five groups (I, II, III, IV and V) of 10 mice each. Mice from group I were fed a normal-fat diet (NFD) (28.05% protein, 12.14% fat and 59.81% carbohydrate), while mice from remain groups had a high-fat diet (HFD) (18.23% protein, 42.89% fat, and 38.88% carbohydrate) for 8 weeks. Fructose (Daesang Corporation, Korea) solution (55%) was added to the food of mice on the high-fat diet. Hyperglycemic mice were obtained by injecting a single intraperitoneal (ip) of 100mg/kg of streptozotocin (STZ), that was dissolved in 0.1M sodium citrate buffer (pH 4.5). At the same time, mice from group I were administered citrate buffer solution as vehicle control. Blood samples to evaluate glycemia were taken by cutting 1mm from the tip of the tails. Blood glucose value was determined by a commercial glucometer (On-Call EZ II, ACON Biotech, USA) and expressed as mmol/L. Three days after STZ injection, the mice exhibiting glycemia levels equal to or greater than 10mmol/L were included in the study as stable diabetic animals. In the second stage, the experimental mice groups were administered orally once daily for 14 days with the following treatment schedule:

Group I (normal control): NFD + distilled water
Group II (diabetic control): HFD + STZ (100mg/kg, ip) + distilled water
Group III (positive control): HFD + STZ (100mg/kg, ip) + gliclazide (80mg/kg/day)
Group IV (treated group): HFD + STZ (100mg/kg, ip) + CD.EAF (100mg/kg/day)
Group V (treated group): HFD + STZ (100mg/kg, ip) + CD.EAF (300mg/kg/day)

To determine the antihyperglycemic activity of the CD.EAF, after overnight fasting, the fasting blood glucose level was evaluated just before treatment (day 0) and then at the 7 and 14 days post-treatment.

To evaluate the influence of the CD.EAF on the lipid profile of STZ-induced diabetic mice, on completion of the 14 days of treatment, the overnight fasted animals were anesthetized with halothane and sacrificed. The blood samples were collected by cardiac puncture into sterilized heparin tubes. The serum was separated from the blood by centrifuging at 3000rpm for 15 minutes. The high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and triglyceride (TG) were examined using the respective commercial diagnostic kit (Erba, Germany). Besides, the livers and pancreas of mice in each group were taken for histopathology evaluations.

## STATISTICAL ANALYSIS

Research data were expressed as X ± SD (X: Mean values, SD: Standard deviation). The SPSS 23.0 software used the independent sample t-test and ANOVA (one-way analysis of variance) tests to analyze the differences between tested groups. $p<0.05$ were considered as statistically significant.

## RESULTS

***Acute toxicity of CD.EAF***

As seen from table 1, there was no mortality in all mice groups that were treated with the CD.EAF at doses from

125mg/kg to 2000mg/kg. When using a maximum dose of 2000mg/kg, mice were still healthy, ate, excreted, and moved naturally. After the testing period, CD.EAF did not generate any visible toxicity in mice. Therefore, the $LD_{50}$ index of CD.EAF was thus above 2000mg/kg.

***Effects of HFD on body weight of mice***

After eight weeks duration, the body weight of mice in the group fed with NFD increased remarkably ($p<0.05$), while a dramatic rise was seen in that of mice groups fed with HFD ($p<0.001$) compared to those before studying (table 2). Furthermore, at all evaluation time points, the body weight of mice groups treated with HFD was significantly higher than that of the group treated with NFD ($p<0.001$).

***Effects of HFD on serum glucose level***

After 72 hours of STZ injection, the serum glucose concentrations of HFD-treated mice groups exhibited a dramatic development ($p<0.001$) when compared to the time points "Before studying" and "After 8 weeks" (table 3). Meanwhile, there was no significant change in the serum glucose values of the NFD-treated mice group at all time points ($p>0.05$). Furthermore, the concentrations of serum glucose in HFD-treated mice increased significantly as compared to those of NFD-treated mice after 8 weeks of treatment ($p<0.01$) and after 72 hours of injection ($p<0.001$), respectively.

***Effect of CD.EAF on blood glucose levels***

Based on the results presented in table 4, diabetic mice had significant increases in blood glucose levels when compared with normoglycemic mice ($p<0.001$). After the treatment period, the repeated daily administration of the CD.EAF at both 100mg/kg/day and 300mg/kg/day doses exposed significant antihyperglycemic activities in comparison with the untreated diabetic group (group II) ($p<0.05$). Similarly, the blood glucose concentrations of the mice group treated with the standard drug (gliclazide 80mg/kg/day) were reduced markedly compared with those at the beginning of the testing period ($p<0.05$). Besides, there was no significant difference in blood glucose concentrations between mice groups treated with the CD.EAF (groups IV and V) and gliclazide (group III).

***Effect of CD.EAF on blood lipid levels***

As shown in table 5, the diabetic animals had remarkably increased concentrations of serum lipid indexes compared to the normal animals ($p<0.05$). No significant difference was observed in the lipid profile between the untreated diabetic mice (group II) and CD.EAF-treated mice (groups IV and V) ($p>0.05$). Therefore, the CD.EAF in both 100mg/kg/day and 300mg/kg/day doses had no effect on the blood lipid levels of STZ-treated mice. In the group treated with gliclazide (group III), there were downward trends in all lipid parameters when compared with group II; however, no significant difference was observed ($p>0.05$).

***Histopathological of pancreas and liver***

As given in fig. 1, the livers of mice in group II (diabetic control group) showed severe hepatic fatty degeneration with necrosis zones and several inflammatory cells in central venules. While the administration of CD.EAF at 100mg/kg/day and 300mg/kg/day doses (groups IV and V) improved significantly histopathological images of livers as compared with the diabetic control group, no clear effect was seen in the glicazide-treated group (group III). Regarding histopathological examination of the pancreas, the islets of Langerhans of the mice groups were treated with the CD.EAF and gliclazide demonstrated a remarkable recovery compared to those of the diabetic control group (fig. 2).

## DISCUSSION

Studies of acute toxicity of medicinal plants aim to find and establish any adverse effects that may occur, their significance, and their dose-dependence responses, including mortality. $LD_{50}$ (the dose that causes death to 50% of the tested animal population) is a major parameter in determining the toxic characteristics of pharmacological agents (Emmanuel *et al.*, 2023). Our present study revealed that the administration of CD.EAF at the highest tested dose (2000mg/kg) using Lorke's method failed to generate any sign of visible toxicity or mortality in mice.

Hence, the $LD_{50}$ value of the CD.EAF was higher than 2000mg/kg, which is supposed to be relatively safe. This finding was consistent with that of previous acute toxicity assessment of the methanolic extract of *C. diffusa*. In particular, although reducing physical activities significantly from 0.5h up to 4h, the mice treated with a 2000mg/kg extract dose were subsequently normal, and the $LD_{50}$ value was thus higher than 2000mg/kg (Sultana *et al.*, 2018).

STZ is an antineoplastic agent that is widely used to produce not only insulin-dependent diabetes mellitus (type 1) but also non-insulin-dependent diabetes mellitus (type 2) in experimental animal models (Ghasemi and Jeddi, 2023). This might be due to the two pathways of diabetes development, which depend on the dosage used of STZ. Generally, STZ penetrates the pancreatic β cells through the binding to the GLUT2 glucose transporter receptor caused by the similarity in its chemical structure with glucose. The privileged accumulation of the chemical in β cells involves the high selective cytotoxicity of STZ to β cells (Kottaisamy *et al.*, 2021). At high doses, STZ damages β cells by alkylating DNA and depleting the nicotinamide adenine dinucleotide level.

**Table 1**: Acute toxicity of CD.EAF

| Doses (mg/kg) | Mortality | Toxicity observed |
|---|---|---|
| 125 | 0/10 | None |
| 250 | 0/10 | None |
| 500 | 0/10 | None |
| 1000 | 0/10 | None |
| 2000 | 0/10 | None |

**Table 2**: Effects of HFD on body weight of mice

| Time points | Body weight (g) | |
|---|---|---|
| | Group I: NFD | Groups II, III, IV, V: HFD |
| Before studying | 25.86 ± 2.64 | 25.34 ± 2.20 |
| After 4 weeks | 28.65 ± 2.12$^{*}$ | 41.02 ± 6.98$^{***###}$ |
| After 6 weeks | 29.78 ± 3.26$^{*}$ | 43.86 ± 9.21$^{***###}$ |
| After 8 weeks | 34.03 ± 3.31$^{*}$ | 49.01 ± 8.84$^{***###}$ |

$^{***}p<0.05$ and $p<0.001$ compared with the time point "Before studying", $^{###}p<0.001$ compared with group I.

**Table 3**: Effects of HFD on blood glucose levels

| Time points | Blood glucose levels (mmol/L) | | *p* (compared with group I) |
|---|---|---|---|
| | Group I: NFD | Groups II, III, IV, V: HFD | |
| Before studying | 5.61 ± 0.69 | 5.79 ± 0.82 | >0.05 |
| After 8 weeks | 5.23 ± 0.54 | 6.41 ± 1.29$^{*}$ | <0.01 |
| 72 hours after injection | 5.83 ± 0.74 | 17.68 ± 4.51$^{***###}$ | <0.001 |

$^{***}p<0.05$ and $p<0.001$ compared with the time point "Before studying", $^{###}p<0.001$ compared with the time point "After 8 weeks".

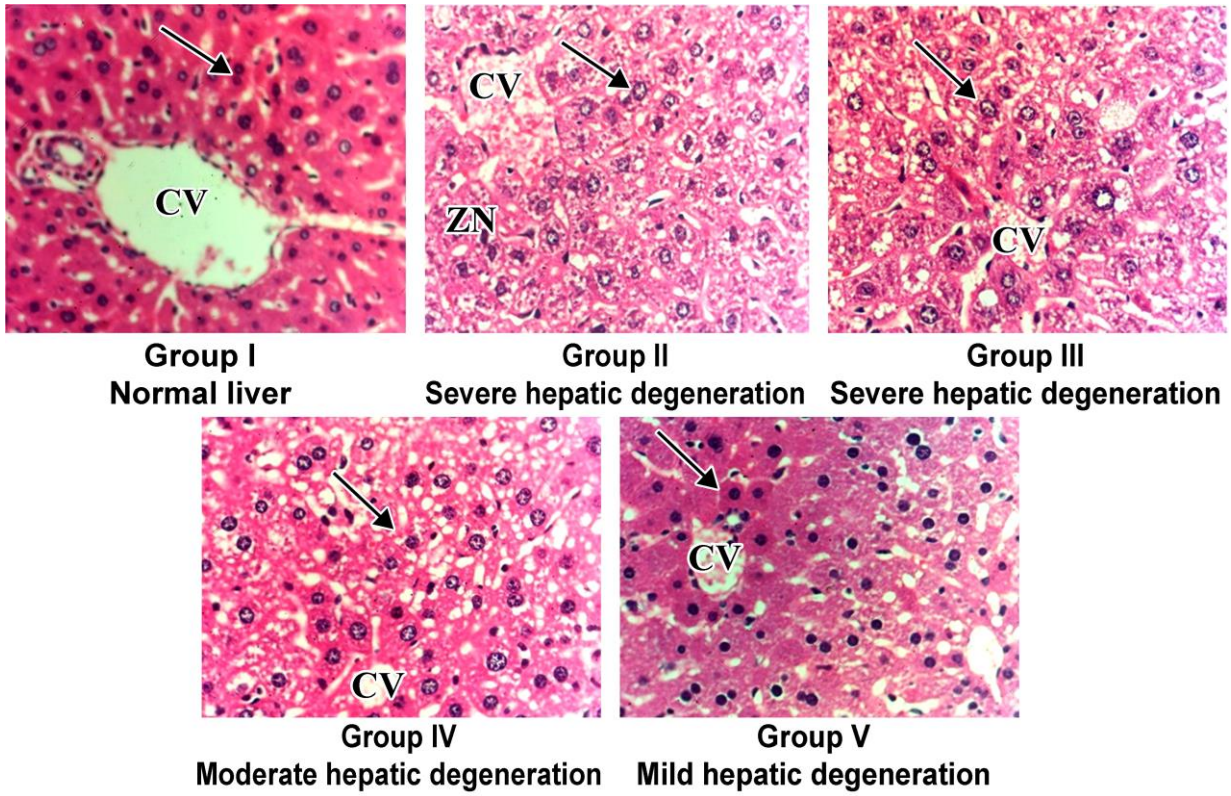


**Fig. 1**: Effects of the CD.EAF on the liver histology of the treated mice (HE x 400). The black arrows indicate hepatocytes. ZN: Zonal necrosis, CV: Central venule.

**Table 4**: Effect of CD.EAF on serum glucose concentrations

| Groups | Serum glucose concentrations (mmol/L) | | |
|---|---|---|---|
| | Day 0 | Day 7 | Day 14 |
| Group I | 5.31 ± 0.71 | 5.38 ± 0.56 | 4.94 ± 0.33 |
| Group II | 16.01 ± 4.96*** | 15.74 ± 5.01*** | 15.81 ± 4.21*** |
| Group III | 17.32 ± 2.11*** | 13.50 ± 4.12*** | 12.68 ± 3.20***# |
| Group IV | 16.78 ± 3.15*** | 15.84 ± 3.36*** | 12.88 ± 3.23***# |
| Group V | 17.16 ± 5.16*** | 14.48 ± 2.72*** | 12.62 ± 3.16***# |

***$p$<0.001 compared with group I, #$p$<0.05 compared with group II.

**Table 5**: Effect of CD.EAF on lipid profile

| Groups | Serum lipid levels (mmol/L) | | | |
|---|---|---|---|---|
| | HDL-C | LDL-C | TC | TG |
| Group I | 0.39 ± 0.06 | 0.79 ± 0.20 | 1.54 ± 0.23 | 0.92 ± 0.18 |
| Group II | 0.54 ± 0.06*** | 1.44 ± 0.36*** | 2.43 ± 0.48*** | 1.12 ± 0.15* |
| Group III | 0.48 ± 0.04*** | 1.20 ± 0.15*** | 2.10 ± 0.21*** | 0.98 ± 0.18 |
| Group IV | 0.42 ± 0.06 | 1.43 ± 0.32*** | 2.32 ± 0.26*** | 1.12 ± 0.16* |
| Group V | 0.46 ± 0.08* | 1.61 ± 0.32*** | 2.55 ± 0.52*** | 1.09 ± 0.30 |

***$p$<0.05 and $p$<0.001 compared with group I (normal control group).

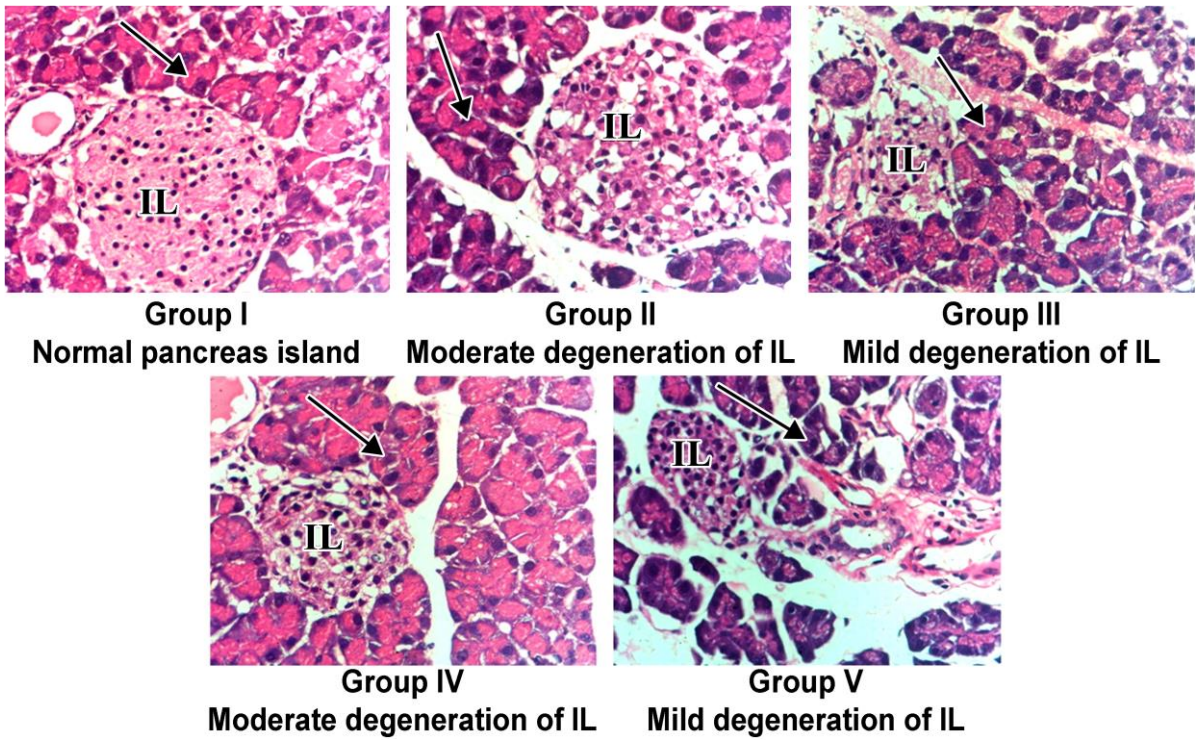


**Fig. 2**: Effects of the CD.EAF on the pancreas histology of the treated mice (HE x 400). The black arrows indicate acinar cells. IL: Islet of Langerhans.

At low doses, the β cell destruction is related to the inflammatory infiltration of autoimmune T lymphocytes in the pancreatic islets due to the reactions of immune and inflammatory, which are probably created by the ability of STZ to generate glutamic acid decarboxylase autoantigens (Zhu, 2022). The experimental model of type 2 diabetes mice induced by a single intraperitoneal injection of 100mg/kg of STZ was described by Hayashi *et al.* (2006). Besides, obesity is proven to elevate permanent plasma-free fatty acid concentrations and, therefore, the muscle reduces the glucose uptake caused by the predominant utilization of lipids. It is the main factor for insulin resistance, which is responsible for the development of type 2 diabetes (Verma and Hussain, 2017). Hence, this study used STZ combined with a high-fat diet for inducing the mice model with type 2 diabetes. After 8

weeks of treating HFD, the body weight of mice improved significantly by 79.2% in comparison with that at the beginning of treatment. Apart from the body weight, the serum glucose concentrations of HFD-fed mice increased markedly compared with those of NFD-fed mice after the eight-week treatment period. Furthermore, after 72 hours of STZ injection, there was a dramatic rise in the blood glucose values of HFD-treated mice groups, which were about three times higher than those at the beginning of the treatment. As a result, it can be assumed that the combination of HFD and STZ injection led to the successful induction of type 2-like diabetes in tested mice.

Our result indicated that the daily oral administration of gliclazide at doses of 80mg/kg/day and the CD.EAF at two tested doses for 14 days could significantly decrease the serum glucose values of high-fed diet and STZ-induced diabetic mice in comparison with the normal mice. The antidiabetic effects of the CD.EAF might be due to the interaction of its main components with enzymes involved in important cellular activities including α-glucosidase and α-amylase (Vu *et al.*, 2023). Regarding lipid parameters, no significant change was observed in the concentrations of lipid indexes between groups treated with the CD.EAF and the model group. However, previous research showed that the administration of 200mg/kg and 400mg/kg doses of ethanolic extract of *C. diffusa* could enhance the HDL-C level and lower the LDL-C, TC and TG levels in doxorubicin-induced cardiomyopathy rats (Sule *et al.*, 2017). The disparity from our current results may be due to the difference in experimental animal models, tested conditions, part of the plant used, and type of extraction solvent.

Diabetes mellitus and obesity are known to have degenerative effects on the liver and kidney through different pathways (Daryabor *et al.*, 2020). In our study, the result of the histopathological evaluation indicated that a significant improvement was observed in both the liver and pancreas structures in the mice treated with the CD.EAF after 2 weeks of the experiment. Therefore, these activities are supposed to contribute to the hypoglycemic effect of the CD.EAF.

## CONCLUSION

In conclusion, the CD.EAF was relatively safe with an $LD_{50}$ value of over 2000mg/kg. The oral administration of the CD.EAF at both 100mg/kg/day and 300mg/kg/day doses had a significant antihyperglycemic effect by reducing the blood glucose levels and improving the hepatocytes and the islets injuries on type 2 diabetic mice induced by a high-fat diet and streptozotocin. Our findings provide a scientific basis for use and a premise for the development of healthcare products based on the hypoglycemic activity of the CD.EAF.

## CONFLICT OF INTEREST

No competing interest was declared by the authors.

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# Chiết xuất, phân lập một số hợp chất từ cây Thài lài trắng (*Commelina diffusa* Burm.f.)

EXTRACTION AND ISOLATION OF SEVERAL COMPOUNDS FROM *COMMELINA DIFFUSA* BURM.F.

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**TÓM TẮT**

***Mục tiêu:*** *Chiết xuất, phân lập một số hợp chất từ cây Thài lài trắng (Commelina diffusa* Burm.f.*).*

***Đối tượng và phương pháp:*** *Cây Thài lài trắng được ngâm chiết bằng MeOH, sau đó cắn MeOH được lắc phân đoạn với n-hexan và ethyl acetat. Sử dụng các phương pháp sắc ký thích hợp để phân lập ba hợp chất sạch, ký hiệu 1-3. Sau đó, đo nhiệt độ nóng chảy, phổ khối (MS), phổ cộng hưởng từ hạt nhân (1H-NMR, 13C-NMR, DEPT) để xác định cấu trúc hóa học của chúng.*

***Kết quả:*** *Ba hợp chất được xác định là stigmasterol (1), daucosterol (2) và quercitrin (3). Trong đó, hợp chất 3 lần đầu tiên được phân lập từ loài thực vật này.*

***Kết luận:*** *Nghiên cứu đã chiết xuất, phân lập được 3 hợp chất từ cắn chiết ethyl acetate của cây Thài lài trắng (Commelina diffusa* Burm.f.*), gồm stigmasterol (1), daucosterol (2) và quercitrin (3).*

***Từ khóa:*** *Commelina diffusa* Burm.f.*, stigmasterol, daucosterol, quercitrin, thành phần hóa học.*

**SUMMARY**

***Objective:*** *Extraction and isolation of several compounds from Commelina diffusa Burm.f.*

***Subjects and methods:*** *Commelina diffusa was extracted by MeOH, then fractionated with n-hexane and ethyl acetate. The appropriate chromatographic methods were utilized to isolate three clean compounds 1-3. Then, measured the melting temperature, mass spectrometry (MS), nuclear magnetic resonance spectroscopy (1H-NMR, 13C-NMR, DEPT) to determine their chemical structure.*

***Results:*** *Three compounds were identified as stigmasterol (1), daucosterol (2) and quercitrin (3). Among them, compound number 3 was isolated for the first time from this plant.*

***Conclusion:*** *The study was extracted and isolated 3 compounds from the ethyl acetate extract of Commelina diffusa* Burm.f.*, including stigmasterol (1), daucosterol (2) and quercitrin (3).*

***Keywords:*** *Commelina diffusa Burm.f., stigmasterol, daucosterol, quercitrin, chemical constituents.*

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## ĐẶT VẤN ĐỀ

Thài lài trắng, tên khoa học là *Commelina diffusa* Burm.f., là một loài thực vật thuộc chi *Commelina* L., được tìm thấy ở khắp các vùng nhiệt đới của châu Mỹ, châu Phi, châu Á và Thái Bình Dương, cũng như ở các vùng cận nhiệt đới phía nam của Hoa Kỳ, Nam Mỹ, Úc và các đảo Nam Á [1]. Tại Việt Nam, loài này thường xuất hiện chủ yếu ở những nơi thoáng và ẩm ướt như các cánh đồng lúa, ven đồi, ven đường. Trong y học cổ truyền, cây được sử dụng để chữa cảm cúm, viêm nhiễm phần trên đường hô hấp, viêm hạnh nhân cấp, viêm hầu, phù thũng, nhiễm khuẩn đường niệu - sinh dục, viêm ruột thừa cấp, lỵ, cao huyết áp và bệnh lậu. Ngoài ra, cây còn được giã tươi để đắp vết thương và điều trị viêm mủ da. Ở dân tộc Mường, người dân sử dụng nước sắc toàn cây để trị bệnh sỏi thận [2,3]. Tuy nhiên, cho đến nay, trên thế giới cũng như ở Việt Nam, các nghiên cứu về thành phần hóa học và hoạt tính sinh học của loài này vẫn còn khá hạn chế. Bằng phương pháp sắc ký lớp mỏng và định lượng sơ bộ, các nhà khoa học đã chứng minh thành phần hóa học của Thài lài trắng bao gồm những nhóm chất như alcaloid, flavonoid, saponin, tanin, coumarin, glycosid tim, phenolic acid, anthraquinon, steroid và terpenoid. Ngoài ra, Thài lài trắng còn chứa các chất có hàm lượng dinh dưỡng cao, bao gồm protein, vitamin C, vitamin $B_2$, vitamin $B_3$, caroten, chất béo và acid hữu cơ [4-6]. Mặc dù cơ chế tác dụng chưa được tìm hiểu rõ ràng, các nghiên cứu dược lý hiện đại đã cho thấy tác dụng kháng khuẩn, kháng nấm, chống oxy hóa, kháng viêm, ức chế thần kinh trung ương, bảo vệ gan, thận và ổn định đường huyết của Thài lài trắng [7,8]. Như vậy, để góp phần cung cấp những cơ sở tiền đề cho việc ứng dụng nguyên liệu cây Thài lài trắng trong chăm sóc sức khỏe, nhóm nghiên cứu đã tiến hành chiết xuất, phân lập và xác định cấu trúc một số hợp chất có trong cây Thài lài trắng.

## ĐỐI TƯỢNG VÀ PHƯƠNG PHÁP NGHIÊN CỨU

### Đối tượng nghiên cứu

Cây Thài lài trắng được thu hái vào tháng 10 năm 2021 tại thị trấn Cổ Lễ, huyện Trực Ninh, tỉnh Nam Định và được giám định tên khoa học bởi Thạc sĩ Nghiêm Đức Trọng, trường Đại học Dược Hà Nội. Mẫu cây được lưu giữ tại Bộ môn Dược liệu - Dược học cổ truyền, Trường Đại học Y Dược, Đại học Quốc gia Hà Nội (Số hiệu tiêu bản: HNIP/18651/22).

### Hóa chất, thuốc thử

Các dung môi hóa chất dùng để chiết xuất và phân lập chất gồm methanol (MeOH), *n*-hexan, ethyl acetat (EtOAc) và nước cất ($H_2O$) đều đạt tiêu chuẩn tinh khiết phân tích.

Pha tĩnh dùng trong sắc ký cột: silica gel (Merck) cỡ hạt 0,040 - 0,063 mm và Sephadex LH-20 (Sigma-Aldrich). Bản mỏng tráng sẵn trên đế nhôm, pha thường silica gel 60 $F_{254}$ (Merck), độ dày 0,2 mm; pha đảo silica gel 60 RP-18 $F_{254s}$ (Merck), độ dày 0,25 mm; hoạt hóa ở 110$^o$C trong 1 giờ.

### Phương pháp nghiên cứu

#### *Phương pháp xử lý mẫu và chiết xuất*

Mẫu cây Thài lài trắng sau khi sấy khô, nghiền nhỏ (5,0 kg) được ngâm chiết ở nhiệt độ phòng bằng 10 L MeOH trong vòng 24 giờ. Quá trình ngâm chiết này được lặp lại 3 lần. Gộp dịch chiết, lọc và cô quay chân không dưới áp suất giảm thu được khoảng 520,0 g cao lỏng toàn phần. Hòa tan cao này trong 2 L nước cất và chiết phân lớp lần lượt với *n*-hexan và EtOAc (mỗi loại chiết 2 L x 3 lần). Các dịch chiết được cất loại dung môi dưới áp suất giảm thu được cắn *n*-hexan (142,1 g), cắn EtOAc (23,4 g) và dịch nước (285,5 g).

***Phương pháp phân lập các hợp chất***

Từ cắn chiết EtOAc (23,4 g) được tiến hành trên sắc ký cột silicagel, rửa giải bằng phương pháp gradient với hệ dung môi là $CH_2Cl_2$–MeOH (100:0→0:100, v/v) thu được 5 phân đoạn, ký hiệu E1→E5. Phân đoạn E1 (322,6 mg) được phân tách trên cột silicagel với hệ dung môi *n*-hexan–EtOAc (4:1, v/v), sau đó được đưa lên cột Sephadex LH-20 và rửa giải bằng dung môi MeOH thu được chất sạch **1** (7,8 mg). Từ phân đoạn E4 (1,23 g), triển khai phương pháp sắc kí cột silicagel pha thường với hệ dung môi rửa giải $CH_2Cl_2$–MeOH (9:1, v/v). Tiếp tục phân tách phân đoạn này bằng cột Sephadex LH-20 với hệ dung môi MeOH–$CH_2Cl_2$ (9:1, v/v) thu được hợp chất **2** (11,0 mg). Từ phân đoạn E5 (1,02 g), triển khai phương pháp sắc kí cột silicagel pha thường với hệ dung môi $CH_2Cl_2$–MeOH–$H_2O$ (5:1:0,1, v/v) thu được 4 phân đoạn nhỏ (E5.1 - E5.4). Phân đoạn nhỏ E5.2 (120 mg) được phân tách bằng phương pháp sắc ký cột silicagel pha thường với hệ dung môi là $CH_2Cl_2$–MeOH–$H_2O$ (5:1:0,1, v/v) thu được chất tinh khiết là hợp chất **3** (5 mg).

***Phương pháp xác định cấu trúc các hợp chất phân lập được***

Xác định cấu trúc các hợp chất phân lập được bằng phương pháp đo nhiệt độ nóng chảy, phổ khối (MS), phổ cộng hưởng từ hạt nhân ($^1$H-NMR, $^{13}$C-NMR, DEPT) và so sánh các số liệu thu được từ thực nghiệm với các số liệu đã công bố [9-11].

## KẾT QUẢ VÀ BÀN LUẬN

**Hợp chất 1: Stigmasterol**

Hợp chất **1** là chất rắn kết tinh, màu trắng, hình kim, nhiệt độ nóng chảy 169 - 170$^o$C. Dựa vào tín hiệu của pic ion phân tử trên phổ khối ESI-MS tại m/z 413 $[M+H]^+$, khối lượng phân tử của hợp chất **1** được xác định là 412, tương ứng với công thức phân tử là $C_{29}H_{48}O$. Phổ $^1$H-NMR của hợp chất **1** cho thấy sự xuất hiện của hai nhóm methyl singlet tại $\delta_H$ 0,85 (3H, s, H-18); 1,02 (3H, s, H-19), ba nhóm methyl doublet tại $\delta_H$ 0,92 (3H, d, J = 6,0 Hz, H-21); 0,83 (3H, d, J = 7,2 Hz, H-26); 0,80 (3H, d, J = 6,8 Hz, H-27) và một nhóm methyl triplet tại $\delta_H$ 0,70 (3H, t, J = 9,5 Hz, H-29). Ngoài ra, phổ $^1$H-NMR cũng cho thấy tín hiệu của các olefinic proton tại $\delta_H$ 5,35 (1H, brs, H-6); 5,15 (1H, m, H-22); 5,03 (1H, m, H-23) chứng tỏ sự có mặt của hai liên kết đôi tại C-5/C-6 và C-22/C-23. Bên cạnh đó, trên phổ còn xuất hiện tín hiệu proton đặc trưng của nhóm –OH trên khung sterol tại $\delta_H$ 3,51 (1H, m, H-3). Phổ $^{13}$C-NMR và DEPT cho biết hợp chất **1** có 29 carbon trong phân tử, bao gồm sáu nhóm methyl ($CH_3$), chín nhóm methylen ($CH_2$), mười một nhóm methin (CH) và ba carbon bậc bốn ($C_q$). Trong đó, xuất hiện tín hiệu carbon của một nhóm oxymethin tại $\delta_C$ 71,8 (C-3), tín hiệu carbon của liên kết đôi tại $\delta_C$ 140,7 (C-5); 121,7 (C-6); 138,3 (C-22); 129,3 (C-23), cùng với tín hiệu carbon của sáu nhóm methyl lần lượt tại $\delta_C$ 12,0 (C-18); 19,1 (C-19); 19,8 (C-21); 21,2 (C-26); 18,9 (C-27); 12,2 (C-29). Các dữ liệu phổ của hợp chất **1** được so sánh với các giá trị tương ứng của hợp chất stigmasterol đã được công bố trong tài liệu tham khảo [9] (Bảng 1). Sự phù hợp giữa các số liệu cho phép khẳng định hợp chất **1** là stigmasterol.

*Bảng 1. Dữ liệu phổ $^1$H-NMR (600 MHz) và $^{13}$C-NMR (150 MHz) của hợp chất **1** và hợp chất tham khảo [9] (dung môi đo $CDCl_3$)*

| Vị trí | Hợp chất 1 | | Stigmasterol [9] | |
|---|---|---|---|---|
| | $\delta_C$ (ppm) | $\delta_H$ (ppm) (J, Hz) | $\delta_C$ (ppm) | $\delta_H$ (ppm) (J, Hz) |
| 1 | 37,3 | - | 37,3 | - |
| 2 | 31,7 | - | 31,6 | - |

| | | | | |
|---|---|---|---|---|
| 3 | 71,8 | 3,51 (1H, m) | 71,8 | 3,52 (1H, m) |
| 4 | 42,2 | - | 42,3 | - |
| 5 | 140,7 | - | 140,7 | - |
| 6 | 121,7 | 5,35 (1H, brs) | 121,7 | 5,36 (1H, brd, *J* = 3,5 Hz) |
| 7 | 31,9 | - | 31,8 | - |
| 8 | 31,8 | - | 31,8 | - |
| 9 | 50,2 | - | 50,1 | - |
| 10 | 36,5 | - | 36,6 | - |
| 11 | 21,0 | - | 21,1 | - |
| 12 | 39,8 | - | 39,7 | - |
| 13 | 42,3 | - | 42,2 | - |
| 14 | 56,8 | - | 56,8 | - |
| 15 | 24,4 | - | 24,5 | - |
| 16 | 28,2 | - | 28,9 | - |
| 17 | 56,1 | - | 56,1 | - |
| 18 | 12,0 | 0,85 (3H, s) | 12,1 | 0,85 (3H, s) |
| 19 | 19,1 | 1,02 (3H, s) | 19,4 | 1,02 (3H, s) |
| 20 | 40,5 | - | 40,6 | - |
| 21 | 19,8 | 0,92 (3H, d, *J* = 6,0 Hz) | 21,1 | 0,92 (3H, d, *J* = 6,5 Hz) |
| 22 | 138,3 | 5,15 (1H, m) | 138,2 | 5,16 (1H, dd, *J* = 15,0; 8,5 Hz) |
| 23 | 129,3 | 5,03 (1H, m) | 129,3 | 5,03 (1H, dd, *J* = 15,0; 8,5 Hz) |
| 24 | 51,2 | - | 51,2 | - |
| 25 | 33,9 | - | 31,9 | - |
| 26 | 21,2 | 0,83 (3H, d, *J* = 7,2 Hz) | 21,2 | 0,83 (3H, t, *J* = 8,5 Hz) |
| 27 | 18,9 | 0,80 (3H, d, *J* = 6,8 Hz) | 19,1 | 0,83 (3H, d, *J* = 6,8 Hz) |
| 28 | 25,4 | - | 25,3 | - |
| 29 | 12,2 | 0,70 (3H, t, *J* = 9,5 Hz) | 12,2 | 0,79 (3H, d, *J* = 9,5 Hz) |

Stigmasterol là một phytosterol được tìm thấy nhiều trong thực vật và có cấu trúc hóa học tương tự như cholesterol. Các nghiên cứu đã xác định hợp chất này có thể ức chế sự phát triển của các tế bào ung thư khác nhau như ung thư vú, ung thư buồng trứng, ung thư ruột kết và ung thư tuyến tiền liệt bằng cách thúc đẩy và gia tăng quá trình apoptosis [12].

**Hợp chất 2: Daucosterol**

Hợp chất **2** là chất rắn màu trắng, dạng bột vô định hình, chuyển sang màu hồng với cerin sulfat, nóng chảy ở 135 - 136°C. Phổ $^{1}$H-NMR của hợp chất **2** cho thấy sáu tín hiệu cộng hưởng của nhóm methyl tại $\delta_H$ 0,62 (3H, s, H-18); 0,84 (3H, m, H-27); 0,88 (3H, m, H-29); 0,89 (3H, s, H-26); 0,92 (3H, m, H-19); 0,95 (3H, m, H-21). Một proton ở C-3 xuất hiện dưới dạng multiplet tại $\delta_H$ 4,25 (1H, m). Ngoài ra, tín hiệu proton anomer tại $\delta_H$ 4,99 (1H, d, *J* = 7,8 Hz, H-1′) chứng tỏ phân tử hợp chất **2** có mặt một gốc đường β-glucopyranose với hằng số tương tác lớn. Từ phổ $^{13}$C-NMR và DEPT chỉ ra rằng hợp chất **2** bao gồm 35 carbon trong cấu trúc, trong đó tín hiệu cộng hưởng của 29 nguyên tử carbon của khung *β*-sitosterol gồm sáu nhóm methyl ($CH_3$) tại $\delta_C$ 12,0 (C-18); 19,3 (C-19); 18,9 (C-21); 19,8 (C-26); 19,1 (C-27); 11,8 (C-29); mười một nhóm methylen ($CH_2$) tại $\delta_C$ 37,3 (C-1); 30,0 (C-2); 39,8 (C-4); 32,0 (C-7); 21,1 (C-11); 39,1 (C-12); 24,3 (C-15); 28,4 (C-16); 34,1 (C-22); 26,3 (C-23); 23,2 (C-28); chín nhóm methin (CH) tại $\delta_C$ 78,1 (C-3); 121,8 (C-6); 31,9 (C-8); 50,2 (C-9); 56,7 (C-14); 56,1 (C-17); 36,2 (C-20); 45,9 (C-24); 29,3 (C-25) và ba carbon bậc bốn ($C_q$) tại $\delta_C$ 140,8 (C-5); 36,8 (C-10); 42,3 (C-13). Bên cạnh đó, xuất hiện sáu tín hiệu cộng hưởng chứng tỏ sự có mặt của một gốc đường β-glucopyranose tại $\delta_C$ 102,3 (C-1′); 75,0 (C-2′); 78,2 (C-3′); 71,4 (C-4′); 78,1 (C-5′); 62,5

(C-6'). Từ dữ liệu phổ thu được, kết hợp so sánh với dữ liệu phổ của hợp chất daucosterol đã được công bố [10] (Bảng 2), có thể kết luận hợp chất **2** là daucosterol (sitosterol-*β*-D-glucosid).

*Bảng 2. Dữ liệu phổ $^{1}$H-NMR (600 MHz) và $^{13}$C-NMR (150 MHz) của hợp chất **2** và hợp chất tham khảo [10] (dung môi đo $C_5D_5N$)*

| Vị trí | Hợp chất 2 | | Daucosterol [10] | |
|---|---|---|---|---|
| | $\delta_C$ (ppm) | $\delta_H$ (ppm) (J, Hz) | $\delta_C$ (ppm) | $\delta_H$ (ppm) (J, Hz) |
| 1 | 37,3 | - | 37,4 | - |
| 2 | 30,0 | - | 30,2 | - |
| 3 | 78,1 | 4,25 (1H, m) | 78,6 | 4,31 (1H, m) |
| 4 | 39,8 | - | 39,3 | - |
| 5 | 140,8 | - | 140,8 | - |
| 6 | 121,8 | 5,30 (1H, overlap) | 121,9 | 5,33 (1H) |
| 7 | 32,0 | - | 32,0 | - |
| 8 | 31,9 | - | 32,1 | - |
| 9 | 50,2 | - | 50,3 | - |
| 10 | 36,8 | - | 36,9 | - |
| 11 | 21,1 | - | 21,2 | - |
| 12 | 39,1 | - | 39,9 | - |
| 13 | 42,3 | - | 42,5 | - |
| 14 | 56,7 | - | 56,8 | - |
| 15 | 24,3 | - | 24,5 | - |
| 16 | 28,4 | - | 28,5 | - |
| 17 | 56,1 | - | 56,2 | - |
| 18 | 12,0 | 0,62 (3H, s) | 11,9 | 0,64 (3H, s) |
| 19 | 19,3 | 0,92 (3H, s) | 19,1 | 0,92 (3H, s) |
| 20 | 36,2 | - | 36,4 | - |
| 21 | 18,9 | 0,95 (3H, m) | 19,0 | 0,97 (3H, d, *J* = 6,5 Hz) |
| 22 | 34,1 | - | 34,2 | - |
| 23 | 26,3 | - | 26,3 | - |
| 24 | 45,9 | - | 46,0 | - |
| 25 | 29,3 | - | 29,4 | - |
| 26 | 19,8 | 0,89 (3H, s) | 19,9 | 0,89 (3H, d, *J* = 5,6 Hz) |
| 27 | 19,1 | 0,84 (3H, m) | 19,2 | 0,84 (3H, d, *J* = 6,5 Hz) |
| 28 | 23,2 | - | 23,3 | - |
| 29 | 11,8 | 0,88 (3H, m) | 12,1 | 0,88 (3H, d, *J* = 3,1 Hz) |
| 1′ | 102,3 | 4,99 (1H, d, *J* = 7,8 Hz) | 102,5 | - |
| 2′ | 75,0 | 3,91 (1H, m) | 75,3 | 3,93 (1H) |
| 3′ | 78,2 | 4,02 (1H, t, *J* = 9,0 Hz) | 78,5 | 4,05 (1H, t, *J* = 8,1 Hz) |
| 4′ | 71,4 | 4,20 (1H, m) | 71,6 | 4,26 (1H, m) |
| 5′ | 78,1 | 3,94 (1H, m) | 78,0 | 3,97 (1H, m) |
| 6′ | 62,5 | 4,33 (1H, m, H-6′b) 4,50 (1H, d, J = 12,0 Hz, H-6′a) | 62,8 | 4,41 (1H, dd, *J* = 5,1; 11,7 Hz, H-6′b) 4,56 (1H, dd, H-6′a) |

Daucosterol, còn gọi là sitogluside, là một sterol khá phổ biến ở các loài thực vật bậc cao. Các nghiên cứu dược lý cho thấy hợp chất này thể hiện nhiều tác dụng sinh học rõ rệt trên *in vitro* và *in vivo* như chống oxy hóa, chống ung thư, bảo vệ thần kinh, kháng viêm, điều hòa miễn dịch, hạ đường huyết và giảm mỡ máu [13].

**Hợp chất 3: Quercitrin**

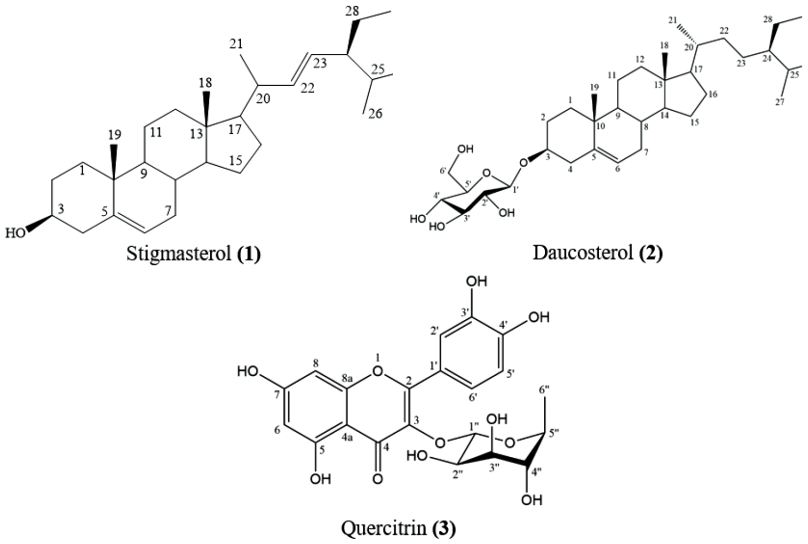
Hợp chất **3** thu được dưới dạng bột vô định hình, màu vàng. Dữ liệu phổ ESI-MS có các phân mảnh ion với *m/z* = 449 $[M+H]^+$. Do đó, khối lượng phân tử của hợp chất **3** là 448 tương ứng với công thức phân tử là $C_{21}H_{20}O_{11}$. Phổ $^{1}$H-NMR của chất **3** xuất hiện tín hiệu của ba proton aromatic tại $\delta_H$ 7,36 (1H, d, *J* = 2,4 Hz, H-2′); 7,33

(1H, dd, *J* = 8,4; 2,4 Hz, H-6′); 6,94 (1H, d, *J* = 8,4 Hz, H-5′) gợi ý sự có mặt của một hệ vòng thơm ABX. Tín hiệu của hai proton aromatic tại $\delta_H$ 6,40 (1H, d, *J* = 2,4 Hz, H-8); 6,23 (1H, d, *J* = 2,4 Hz, H-6) cũng gợi ý sự có mặt của hệ vòng thơm AX trong cấu trúc. Các dữ liệu này đặc trưng cho một hợp chất flavonoid. Ngoài ra, trên phổ $^1$H-NMR cũng xuất hiện tín hiệu proton anomeric tại $\delta_H$ 5,37 (1H, d, *J* = 1,2 Hz, H-1″) cùng với các tín hiệu proton của nhóm oxymethin tại $\delta_H$ 4,23 (1H, dd; *J* = 3,6; 1,8 Hz, H-2″); 3,77 (1H, dd; *J* = 9,6; 3,6 Hz, H-3″); 3,43 (1H, m, H-4″); 3,36 (1H, m, H-5″) và doublet của nhóm methyl tại $\delta_H$ 0,97 (3H, d; *J* = 6,0 Hz, H-6″) gợi ý cấu trúc của một gốc đường α-L-rhamnopyranosid. Phổ $^{13}$C-NMR và DEPT của hợp chất **3** xuất hiện tín hiệu của 21 carbon, bao gồm một nhóm methyl ($CH_3$), mười nhóm methin (CH), và mười carbon bậc bốn ($C_q$). Hai hệ vòng thơm ABX và AX được khẳng định bởi tín hiệu của năm nhóm methin tại $\delta_C$ 116,96 (C-2′); 116,39 (C-5′); 123,00 (C-6′); 99,84 (C-6); 94,73 (C-8). Trong khi đó, sự xuất hiện của một tín hiệu nhóm carbonyl tại $\delta_C$ 179,68 (C-4) cùng các tín hiệu carbon của vòng thơm liên kết với oxy tại $\delta_C$ 136,25 (C-3); 163,24 (C-5); 165,94 (C-7); 146,45 (C-3′); 149,82 (C-4′) cho phép xác định cấu trúc của một hợp chất khung flavonol. Năm tín hiệu methin liên kết với oxy tại $\delta_C$ 103,57 (C-1″); 71,92 (C-2″); 72,15 (C-3″); 72,04 (C-4″); 73,28 (C-5″) và một tín hiệu methyl tại $\delta_C$ 17,65 (C-6″) cho phép khẳng định sự xuất hiện của một gốc rhamnopyranosid. Như vậy, có thể xác định hợp chất **3** là một flavonol monoglycosid. Dữ liệu phổ NMR của hợp chất **3** tương đồng với các dữ liệu công bố trong tài liệu tham khảo của hợp chất quercitrin [11] (Bảng 3). Từ đó, có thể khẳng định hợp chất **3** chính là Quercitrin (quercetin-3-*O*-*α*-L-rhamnopyranosid).

*Bảng 3. Dữ liệu phổ $^1$H-NMR (500 MHz) và $^{13}$C-NMR (125 MHz) của hợp chất **3** và hợp chất tham khảo [11] (dung môi đo $CD_3OD$)*

| Vị trí | Hợp chất 3 | | Quercitrin [11] | |
|---|---|---|---|---|
| | $\delta_C$ (ppm) | $\delta_H$ (ppm) (J, Hz) | $\delta_C$ (ppm) | $\delta_H$ (ppm) (J, Hz) |
| 2 | 159,34 | - | 159,31 | - |
| 3 | 136,25 | - | 136,23 | - |
| 4 | 179,68 | - | 179,65 | - |
| 4a | 105,91 | - | 105,90 | - |
| 5 | 163,24 | - | 163,21 | - |
| 6 | 99,84 | 6,23 (1H, d, *J* = 2,4 Hz) | 99,82 | 6,20 (1H, d, *J* = 2,1 Hz) |
| 7 | 165,94 | - | 165,89 | - |
| 8 | 94,73 | 6,40 (1H, d, *J* = 2,4 Hz) | 94,71 | 6,37 (1H, d, *J* = 2,1 Hz) |
| 8a | 158,57 | - | 158,53 | - |
| 1′ | 122,86 | - | 122,85 | - |
| 2′ | 116,96 | 7,36 (1H, d, *J* = 2,4 Hz) | 116,93 | 7,34 (1H, d, *J* = 2,1 Hz) |
| 3′ | 146,45 | - | 146,41 | - |
| 4′ | 149,82 | - | 149,80 | - |
| 5′ | 116,39 | 6,94 (1H, d, *J* = 8,4 Hz) | 116,36 | 6,91 (1H, d, *J* = 8,3 Hz) |
| 6′ | 123,00 | 7,33 (1H, dd, *J* = 8,4; 2,4 Hz) | 122,96 | 7,31 (1H, dd; *J* = 8,3; 2,1 Hz) |
| 1″ | 103,57 | 5,37 (1H, d, *J* = 1,2 Hz) | 103,54 | 5,35 (1H, d; *J* = 1,4 Hz) |
| 2″ | 71,92 | 4,23 (1H, dd; *J* = 3,6; 1,8 Hz) | 71,89 | 4,22 (1H, dd; *J* = 3,3; 1,4 Hz) |
| 3″ | 72,15 | 3,77 (1H, dd; *J* = 9,6; 3,6 Hz) | 72,11 | 3,75 (1H, dd; *J* = 9,5; 3,3 Hz) |
| 4″ | 72,04 | 3,43 (1H, m) | 72,02 | 3,41 (1H, m) |
| 5″ | 73,28 | 3,36 (1H, m) | 73,25 | 3,15 (1H, m) |
| 6″ | 17,65 | 0,97 (3H, d; *J* = 6,0 Hz) | 17,65 | 0,94 (3H, d; *J* = 6,2 Hz) |

Quercitrin là một flavonoid glycosid với phần aglycon là quercetin, được tìm thấy trong nhiều loài thực vật. Hợp chất này đã được chứng minh có khả năng chống oxy hóa, kháng viêm, khôi phục hoạt tính của flavin monooxygenase ở những bệnh nhân bị viêm gan, xơ gan, ung thư gan do tăng sản xuất nitric oxide và ngăn chặn sự tổn thương của tế bào β thông qua con đường ty thể và truyền tín hiệu NF-κB [11,14,15].




*Hình 1. Cấu trúc hóa học của các hợp chất **1 - 3***

## KẾT LUẬN

Nghiên cứu đã chiết xuất, phân lập được 3 hợp chất từ cắn chiết ethyl acetate của cây Thài lài trắng (*Commelina diffusa* Burm.f.), gồm stigmasterol (**1**), daucosterol (**2**) và quercitrin (**3**). Cấu trúc hóa học của các hợp chất đã được xác định thông qua các kết quả đo nhiệt độ nóng chảy, phổ khối, phổ cộng hưởng từ hạt nhân và so sánh với các dữ liệu công bố của các hợp chất liên quan. Đây là lần đầu tiên hợp chất **3** được phân lập từ cây Thài lài trắng. Kết quả của nghiên cứu đã góp phần cung cấp thông tin, làm phong phú thêm tri thức về thành phần hóa học của loài *C. diffusa*. Từ đó, mở ra những hướng nghiên cứu tiếp theo nhằm tìm ra những hoạt chất chính có khả năng ứng dụng trong nghiên cứu và phát triển thuốc.

## LỜI CẢM ƠN

Nghiên cứu này được tài trợ bởi đề tài cấp Đại học Quốc gia Hà Nội, mã số: QG.20.81.

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Original Article

# Evaluation of the Inhibitory Effect on α-glucosidase and α-amylase Enzymes of Triterpenes Isolated from *Commelina diffusa* Burm.f.

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**Abstract:** By using column chromatography and thin layer chromatography, three triterpene compounds were isolated from the *n*-hexane fraction of the methanol extract of *Commelina diffusa*, including corosolic acid (**1**), 3α-acetyl-20S,24S-epoxydammarane-25-ol (**2**) and 11-oxo-α-amyrin acetate (**3**). The structures of the isolated compounds were determined by spectroscopic methods and compared with references. This is the first time compounds **2** and **3** have been isolated from the genus *Commelina*. For enzyme inhibition assays, among three tested compounds, compound **1** had the highest inhibitory effect on α-glucosidase enzyme with an $IC_{50}$ value of 93.68 ± 4.1 µg/mL while compound **2** showed the strongest ability to inhibit α-amylase enzyme with an $IC_{50}$ value of 100.64 ± 3.6 µg/mL. Meanwhile, the $IC_{50}$ values of positive control acarbose in these two assays were 135.73 ± 1.8 µg/mL against α-glucosidase enzyme and 83.33 ± 2.0 µg/mL against α-amylase enzyme, respectively.

*Keywords: Commelina diffusa* Burm.f., corosolic acid, 3*α*-acetyl-20*S*,24*S*-epoxydammarane-25-ol, 11-oxo-α-amyrin acetate, α-glucosidase, α-amylase.

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# Đánh giá khả năng ức chế enzym α-glucosidase và α-amylase của các hợp chất triterpene phân lập từ cây Thài lài trắng (*Commelina diffusa* Burm.f.)

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**Tóm tắt:** Bằng phương pháp phân tách sắc ký cột và sắc ký lớp mỏng, ba hợp chất triterpen đã được phân lập từ phân đoạn *n*-hexan của dịch chiết methanol cây Thài lài trắng, gồm axit corosolic (**1**), 3*α*-acetyl-20*S*,24*S*-epoxydammaran-25-ol (**2**) và 11-oxo-α-amyrin acetat (**3**). Cấu trúc của các hợp chất được xác định bằng các phương pháp phổ nghiệm và so sánh với các tài liệu tham khảo. Đây là lần đầu tiên hợp chất **2** và **3** được phân lập từ chi *Commelina*. Đối với các thử nghiệm ức chế enzym, trong ba hợp chất được khảo sát, hợp chất **1** có tác dụng ức chế enzym α-glucosidase cao nhất với giá trị $IC_{50}$ là 93,68 ± 4,1 µg/mL và hợp chất **2** thể hiện khả năng ức chế enzym α-amylase mạnh nhất với giá trị $IC_{50}$ là 100,64 ± 3,6 µg/mL. Trong khi đó, giá trị $IC_{50}$ thu được của chứng dương acarbose ở hai thử nghiệm này lần lượt là 135,73 ± 1,8 µg/mL đối với enzym α-glucosidase và 83,33 ± 2,0 µg/mL đối với enzym α-amylase.

*Từ khóa: Commelina diffusa* Burm.f., axit corosolic, 3*α*-acetyl-20*S*,24*S*-epoxydammaran-25-ol, 11-oxo-α-amyrin acetat, α-glucosidase, α-amylase.

## 1. Mở đầu

Đái tháo đường là một trong những bệnh không lây nhiễm phổ biến nhất trên toàn cầu. Trong đó, đái tháo đường type 2, còn được gọi là đái tháo đường không phụ thuộc insulin, chịu trách nhiệm cho 90 – 95% tất cả các trường hợp đái tháo đường [1]. Nhiều nghiên cứu đã chứng minh tăng đường huyết sau ăn là một yếu tố góp phần quan trọng cho sự phát triển của bệnh đái tháo đường type 2. Do đó, kiểm soát tăng đường huyết sau ăn là một trong những phương pháp chính để điều trị căn bệnh này [2]. α-Amylase và α-glucosidase là hai enzym chuyển hóa carbohydrate quan trọng liên quan đến tăng đường huyết sau ăn ở bệnh nhân mắc đái tháo đường type 2. α-Amylase có khả năng xúc tác sự phân cắt các liên kết α-1,4-glycosidic để chuyển hóa các polysaccharide thành các đoạn oligosaccharide nhỏ hơn, sau đó bị phân hủy bởi α-glucosidase. Trong khi đó, α-glucosidase chịu trách nhiệm thủy phân các gốc α-1,4-glycoside ở đầu tận cùng không khử của carbohydrate để giải phóng các monosaccharide có thể hấp thụ vào

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máu. Do đó, việc ức chế hai enzym này có thể làm giảm quá trình thủy phân carbohydrate và làm chậm sự thẩm thấu glucose vào máu [3, 4].

Thài lài trắng (*Commelina diffusa* Burm.f.) là một loài cỏ dại ở vùng nhiệt đới và cận nhiệt đới, xuất hiện chủ yếu ở các môi trường sống thoáng và ẩm. Ở nước ta, cây phân bố ở khắp các tỉnh thành và thường mọc ở những nơi ẩm ướt như ven sông, ven đường và các vùng đất canh tác [5]. Theo y học cổ truyền, Thài lài trắng có vị ngọt, tính hàn; quy vào các kinh tâm, can, tỳ, thận, đại tiểu trường; công năng thanh nhiệt, giải độc, lợi thủy, tiêu thũng. Loài này được sử dụng trong dân gian để chữa viêm nhiễm phần trên đường hô hấp, viêm họng, cảm cúm, viêm hạnh nhân cấp, phù thũng, viêm ruột, kiết lỵ, nhiễm khuẩn đường niệu – sinh dục, cao huyết áp, bệnh lậu và tăng đường huyết [6]. Thành phần hóa học của Thài lài trắng đã được xác định bằng phương pháp sắc ký lớp mỏng và định lượng sơ bộ, bao gồm các nhóm chất chính như alcaloid, flavonoid, terpenoid, steroid, tanin, coumarin, saponin, glycosid tim, phenolic acid và anthraquinon [7]. Các nghiên cứu dược lý đã cho thấy Thài lài trắng thể hiện nổi bật tác dụng kháng khuẩn, kháng nấm, chống viêm, chống oxy hóa, bảo vệ gan, lợi tiểu và ức chế hệ thần kinh trung ương [8]. Tuy nhiên, các bằng chứng khoa học về tác dụng hạ đường huyết của loài này còn hạn chế. Gần đây, nhóm nghiên cứu của Trường Đại học Y Dược, Đại học Quốc gia Hà Nội đã chứng minh phân đoạn *n*-hexan của cây Thài lài trắng thể hiện hoạt tính ức chế tốt đối với enzym α-glucosidase và α-amylase khi so sánh với chứng dương acarbose [9]. Do đó, trong nghiên cứu này, chúng tôi tiến hành phân lập một số hợp chất từ phân đoạn *n*-hexan của cây Thài lài trắng và đánh giá khả năng ức chế hai enzym α-glucosidase và α-amylase của các hợp chất này.

## 2. Đối tượng và phương pháp nghiên cứu

### *2.1. Đối tượng nghiên cứu*

Trong nghiên cứu trước đây, chúng tôi đã tiến hành chiết xuất các phân đoạn, dịch chiết của cây Thài lài trắng [5]. Cụ thể, mẫu dược liệu được thu hái và được định danh bởi nhà thực vật học. Mẫu vật có đầy đủ các bộ phận và đặc biệt là có hoa. Tiêu bản của mẫu thu hái được lưu trữ tại Trường Đại học Y Dược, Đại học Quốc gia Hà Nội (số hiệu tiêu bản: HNIP/18651/22). Sau khi định danh, dược liệu được rửa sạch, cắt nhỏ, phơi khô. Nguyên liệu Thài lài trắng sau khi sấy khô được nghiền nhỏ, ngâm chiết 3 lần trong dung môi methanol ở nhiệt độ phòng. Lọc loại bã dược liệu, gộp các dịch chiết và cất thu hồi dung môi dưới áp suất giảm thu được cao chiết toàn phần methanol. Cao chiết này được phân tán vào nước cất với tỉ lệ 1:1 và chiết phân bố lần lượt với *n*-hexan và ethyl acetat (mỗi dung môi 3 lần). Các dịch chiết được cất loại dung môi dưới áp suất giảm thu được các phân đoạn tương ứng.

### *2.2. Dung môi, hóa chất và thiết bị*

Các dung môi, hóa chất dùng trong chiết xuất và phân lập gồm methanol (MeOH), *n*-hexan, dichloromethan ($CH_2Cl_2$), ethyl acetat (EtOAc) và nước cất ($H_2O$) đều đạt tiêu chuẩn công nghiệp. Sắc ký cột được tiến hành trên silica gel (Merck) cỡ hạt 0,040 – 0,063 mm. Sắc ký lớp mỏng được tiến hành trên bản mỏng tráng sẵn trên đế nhôm, pha thường silica gel 60 $F_{254}$ (Merck), độ dày 0,2 mm; pha đảo silica gel 60 RP-18 $F_{254s}$ (Merck), độ dày 0,25 mm; hoạt hóa ở 110 ºC trong 1 giờ. Các hợp chất được phát hiện bằng đèn tử ngoại bước sóng 254 nm và sử dụng thuốc thử là dung dịch $H_2SO_4$ 10% hơ nóng để phát hiện vết chất.

Phổ cộng hưởng từ hạt nhân (NMR) được ghi trên máy Bruker Avance 500 MHz (Bruker) tại Viện Hóa học, Viện Hàn lâm Khoa học và Công nghệ Việt Nam. Nhiệt độ nóng chảy được đo trên máy Stuart SMP10 tại Trường Đại học Y Dược, Đại học Quốc gia Hà Nội.

Các hóa chất được sử dụng trong nghiên cứu đánh giá khả năng ức chế enzym bao gồm enzym α-glucosidase và α-amylase (Sigma-Aldrich, Mỹ); tinh bột, p-nitrophenyl-α-D-glucopyranoside (pNPG), kali photphat, natri clorid, natri carbonat, axit dinitrosalicylic, dimethyl sulfoxide (Merck, Đức); acarbose (Chemcruz, Mỹ). Các loại hóa chất khác đều đạt tiêu chuẩn dược dụng hoặc tinh khiết phân tích.

### *2.3. Phương pháp nghiên cứu*

#### *2.3.1. Chiết xuất và phân lập các hợp chất*

Cắn *n*-hexan (65,0 g) được phân lập trên cột sắc ký với chất hấp phụ silicagel sử dụng hệ dung môi *n*-hexan:$CH_2Cl_2$ (15:1, v/v). Hứng dịch rửa giải vào các ống và kiểm tra bằng sắc ký lớp mỏng. Gộp các ống từ 2 – 16 thu được phân đoạn H1, gộp các ống từ 17 –25 thu được phân đoạn H2 và gộp các ống từ 26 – 40 thu được phân đoạn H3.

Tiến hành sắc ký cột cắn phân đoạn H1 với chất hấp phụ silicagel, hệ dung môi *n*-hexan:$CH_2Cl_2$ (10:1; v/v). Kiểm tra các ống hứng dịch rửa giải bằng sắc ký lớp mỏng, gộp các ống có cùng thành phần và bốc hơi dung môi thu được 4 phân đoạn nhỏ gồm: H1.1, H1.2, H1.3, H1.4. Phân đoạn H1.3 được phân lập trên cột silicagel với hệ dung môi *n*-hexan:EtOAc (7:3; v/v) thu được hợp chất **1** (21,3 mg). Từ phân đoạn H1.4, triển khai phương pháp sắc ký cột silicagel pha thường với hệ dung môi *n*-hexan:EtOAc (9:1; v/v) thu được hợp chất **2 (**26,0 mg).

Tiến hành sắc ký cột cắn phân đoạn H2 với chất hấp phụ silicagel, hệ dung môi rửa giải *n*-hexan:EtOAc (10:1, 5:1; v/v), thu được 2 phân đoạn nhỏ ký hiệu là H2.1 và H2.2. Phân đoạn H2.2 được phân tách bằng phương pháp sắc ký cột silicagel pha thường với hệ dung môi là *n*-hexan:EtOAc (20:1; v/v) thu được hợp chất **3** (27,5 mg).

Sử dụng phương pháp đo nhiệt độ nóng chảy, phổ cộng hưởng từ hạt nhân ($^1$H-NMR, $^{13}$C-NMR, DEPT, HMBC, HSBC) và so sánh các số liệu thu được từ thực nghiệm với các số liệu đã công bố để xác định cấu trúc của các hợp chất phân lập được.

#### *2.3.2. Đánh giá khả năng ức chế enzym α-glucosidase và α-amylase*

Khả năng hạ đường huyết *in vitro* của các hợp chất triterpen phân lập được từ cây Thài lài trắng được đánh giá thông qua tác dụng ức chế enzym α-glucosidase và α-amylase. Các thử nghiệm ức chế enzym được thực hiện ba lần theo các phương pháp đã được mô tả trong nghiên cứu trước đây [9]. Các dung dịch mẫu thử được chuẩn bị bằng cách hòa tan các hợp chất tinh khiết trong dimethyl sulfoxide (DMSO) và pha loãng thành dãy các nồng độ khác nhau. Phép đo hoạt tính ức chế enzym α-glucosidase sử dụng dung dịch đệm photphat (kali photphat 100 mM; pH 6,8) và dung dịch cơ chất (*p*-nitrophenyl-α-D-glucopyranoside 5 mM). Đầu tiên, 50 µL dung dịch mẫu thử được trộn với 50 µL dung dịch enzyme (0,2 U/mL) và ủ trong đĩa 96 giếng trong 10 phút ở 37 $^o$C. Sau đó, thêm 100 µL dung dịch cơ chất vào hỗn hợp và ủ trong 20 phút ở 37 $^o$C. Cuối cùng, 80 µL dung dịch $Na_2CO_3$ 0,2 M được thêm vào để kết thúc phản ứng. Độ hấp thụ quang của hỗn hợp được đo ở bước sóng 405 nm bằng máy quang phổ Microplate (xMark, Bio-Rad). Đối với thử nghiệm α-amylase, dung dịch đệm natri photphat (NaCl 6,7 mM; pH 6,9) và dung dịch cơ chất (tinh bột 1% pha trong dung dịch đệm 20 mM) được sử dụng. Trong mỗi thí nghiệm, 10 µL dung dịch mẫu thử được trộn với 10 µL dung dịch enzym (50 U/mL) trong các ống nghiệm và ủ trong 10 phút ở 25 $^o$C. Sau quá trình ủ sơ bộ, 10 µL dung dịch cơ chất được thêm vào mỗi ống và hỗn hợp này được ủ tiếp trong 10 phút ở 25 $^o$C. Kết thúc phản ứng bằng cách thêm 20 µL dung dịch thuốc thử DNSA (dinitrosalicylic acid) và đun nóng trong nồi cách thủy ở 90 – 100 $^o$C trong 10 phút. Cuối cùng, dung dịch phản ứng được làm nguội ở nhiệt độ phòng và được pha loãng với 300 µL nước cất. Độ hấp thụ quang của hỗn hợp được đo ở bước sóng 540 nm bằng máy quang phổ UV-VIS. Acarbose được sử dụng làm chứng dương trong cả hai thử nghiệm. Hỗn hợp phản ứng không có enzyme được sử dụng làm đối chứng trắng (blank), trong khi hỗn hợp không có dung dịch thử được sử dụng làm đối chứng. Khả năng ức chế enzym của dung dịch thử được đánh giá thông qua giá trị phần trăm ức chế và được tính theo công thức:

$$I\ (\%) = (1 - \frac{A_t}{A_c}) \times 100\%$$

Trong đó:
I (%): phần trăm ức chế;
$A_t$: độ hấp thụ quang của mẫu thử;
$A_C$: độ hấp thụ quang của mẫu đối chứng.

Giá trị $IC_{50}$ tương ứng với nồng độ ức chế 50% hoạt tính enzym của mẫu thử được tính dựa theo đồ thị tuyến tính thể hiện mối tương quan giữa nồng độ (C) và phần trăm ức chế (I%); từ đó xây dựng được phương trình hồi quy tuyến tính.

*2.4. Phương pháp xử lý số liệu*

Các dữ liệu nghiên cứu được biểu diễn ở dạng giá trị trung bình ± SD (SD: độ lệch chuẩn). Số liệu được xử lý thống kê bằng phần mềm Microsoft Excel 2016 và SigmaPlot 12.

## 3. Kết quả nghiên cứu

*3.1. Kết quả phân lập và xác định cấu trúc các hợp chất*

**Hợp chất 1:** Axit corosolic

$^1$H-NMR (500 MHz, DMSO-$d_6$, $\delta_H$ ppm): 0,71 (3H, s, H-29); 0,75 (3H, s, H-30); 0,82 (3H, s, H-25); 0,88 (3H, s, H-24); 0,91 (3H, s, H-26); 0,92 (3H, s, H-27); 1,03 (3H, s, H-23); 2,11 (1H, d, *J* = 11,5 Hz, H-20); 2,75 (1H, dd, *J* = 4,0; 9,5 Hz, H-19); 3,42 (1H, t, *J* = 9,5 Hz, H-18); 4,24 (1H, d, *J* = 4,0 Hz, H-2); 4,35 (1H, d, *J* = 9,0 Hz, H-3); 5.14 (1H, brs, H-12).

$^{13}$C-NMR (125 MHz, DMSO-$d_6$, $\delta_C$ ppm): 16,5 (C-25); 16,9 (C-26); 17,1 (C-30); 17,2 (C-24); 18,0 (C-6); 21,1 (C-29); 22,9 (C-11); 23,3 (C-27); 23,8 (C-16); 27,6 (C-15); 28,8 (C-23); 30,2 (C-21); 32,5 (C-7); 36,3 (C-22); 37,6 (C-10); 38,4 (C-19); 38,5 (C-20); 39,0 (C-4); 39,3 (C-8); 41,7 (C-14); 46,9 (C-9); 47,0 (C-17); 47,1 (C-1); 52,2 (C-18); 54,6 (C-5); 67,2 (C-2); 82,1 (C-3); 124,5 (C-12); 138,3 (C-13); 178,2 (C-28).

Hợp chất **1** được phân lập dưới dạng bột màu trắng, có nhiệt độ nóng chảy ở 243-245 $^o$C. Phổ $^1$H-NMR của hợp chất **1** cho thấy có tổng cộng 7 nhóm methyl với các tín hiệu singlet tại $\delta_H$ 0,71 (H-29); 0,75 (H-30); 0,82 (H-25); 0,88 (H-24); 0,91 (H-26); 0,92 (H-27); 1,03 (H-23). Ngoài ra, phổ $^1$H-NMR cũng cho thấy tín hiệu của 2 nhóm hydroxy gắn với cacbon bậc 3 tại $\delta_H$ 4,24 (1H, d, *J* = 4,0 Hz, H-2) và 4,35 (1H, d, *J* = 9,0 Hz, H-3), cùng với 1 tín hiệu của nối đôi tại $\delta_H$ 5,14 (1H, brs, H-12). Đây là các tín hiệu đặc trưng của một triterpen khung ursan. Phổ $^{13}$C-NMR và DEPT của hợp chất này cho thấy sự có mặt của 30 cacbon, bao gồm 7 nhóm methyl tại $\delta_C$ 16,5 (C-25), 16,9 (C-26), 17,1 (C-30), 17,2 (C-24), 21,1 (C-29), 23,3 (C-27), 28,8 (C-23); 8 nhóm methylen tại $\delta_C$ 18,0 (C-6), 22,9 (C-11), 23,8 (C-16), 27,6 (C-15), 30,2 (C-21), 32,5 (C-7), 36,3 (C-22), 47,1 (C-1); 8 nhóm methin tại $\delta_C$ 38,4 (C-19), 38,5 (C-20), 46,9 (C-9), 52,2 (C-18), 54,6 (C-5), 67,2 (C-2), 82,1 (C-3), 124,5 (C-12) và 7 nhóm carbon bậc 4 tại $\delta_C$ 37,6 (C-10), 39,0 (C-4), 39,3 (C-8), 41,7 (C-14), 47,0 (C-17), 138,3 (C-13), 178,2 (C-28). So sánh các số liệu phổ $^1$H-NMR và $^{13}$C-NMR của hợp chất **1** với số liệu của axit corosolic trong tài liệu tham khảo [10] thấy hoàn toàn trùng khớp. Do vậy, có thể khẳng định hợp chất **1** chính là axit corosolic.

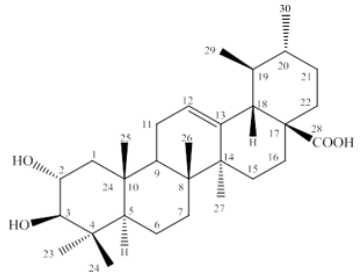
**Hợp chất 2**: 3*α*-acetyl-20*S*,24*S*-epoxydammaran-25-ol

$^1$H-NMR (500 MHz, $CDCl_3$, $\delta_H$ ppm): 0,85 (3H, s, H-28); 0,87 (3H, s, H-19); 0,88 (3H, s, H-29); 0,90 (3H, s, H-30); 0,96 (3H, s, H-18); 1,11 (3H, s, H-17); 1,13 (3H, s, H-21); 1,20 (3H, s, H-26); 2,07 (3H, s, Ac-Me); 3,72 (1H, t, *J* = 7,5; 14,5 Hz, H-24); 4,48 (1H, m, H-3).

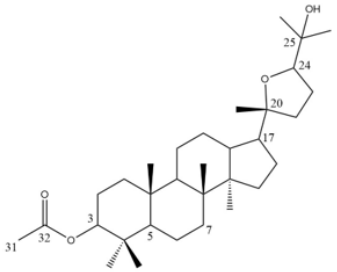
$^{13}$C-NMR (125 MHz, $CDCl_3$, $\delta_C$ ppm): 15,5 (C-18); 16,2 (C-19); 16,6 (C-28); 18,2 (C-6); 21,3 (Ac-Me); 21,6 (C-11); 23,5 (C-30); 23,7 (C-2); 24,3 (C-27); 25,7 (C-16); 26,1 (C-23); 27,1 (C-21); 27,3 (C-12); 27,5 (C-26); 28,0 (C-29); 31,4 (C-15); 35,2 (C-1); 35,8 (C-22); 37,1 (C-7); 37,9 (C-4); 38,7 (C-10); 40,4 (C-8); 42,9 (C-13); 49,5 (C-17); 50,0 (C-14); 50,7 (C-9); 56,0 (C-5); 71,4 (C-25); 80,9 (C-3); 83,3 (C-24); 86,4 (C-20); 171,0 (C-32).

Hợp chất **2** được phân lập dưới dạng tinh thể hình kim, không màu, có nhiệt độ nóng chảy ở 148-150$^o$ C. Phổ $^1$H-NMR của hợp chất **2** xuất hiện tín hiệu proton của hai nhóm methin gắn oxy tại $\delta_H$ 4,48 (1H, m, H-3) và 3,72 (1H, t, *J* = 7,5; 14,5 Hz, H-24). Trên phổ $^1$H-NMR còn cho thấy tín hiệu proton của tám nhóm methyl tại $\delta_H$ 0,85 (3H, s, H-28); 0,87 (3H, s, H-19); 0,88 (3H, s, H-29); 0,90 (3H, s, H-30); 0,96 (3H, s, H-18); 1,11 (3H, s, H-17); 1,13 (3H, s, H-21); 1,20 (3H, s, H-26) và tín hiệu proton của nhóm methyl gắn nhóm rút điện tử tại $\delta_H$ 2,07 (3H, s, Ac-Me).

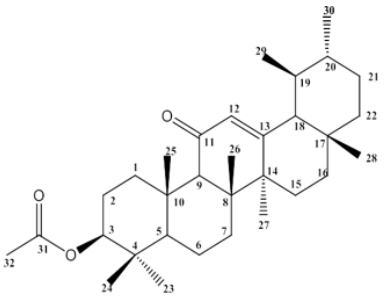
Ngoài ra, còn có sự hiện diện của các tín hiệu proton nhóm methylen và methin trong khoảng $\delta_H$ từ 1,2 đến 1,9 ppm. Phổ $^{13}$C-NMR của hợp chất **2** xuất hiện tín hiệu của 32 carbon, bao gồm 9 nhóm methyl, 10 nhóm methylen, 6 nhóm methin và 7 carbon không liên kết với hydro. Hai tín hiệu carbon methin liên kết với oxy tại $\delta_C$ 80,9 (C-3); 83,3 (C-24) và hai tín hiệu carbon bậc bốn liên kết với oxy tại $\delta_C$ 83,3 (C-24); 86,4 (C-20) cho phép khẳng định hợp chất **2** là triterpenoid có khung dammaran. Các tương tác trên phổ HMBC của $\delta_H$ 4.48 (1H, m, H-3) với $\delta_C$ 16,6 (C-28); 23,7 (C-2); 37,9 (C-4); 171,0 (C-32) và của $\delta_H$ 2,07 (3H, s, Ac-Me) với $\delta_C$ 171,0 (C-32); 80,9 (C-3) chứng minh sự hiện diện của nhóm acetoxyl tại vị trí C-3. Ngoài ra, phổ HMBC còn cho thấy sự tương tác của $\delta_H$ 0,85 (3H, s, H-28) với $\delta_C$ 16,6 (C-28); 80,9 (C-3); 37,9 (C-4); 56,0 (C-5); của $\delta_H$ 1,11 (3H, s, H-17) với $\delta_C$ 86,4 (C-20); 49,5 (C-17); của $\delta_H$ 1,20 (3H, s, H-26) với $\delta_C$ 27,5 (C-26); 83,3 (C-24); 71,4 (C-25) và của $\delta_H$ 3,72 (1H, t, *J* = 7,5; 14,5 Hz, H-24) với $\delta_C$ 27,5 (C-26); 24,3 (C-27). Từ các số liệu phổ trên kết hợp với so sánh tài liệu tham khảo [11], cấu trúc của **2** được xác định là 3*α*-acetyl-20*S*,24*S*-epoxydammaran-25-ol.




Axit corosolic **(1)**




3*α*-acetyl-20*S*,24*S*-epoxydammaran-25-ol **(2)**




11-oxo-α-amyrin acetat **(3)**

Hình 1. Cấu trúc hóa học của các hợp chất **1 – 3**.

**Hợp chất 3**: 11-oxo-α-amyrin acetat

$^1$H-NMR (500 MHz, $CDCl_3$, $\delta_H$ ppm): 0,81 (3H, d, *J* = 2,0 Hz, H-29); 0,82 (3H, s, H-28); 0,88 (3H, s, H-23); 0,89 (3H, s, H-24); 0,95 (3H, d, *J* = 6,5 Hz, H-30); 1,17 (3H, s, H-26); 1,19 (3H, s, H-25); 1,29 (3H, s, H-27); 2,05 (3H, s, H-32); 2,35 (1H, brs, H-9); 4,52 (1H, dd, *J* = 4,5; 12,0 Hz, H-3); 5,54 (1H, s, H-12).

$^{13}$C-NMR (125 MHz, $CDCl_3$, $\delta_C$ ppm): 16,6 (C-26); 16,7 (C-24); 17,5 (C-29); 17,5 (C-6); 18,5 (C-25); 20,5 (C-30); 20,5 (C-27); 21,2 (COO<u>C</u>H$_3$); 23,6 (C-2); 27,2 (C-15); 27,5 (C-22); 28,1 (C-23); 28,8 (C-28); 30,9 (C-21); 32,8 (C-7); 33,9 (C-17); 36,8 (C-10); 38,0 (C-4); 38,9 (C-1); 39,2 (C-19); 39,3 (C-20); 40,9 (C-16); 43,7 (C-14); 45,1 (C-8); 55,0 (C-5); 59,0 (C-18); 61,5 (C-9); 80,8 (C-3); 130,4 (C-12); 165,0 (C-13); 171,0 (<u>C</u>OOCH$_3$); 199,6 (C-11).

Hợp chất **3** thu được tồn tại ở dạng chất rắn vô định hình màu trắng, nóng chảy ở nhiệt độ

278-281 °C. Phổ $^{1}$H-NMR của hợp chất **3** mang đặc trưng của hợp chất triterpen khung ursan với sự hiện diện của 8 nhóm methyl tại $\delta_H$ 0,81 (3H, *d*, *J* = 2,0 Hz, H-29); 0,82 (3H, s, H-28); 0,88 (3H, s, H-23); 0,89 (3H, s, H-24); 0,95 (3H, s, H-30); 1,17 (3H, s, H-26); 1,19 (3H, s, H-25), 1,29 (3H, s, H-27), một nhóm acetoxy tại $\delta_H$ 2,05 (3H, s, H-32) và một olefinic proton tại $\delta_H$ 5,54 (1H, s, H-12). Phổ $^{13}$C-NMR và HSQC của hợp chất **3** cho thấy sự hiện diện của 32 carbon bao gồm chín nhóm methyl, tám nhóm methylen, năm nhóm methin, sáu nhóm carbon bậc bốn bão hòa, một nhóm acetoxyl tại $\delta_C$ 21,2 (COO<u>C</u>H$_3$) và 171,0 (<u>C</u>OOCH$_3$), một nhóm oxymethin tại $\delta_C$ 80,8 (C-3) và một nhóm carbonyl tại $\delta_C$ 199,6 (C-11). Trên phổ HMBC, tương tác giữa H-3 ($\delta_H$ 4,52) với C-23 ($\delta_C$ 28,1), C-24 ($\delta_C$ 16,7), C-31 ($\delta_C$ 171,0) và giữa H-32 ($\delta_H$ 2,05) với C-31 ($\delta_C$ 171,0) cho phép xác định vị trí của nhóm acetoxy tại C-3. Bên cạnh đó, vị trí của nhóm carbonyl tại C-11 được xác định dựa trên tương tác giữa H-9 ($\delta_H$ 2,35) với C-8 ($\delta_C$ 45,1), C-10 ($\delta_C$ 36,8), C-11 ($\delta_C$ 199,6), C-25 ($\delta_C$ 18,5) và C-26 ($\delta_C$ 16,6). Cấu hình α của nhóm acetoxy tại vị trí C-3 được xác định thông qua dạng phân tách của proton H-3 dưới dạng doublet doublet với một hằng số tương tác nhỏ (*J* = 4,5 Hz) và một hằng số tương tác lớn (*J* = 12,0 Hz). Trên cơ sở phân tích các dữ liệu phổ và so sánh với tài liệu tham khảo [12], cấu trúc của hợp chất **3** được xác định là 11-oxo-α-amyrin acetat.

### *3.2. Khả năng ức chế enzym α-glucosidase và α-amylase của các hợp chất phân lập được*

Tác dụng ức chế enzym α-glucosidase và α-amylase của các hợp chất triterpen phân lập từ cây Thài lài trắng được thể hiện trong Bảng 1. Trong ba hợp chất triterpene phân lập được, hợp chất **1** thể hiện khả năng ức chế enzym α-glucosidase tốt nhất với giá trị $IC_{50}$ là 93,68 ± 4,1 µg/mL và tác dụng này mạnh hơn so với chứng dương acarbose ($IC_{50}$ = 135,73 ± 1,8 µg/mL). Tuy nhiên, đối với -amylase, hợp chất **1** có khả năng ức chế kém nhất với giá trị $IC_{50}$ thu được là cao nhất (245,41 ± 2,8 µg/mL), gấp gần 3 lần so với mẫu chứng ($IC_{50}$ = 83,33 ± 2,0 µg/mL). Bên cạnh đó, kết quả nghiên cứu cho thấy mặc dù có khả năng ức chế enzym α-glucosidase thấp nhất trong ba hợp chất thử nghiệm ($IC_{50}$ = 247,03 ± 5,6 µg/mL), hợp chất **2** có hoạt tính ức chế enzym α-amylase mạnh nhất với giá trị $IC_{50}$ thu được là 100,64 ± 3,6 µg/mL. Trong cả hai thử nghiệm, hợp chất **3** thể hiện tác dụng ức chế trung bình với $IC_{50}$ lần lượt là 193,55 ± 2,5 µg/mL đối với enzym α-glucosidase và 242,35 ± 4,3 µg/mL đối với enzym α-amylase.

Bảng 1. Tác dụng ức chế enzym α-glucosidase và α-amylase của các hợp chất triterpene từ cây Thài lài trắng và chứng dương acarbose

| Mẫu | $IC_{50}$ (µg/mL) | |
|---|---|---|
| | α-glucosidase | α-amylase |
| Axit corosolic (**1**) | 93,68 ± 4,1 | 245,41 ± 2,8 |
| 3*α*-acetyl-20*S*,24*S*-epoxydammaran-25-ol (**2**) | 247,03 ± 5,6 | 100,64 ± 3,6 |
| 11-oxo-α-amyrin acetat (**3**) | 193,55 ± 2,5 | 242,35 ± 4,3 |
| Acarbose | 135,73 ± 1,8 | 83,33 ± 2,0 |

## 4. Bàn luận

Bằng phương pháp sắc ký cột, từ cắn chiết phân đoạn *n*-hexan của dịch chiết methanol cây Thài lài trắng (*Commelina diffusa* Burm.f.) đã phân lập được ba hợp chất triterpen tinh khiết gồm axit corosolic (**1**), 3*α*-acetyl-20*S*,24*S*-epoxydammaran-25-ol (**2**) và 11-oxo-α-amyrin acetat (**3**). Trong đó, hợp chất 2 và 3 lần đầu tiên được phân lập từ chi *Commelina*. Axit corosolic là một hợp chất triterpen khung ursan được tìm thấy ở một số loài thực vật trong tự nhiên. Các nghiên cứu đã chứng minh hợp chất này sở hữu nhiều hoạt tính sinh học quý như hạ đường huyết,

chống béo phì, kháng viêm, giảm lipid máu và chống khối u. Axit corosolic cho thấy đặc tính hạ đường huyết đầy hứa hẹn với khả năng tăng cường sự hấp thu glucose, tăng độ nhạy insulin và ức chế các enzym liên quan đến sự hấp thụ carbohydrat, chẳng hạn như α-glucosidase và α-amylase. Những cơ chế này góp phần cải thiện việc kiểm soát đường huyết và có ý nghĩa tiềm năng trong việc quản lý bệnh đái tháo đường và các biến chứng liên quan của nó [13]. Trong khi đó, 3*α*-acetyl-20*S*,24*S*-epoxydammaran-25-ol, còn được gọi là cabraleadiol monoacetate, là một hợp chất triterpen khung dammaran được phân lập từ cây gội bốn cánh (*Aglaia lawii*) thu hái tại Việt Nam [14]. Tương tự như axit corosolic, 11-oxo-α-amyrin acetat là một hợp chất triterpen khung ursan. Hợp chất này đã được chứng minh có hoạt tính kháng khuẩn trên chủng vi khuẩn *S. typhi*, *P. mirabilis* và *E. coli* [15, 16].

Triterpen là nhóm các chất chuyển hóa thứ cấp phân bố rộng rãi trong giới thực vật, hiện diện ở dạng tự do hoặc glycosid. Cấu trúc hóa học của các triterpen có thể là mạch hở 3, 4 hoặc 5 vòng với 33 khung sườn carbon cơ bản chính [17]. Nhiều nghiên cứu dược lý đã chứng minh các tác dụng sinh học nổi bật của triterpen bao gồm chống ung thư, chống oxy hóa, chống viêm, chống xơ vữa động mạch, kháng virut, kháng khuẩn và hạ đường huyết. Triterpen thể hiện hoạt tính hạ đường huyết bằng cách ức chế các enzym liên quan đến chuyển hóa glucose, ngăn ngừa sự phát triển của tình trạng kháng insulin và bình thường hóa lượng glucose và insulin trong huyết tương. Khác với các loại thuốc tổng hợp, các hợp chất tự nhiên này ngoài việc gây tác dụng hạ đường huyết, chúng còn có tác dụng hạ mỡ máu và chống béo phì. Triterpen cũng là tác nhân đầy tiềm năng để ứng dụng trong việc ngăn ngừa các biến chứng của bệnh tiểu đường. Cụ thể, chúng có hoạt tính chống oxy hóa mạnh và ức chế sự hình thành các sản phẩm cuối cùng của quá trình glycation, liên quan đến cơ chế bệnh sinh của bệnh thận, bệnh lý phôi và bệnh thần kinh do đái tháo đường [18]. Trong nghiên cứu này, tác dụng hạ đường huyết của các hợp chất triterpen phân lập từ cây Thài lài trắng được đánh giá thông qua khả năng ức chế *in vitro* enzym α-glucosidase và α-amylase. Kết quả thực nghiệm cho thấy axit corosolic có tác dụng ức chế enzym α-glucosidase mạnh nhất nhưng thể hiện khả năng ức chế enzym α-amylase thấp nhất khi so sánh với chứng dương acarbose. Kết quả này tương đồng với các dữ liệu đã được báo cáo trong công bố trước đây của Zhang và cộng sự [19]. Trong khi đó, đây là nghiên cứu đầu tiên về tác dụng ức chế enzym α-glucosidase và α-amylase của 3*α*-acetyl-20*S*,24*S*-epoxydammaran-25-ol và 11-oxo-α-amyrin acetat. Mặc dù có tác dụng ức chế yếu đối với enzym α-glucosidase, 3*α*-acetyl-20*S*,24*S*-epoxydammaran-25-ol có khả năng ức chế mạnh enzym α-amylase với giá trị $IC_{50}$ thu được là thấp nhất trong ba hợp chất được khảo sát. Trong khi đó, 11-oxo-α-amyrin acetat thể hiện khả năng ức chế trung bình đối với cả hai enzym thử nghiệm.

## 5. Kết luận

Nghiên cứu đã phân lập được ba hợp chất triterpen từ phân đoạn *n*-hexan của dịch chiết methanol cây Thài lài trắng (*Commelina diffusa* Burm.f.), bao gồm axit corosolic (**1**), 3*α*-acetyl-20*S*,24*S*-epoxydammaran-25-ol (**2**) và 11-oxo-α-amyrin acetat (**3**). Cấu trúc hóa học của các hợp chất đã được chứng minh bằng các phương pháp phổ nghiệm và kết hợp với các tài liệu tham khảo đã được công bố trước đó. Đây là nghiên cứu đầu tiên phân lập được hợp chất **2** và **3** từ loài Thài lài trắng nói riêng cũng như chi *Commelina* nói chung. Bên cạnh đó, hoạt tính ức chế enzym α-glucosidase và α-amylase của 3 hợp chất này đã được khảo sát. Kết quả thử nghiệm cho thấy trong ba hợp chất khảo sát, hợp chất **1** có khả năng ức chế enzym α-glucosidase mạnh nhất với giá trị $IC_{50}$ là 93,68 ± 4,1 µg/mL và hợp chất **2** có tác dụng ức chế enzym α-amylase tốt nhất với $IC_{50}$ thu được là 100,64 ± 3,6 µg/mL.

## Lời cảm ơn

Nghiên cứu này được tài trợ bởi Trường Đại học Y Dược, Đại học Quốc gia Hà Nội, mã số đề tài CS.23.03.

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BỘ KHOA HỌC VÀ CÔNG NGHỆ
**CỤC SỞ HỮU TRÍ TUỆ**

**CỘNG HÒA XÃ HỘI CHỦ NGHĨA VIỆT NAM**
**Độc lập – Tự do – Hạnh phúc**

# BẰNG ĐỘC QUYỀN
# GIẢI PHÁP HỮU ÍCH
# Số: 3900

**Tên giải pháp hữu ích:** QUY TRÌNH CHIẾT HỖN HỢP HOẠT CHẤT CÓ TÁC DỤNG HẠ GLUCOZA HUYẾT TỪ CÂY THÀI LÀI TRẮNG (COMMELINA DIFFUSA BURM.F.)

**Chủ Bằng độc quyền:** TRƯỜNG ĐẠI HỌC Y DƯỢC, ĐẠI HỌC QUỐC GIA HÀ NỘI (VN)
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**Tác giả:** 1. Vũ Đức Lợi (VN)
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2. (Danh sách kèm theo)

**Số đơn:** 2-2022-00481

**Ngày nộp đơn:** 08/11/2022

**Số điểm yêu cầu bảo hộ:** 02 **Số trang mô tả:** 39

Cấp theo Quyết định số: 142452/QĐ-SHTT.IP, ngày: 05/12/2024

Có hiệu lực từ ngày cấp đến hết 10 năm tính từ ngày nộp đơn (Hiệu lực bảo hộ cần duy trì hàng năm).

**KT. CỤC TRƯỞNG**
**PHÓ CỤC TRƯỞNG**

**Lê Huy Anh**

V 003900

**BẰNG ĐỘC QUYỀN GIẢI PHÁP HỮU ÍCH SỐ: 3900**

**Tác giả khác:**

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3. LÊ HỒNG DƯƠNG (VN)
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(12) **BẢN MÔ TẢ GIẢI PHÁP HỮU ÍCH THUỘC BẰNG ĐỘC QUYỀN**
**GIẢI PHÁP HỮU ÍCH**

(19) **Cộng hòa xã hội chủ nghĩa Việt Nam (VN)**
CỤC SỞ HỮU TRÍ TUỆ

(11) **2-0003900**

(51) **A61K 36/286**; A61P 3/10; A61P 3/04
2022.01

(13) **Y**

(21) 2-2022-00481
(22) 08/11/2022
(45) 27/01/2025 442
(43) 27/01/2023 418
(73) Trường Đại học Y Dược, Đại học Quốc gia Hà Nội (VN)
Nhà Y1, số 144 Xuân Thủy, quận Cầu Giấy, Hà Nội
(72) Vũ Đức Lợi (VN); Nguyễn Xuân Tùng (VN); Lê Hồng Dương (VN).

(54) QUY TRÌNH CHIẾT HỖN HỢP HOẠT CHẤT CÓ TÁC DỤNG HẠ GLUCOZA HUYẾT TỪ CÂY THÀI LÀI TRẮNG (COMMELINA DIFFUSA BURM.F.)

# SUPPLEMENTARY DOCUMENTS

VIỆN HÀN LÂM
KHOA HỌC VÀ CÔNG NGHỆ VN
**TRƯỜNG ĐẠI HỌC KHOA HỌC VÀ CÔNG NGHỆ HÀ NỘI**
*

**ĐẢNG CỘNG SẢN VIỆT NAM**

Số: 107 -QĐ/ĐHKHCN

*Hà Nội, ngày 06 tháng 5 năm 2026*

# QUYẾT ĐỊNH
## Về việc thành lập Hội đồng đánh giá luận án Tiến sĩ cấp trường đối với nghiên cứu sinh Nguyễn Xuân Tùng

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- Căn cứ Quyết định số 2067/QĐ-TTg ngày 09/12/2009 của Thủ tướng Chính phủ về việc thành lập Trường Đại học Khoa học và Công nghệ Hà Nội (ĐHKHCNHN);
- Căn cứ Quyết định số 2557/QĐ-TTg ngày 30/12/2016 của Thủ tướng Chính phủ về việc ban hành Quy chế tổ chức và hoạt động của Trường ĐHKHCNHN;
- Căn cứ Quyết định số 307/QĐ-ĐHKHCN ngày 04/04/2025 của Hiệu trưởng Trường ĐHKHCNHN về việc ban hành Quy chế đào tạo trình độ tiến sĩ;
- Căn cứ Quyết định số 632/QĐ-ĐHKHCN ngày 30/6/2022 của Hiệu trưởng Trường ĐHKHCNHN về việc công nhận nghiên cứu sinh Nguyễn Xuân Tùng, tên đề tài và người hướng dẫn luận án tiến sĩ;
- Căn cứ Quyết định số 1174/QĐ-ĐHKHCN ngày 24/11/2025 của Hiệu trưởng Trường ĐHKHCNHN về việc thành lập Hội đồng đánh giá luận án tiến sĩ cấp cơ sở đối với nghiên cứu sinh Nguyễn Xuân Tùng;
- Căn cứ Biên bản họp Hội đồng đánh giá luận án tiến sĩ cấp cơ sở đối với nghiên cứu sinh Nguyễn Xuân Tùng ngày 26/01/2026;
- Xét đề nghị của Trưởng phòng Quản lý đào tạo,

**HIỆU TRƯỞNG TRƯỜNG ĐẠI HỌC KHOA HỌC VÀ CÔNG NGHỆ HÀ NỘI**

**QUYẾT ĐỊNH**

**Điều 1.** Thành lập Hội đồng đánh giá luận án tiến sĩ cấp trường đối với nghiên cứu sinh Nguyễn Xuân Tùng, mã số nghiên cứu sinh D22.PMAB.003, ngành Công nghệ Sinh học nông, y, dược.

Tên đề tài luận án: Chemical constituents and hypoglycemic effects of Commelina diffusa Burm.F.

Danh sách thành viên hội đồng tại Phụ lục kèm theo Quyết định này.

**Điều 2.** Hội đồng có trách nhiệm tổ chức đánh giá luận án tiến sĩ của Nghiên cứu sinh theo quy chế đào tạo hiện hành và tự giải thể sau khi hoàn thành nhiệm vụ.

**Điều 3.** Trưởng phòng Quản lý đào tạo, Trưởng khoa Khoa học Sự sống, Chánh văn phòng, Trưởng phòng Bảo đảm chất lượng và Khảo thí, Trưởng phòng Kế toán – Tài chính và các thành viên có tên tại Điều 1 chịu trách nhiệm thi hành quyết định này./.

Nơi nhận:
- Như Điều 3,
- HT và các PHT,
- Lưu: VT, KTTC, KHSS, QLĐT.L5.

**HIỆU TRƯỞNG CHÍNH**

TRƯỜNG ĐẠI HỌC KHOA HỌC VÀ CÔNG NGHỆ HÀ NỘI

**Jean-Marc Lavest**

**DANH SÁCH HỘI ĐỒNG ĐÁNH GIÁ LUẬN ÁN TIẾN SĨ CẤP TRƯỜNG**
**ĐỐI VỚI NGHIÊN CỨU SINH NGUYỄN XUÂN TÙNG**

*(Kèm theo Quyết định số 107-QĐ/ĐHKHCN ngày 06 / 5 /2026)*

| STT | Thành viên Hội đồng | Nơi công tác | Chức danh Hội đồng |
|---|---|---|---|
| 1 | PGS.TS. Nguyễn Hải Đăng | Trường ĐHKHCNHN, Viện Hàn lâm Khoa học và Công nghệ Việt Nam | Chủ tịch |
| 2 | GS.TS. Byung Sun Min | Đại học Công giáo Daegu, Hàn Quốc | Phản biện |
| 3 | PGS.TS. Phạm Thị Nguyệt Hằng | Viện dược liệu | Phản biện |
| 4 | PGS. TS. Bùi Hữu Tài | Viện Hóa học, Viện Hàn lâm Khoa học và Công nghệ Việt Nam | Phản biện |
| 5 | PGS. TS. Nguyễn Xuân Nhiệm | Học viện Khoa học và Công nghệ, Viện Hàn lâm Khoa học và Công nghệ Việt Nam | Ủy viên |
| 6 | TS. Trần Thị Thu Phương | Trường ĐHKHCNHN, Viện Hàn lâm Khoa học và Công nghệ Việt Nam | Ủy viên |
| 7 | TS. Lê Thanh Hương | Trường ĐHKHCNHN, Viện Hàn lâm Khoa học và Công nghệ Việt Nam | Ủy viên, Thư ký |

*Danh sách gồm 07 thành viên./.*

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No. 107 -QĐ/ĐHKHCN

*Hanoi, May 6 , 2026*

# DECISION

## on establishing the Thesis Examination Jury to examine the doctoral thesis of PhD student Nguyen Xuan Tung

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- Pursuant to Decision No. 2067/QĐ-TTg dated December 09, 2009 of the Prime Minister on the establishment of University of Science and Technology of Hanoi (USTH);

- Pursuant to Decision No. 2557/QĐ-TTg dated December 30, 2016 of the Prime Minister on Regulations on organization and operation of USTH;

- Pursuant to Decision No. 307/QĐ-ĐHKHCN dated April 04, 2025 of the Rector of USTH on issuing the Regulation of PhD training;

- Pursuant to Decision No. 632/QĐ-ĐHKHCN dated June 30, 2022 of the Rector of USTH on recognizing doctoral student Nguyen Xuan Tung, thesis topic and thesis supervisor;

- Pursuant to Decision No. 1174/QĐ-ĐHKHCN dated November 24, 2025 of the Rector of USTH on the establishment of Internal Jury to examine the doctoral thesis of PhD student Nguyen Xuan Tung;

- Pursuant to Jury report of the Internal Jury for PhD thesis examination for PhD student Nguyen Xuan Tung dated January 26, 2026;

- At the proposal of Director of Department of Academic Affairs.

**RECTOR OF USTH**

**DECIDES**

**Article 1.** To establish the Thesis Examination Jury to examine the doctoral thesis of PhD student Nguyen Xuan Tung, student ID D22.PMAB.003, program Pharmacological, Medical and Agronomical Biotechnology.

Thesis title: Chemical constituents and hypoglycemic effects of Commelina diffusa Burm.F.

The list of members is attached in the Annex attached to this Decision.

**Article 2.** The Thesis Examination Jury shall be responsible for examining the doctoral thesis according to current training regulations and will be automatically dissolved after completing all tasks.

**Article 3.** The Director of Academic Affairs, the Director of the Department of Life Sciences, the Director of Administration, the Director of Department of Quality Assurance and Examination, the Head of Accounting and Finance Department and the members mentioned in Article 1 shall be in charge of implementing this Decision./.

Recipients:
- As Article 3,
- Rector and Vice-Rectors,
- Archive: Admin, AF, LS, DAA.L5.

**PRINCIPAL RECTOR**
*(Signed and sealed)*
**Jean-Marc Lavest**

**LIST OF MEMBERS OF THE THESIS EXAMINATION JURY FOR PHD STUDENT NGUYEN XUAN TUNG**

*(Attached with the Decision 107-QĐ/ĐHKHCN dated May 6, 2026)*

| No. | Members | Institutions | Position in the jury |
|---|---|---|---|
| 1 | Assoc.Prof. Nguyen Hai Dang | USTH, Vietnam Academy of Science and Technology | Chairman |
| 2 | Prof. Dr. Byung Sun Min | Daegu Catholic University, South Korea | Reviewer |
| 3 | Assoc.Prof. Pham Thi Nguyet Hang | National Institute of Medicinal Materials | Reviewer |
| 4 | Assoc.Prof. Bui Huu Tai | Institute of Chemistry, Vietnam Academy of Science and Technology | Reviewer |
| 5 | Assoc.Prof. Nguyen Xuan Nhiem | Graduate University of Science and Technology, Vietnam Academy of Science and Technology | Member |
| 6 | Dr. Tran Thi Thu Phuong | USTH, Vietnam Academy of Science and Technology | Member |
| 7 | Dr. Le Thanh Huong | USTH, Vietnam Academy of Science and Technology | Member, Secretary |

*The list includes 7 members./.*

# PhD Thesis Examination Jury
# Jury Report

**Time**: From 2:00 PM- 5:00 PM, April 24, 2026
**Location**: Meeting Room 402, USTH Building

## I. JURY MEMBERS:

| No. | Members | Institutions | Position in the jury |
|---|---|---|---|
| 1 | Assoc.Prof. Nguyen Hai Dang | USTH, Vietnam Academy of Science and Technology | Chairman |
| 2 | Prof. Dr. Byung Sun Min | Daegu Catholic University, South Korea | Reviewer |
| 3 | Assoc.Prof. Pham Thi Nguyet Hang | National Institute of Medicinal Materials | Reviewer |
| 4 | Assoc.Prof. Bui Huu Tai | Institute of Chemistry, Vietnam Academy of Science and Technology | Reviewer |
| 5 | Assoc.Prof. Nguyen Xuan Nhiem | Graduate University of Science and Technology, Vietnam Academy of Science and Technology | Member |
| 6 | Dr. Tran Thi Thu Phuong | USTH, Vietnam Academy of Science and Technology | Member |
| 7 | Dr. Le Thanh Huong | USTH, Vietnam Academy of Science and Technology | Member, Secretary |

## II. PHD STUDENT:

| | |
|---|---|
| **Full name** | **Nguyen Xuan Tung** |
| **Student ID** | D22.PMAB.003 |
| **Program** | Pharmacological, Medical and Agronomical Biotechnology |
| **Thesis title** | Chemical constituents and hypoglycemic effects of Commelina diffusa Burm.F./ *Nghiên cứu thành phần hóa học và tác dụng hạ đường huyết của cây Thài lài trắng (Commelina diffusa Burm.F.).* |
| **Supervisor** | Assoc. Prof. Vu Duc Loi, Vietnam University of Traditional Medicine |
| **Co-supervisor** | Assoc.Prof. Nguyen Thi Van Anh, USTH, VAST |

## III. JURY COMMENTS:

- Assoc. Prof. Phạm Thị Nguyệt Hằng

+ Revise and clarify the Materials and Methods section, including the rationale for the selection of the experimental models, the addition of appropriate supporting references, and the completion of methodological information such as chemicals, equipment, the statistical software version (SPSS), the sample size for histopathological evaluation, and the study site.
+ Review and improve the presentation of the results, including the addition of scale bars to gross images and the use of descriptive names for experimental groups in tables and figures instead of numerical labels (e.g., Group 1–5) to improve clarity.
+ Review the reference list by removing unnecessary references and retaining the most relevant and scientifically valuable citations directly related to the research.
+ Standardize scientific terminology, botanical nomenclature, and formatting throughout the dissertation, including the correct use of scientific names (italicization and capitalization) and ensuring consistency in technical terminology and formatting.

+ Revise the Vietnamese summary by using standardized scientific terminology, translating technical terms into appropriate Vietnamese where applicable, and providing complete information on the experimental conditions (e.g., treatment dose and duration) to avoid ambiguity.

- Assoc. Prof. Bui Huu Tai

+ Revise the Materials and Methods section by correcting and completing experimental information, including the particle size of the reverse phase, the length of the chromatography column, stock solution concentrations for the enzymatic inhibition assays, and confirmation of the enzyme solution unit.
+ Carefully verify the structural elucidation data by checking the multiplicity of proton signals (H-20, H-19, H18, H-2), stereochemical assignments for compound 2, compound 4, compound 12, carbon chemical shift assignments C-8' and C-7', NMR data, and the consistency of NMR solvent descriptions throughout the dissertation and the Appendix.
+ Improve the presentation of the phytochemical results by adding a figure summarizing the chemical structures of all isolated compounds.
+ Strengthen the molecular docking section by providing a clearer rationale for the selection of proteins (PDB IDs: 3W37 and 2QV4) and discussing their relationship with the *in vitro* experimental findings.

+ Revise the Conclusion and Recommendations sections by improving the academic writing style, using appropriate passive voice where applicable, and expanding the recommendations to include the limitations of the current study and future research directions.

- Prof. Byung Sun Min

+ Clarify the novelty and scientific significance of the newly identified compounds and better highlight their contributions to the field.

+ Provide more detailed phytochemical methodology, including chromatographic purification procedures, compound yields, and purity assessment.

+ Strengthen the discussion of the molecular docking results by clearly acknowledging the limitations of docking-based predictions and the need for experimental validation.

+ Revise the English language throughout the thesis to correct grammatical, stylistic, and typographical errors and improve readability.

+ Strengthen the Conclusion by emphasizing the practical significance of the findings and highlighting the potential of the most promising compounds (e.g., compounds 9, 10, and 14) as lead candidates for future antidiabetic drug development.

- Assoc. Prof. Nguyen Xuan Nhiem

Several minor issues should be addressed, including clarification of the extraction and *in vivo* formulation procedures, interpretation of histopathological and biochemical findings, improvement of phytochemical data presentation, consistency of terminology, reference formatting, and English language editing

- Dr. Tran Thi Thu Phuong

+ Clarify the novelty and scientific contributions of the study by better defining the research gap and the originality of the thesis.

+ Improve the interpretation of the results by strengthening the discussion of structure–activity relationships and the significance of the enzyme inhibition and molecular docking findings.

+ Strengthen the Discussion by incorporating more recent literature, discussing study limitations, and better integrating the *in vitro*, *in vivo*, and *in silico* results.

+ Provide more explicit methodological information, particularly regarding ethical approval and experimental procedures.

+ Revise the overall organization and language by reducing repetitive descriptions and improving the academic writing style.

- Dr. Le Thanh Huong

+ Strengthen the interpretation of the biological activity by discussing the limitations of the current evidence and avoiding overstatement of the proposed antidiabetic mechanism (e.g., distinguish antihyperglycemic from antidiabetic activity where appropriate).

+ Improve the histopathological evaluation by including quantitative or semi-quantitative analyses (e.g., histological scoring, morphometric analysis, pancreatic islet quantification) and adding scale bars to histological images.

+ Provide a more critical discussion of the results, particularly where discrepancies exist between different findings (e.g., improved liver histology versus unchanged AST and ALT levels).

+ Clarify the limitations of the computational analysis by emphasizing that molecular docking and ADMET predictions require experimental validation.

+ Improve the consistency and accuracy of scientific writing by using terminology, abbreviations, and wording consistently throughout the thesis (e.g., antihyperglycemic vs. hypoglycemic vs. antidiabetic), and revising statements in the Abstract that imply quantitative significance when only qualitative observations were presented.

- Assoc. Prof. Nguyen Hai Dang

+ The thesis is well structured and the research objectives have been achieved. The study provides valuable phytochemical information on Commelina diffusa and demonstrates the antihyperglycemic potential of its ethyl acetate fraction through both in vitro and in vivo experiments

+ Use the uM with the pure compound instead of ug/ml

+ The candidate should address the comments and recommendations provided by the reviewers and members of the examination jury to further improve the quality of the thesis.

## IV. CONCLUSION:

☑ The title of the thesis is appropriate for the program of Pharmacological, Medical and Agronomical Biotechnology (Code: 9420201).

☑ The thesis does not overlap with any previously published works or theses.

☑ The main scientific conclusions, new findings, and contributions of the thesis are as follows:

- Successfully isolated and structurally elucidated fourteen compounds from *Commelina diffusa* Burm.f., including several compounds reported for the first time from this species and the genus *Commelina*, thereby expanding the phytochemical knowledge of this medicinal plant.

- Identified the ethyl acetate fraction (CD.EAF) as the most active fraction against α-glucosidase and α-amylase and identified several compounds with potent enzyme inhibitory activities through bioactivity-guided fractionation.
- Demonstrated the antihyperglycemic potential and acute safety of CD.EAF in a high-fat diet/streptozotocin-induced diabetic mouse model, providing a scientific basis for further pharmacological investigation of *C. diffusa*.

Comments and suggestions

Clarify the preparation of CD.EAF for the in vivo experiments. Since the ethyl acetate fraction is not readily soluble in water, the manuscript should clearly describe how the extract was dispersed or solubilized prior to oral administration, and justify the choice of the tested doses (100 and 300 mg/kg). In addition, please ensure consistency regarding the positive controls used in the *in vitro* and *in vivo* studies.

Expand the discussion regarding the discrepancy between histopathological observations and biochemical findings. Although liver histology suggested marked tissue injury in diabetic mice, serum AST and ALT levels were not significantly different between the normal and diabetic groups. This inconsistency should be discussed in greater detail.

The reference list is excessively long. It is recommended to remove redundant or less relevant citations and retain the most representative and up-to-date references.

For previously reported compounds, appropriate literature citations should be provided for structural identification. The NMR data should be presented together with comparison tables against published values to better support compound identification.

☐ Agree to award the PhD diploma to the candidate.

☑ Agree to award the PhD diploma to the candidate after revision.

☐ The thesis is not qualified for the PhD diploma. A second jury could be considered.

## V. SIGNATURES:

| Chairman | Secretary |
|---|---|
| Assoc.Prof. Nguyen Hai Dang | Dr. Le Thanh Huong |

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# Examination Jury for PhD thesis
## Jury Member's Assessment Form

| PhD Student | |
|---|---|
| **Full name** | Nguyen Xuan Tung |
| **Program** | Pharmacological, Medical and Agronomical Biotechnology |
| **PhD ID** | D22.PMAB.003 |
| **PhD thesis** | |
| **Title** | Chemical constituents and hypoglycemic effects of *Commelina diffusa* Burm.F./ *Nghiên cứu thành phần hóa học và tác dụng hạ đường huyết của cây Thài lài trắng (Commelina diffusa Burm.F.)* |
| **Supervisor** | Assoc. Prof. Vu Duc Loi, Vietnam University of Traditional Medicine |
| **Co-supervisor** | Assoc.Prof. Nguyen Thi Van Anh, USTH, VAST |
| **Jury Member** | |
| **Full name** | Nguyen Hai Dang |
| **Title** | Assoc. Prof. Dr. |
| **Institution** | University of Science and Technology of Hanoi |
| **Contact (Email/SMS)** | nguyen-hai.dang@usth.edu.vn |
| **Role (Chairman/ Reviewer/ Member)** | Chairman |

**Technical comments (e.g. on the thesis topic, research methodology, finding, discussion, thesis structure, wording, references, etc). Please note that the reviewers can fill this part as "please refer to my review report" and/or with additional comments on the revised manuscript and/ or on the presentation made by the candidate**

**1. Research Topic and Relevance**

The thesis addresses a timely and clinically relevant topic — the phytochemical investigation and antidiabetic potential of *Commelina diffusa* Burm.f., a plant with traditional use in Vietnamese medicine. Given the rapid global increase in type 2 diabetes prevalence and the growing interest in plant-derived enzyme inhibitors as therapeutic agents, the research question is scientifically

justified and of significant public health importance. The dual focus on chemical characterization and biological activity, complemented by *in silico* study, represents a comprehensive and well-conceived research framework.

**2. Research Methodology**

The methodology is generally sound and appropriate for the research objectives. The extraction and isolation procedures (maceration, TLC, column chromatography with silica gel and Sephadex LH-20) are standard and well-established for phytochemical work. Structure elucidation by NMR (1H, 13C, HSQC, HMBC), ESI-MS, and comparison with published literature is rigorous and appropriate. The *in vitro* enzyme inhibition assays using pNPG (α-glucosidase) and DNSA (α-amylase) methods are validated and widely accepted. The *in vivo* model of HFD/STZ-induced type 2 diabetic mice and the acute toxicity study (OECD 423/425 guidelines) are appropriately designed. The molecular docking validation using RMSD (both < 1.5 Å) adds credibility to the computational analysis. Statistical analysis using ANOVA with Tukey's post-hoc test is appropriate.

**3. Key Findings**

The study yielded 14 isolated compounds, of which 8 (compounds 1–3, 5, 8–10, and 13) are reported for the first time from *C. diffusa* and the entire *Commelina* genus, a notable original contribution. The identification of N-trans-p-coumaroyl-3',4'-dihydroxyphenylethylamine (compound 9) as the most potent α-glucosidase inhibitor (IC50 = 19.63 μg/mL, ~7-fold more active than acarbose) and compound 10 as the strongest α-amylase inhibitor (IC50 = 26.15 μg/mL) are significant. The CD.EAF demonstrated in vivo antihyperglycemic efficacy and histoprotective effects on liver and pancreatic tissues in the HFD/STZ mouse model, with a favorable acute toxicity profile (LD50 > 2000 mg/kg). These findings are internally consistent and well-supported by the data presented.

**4. Discussion**

The discussion chapter is thorough and places the findings in appropriate context with the existing literature. The comparison of isolated compounds with analogues from related species, the mechanistic discussion of enzyme inhibition, and the acknowledgement of limitations of the molecular docking approach (scoring function subjectivity, discrepancy between in silico and in vivo conditions) are commendable. However, the discussion could be strengthened by a more explicit mechanistic explanation for why the CD.EAF did not significantly improve lipid parameters despite reducing blood glucose levels. A deeper interpretation of the ADMET predictions in relation to drug development potential would also be beneficial.

**5. Thesis Structure and Presentation**

The thesis is logically structured, progressing from literature review through methodology, results, and discussion to conclusions and recommendations. Tables and figures are clearly labeled and informative. However, there are several minor issues that warrant attention: (a) the conclusion section numbering references "References" on page 135 and "List of publications" on page 136 appear reversed in the table of contents; (b) some compound names are exceptionally long — abbreviations or systematic short names should be used consistently in the text; (c) the sentence at the end of the abstract ("orienting the application in the prevention and treatment of type diabetes") contains a typographical omission ("type 2 diabetes"); (d) minor grammatical issues are present throughout and should be corrected in a revised version.

**6. References**

The reference list is comprehensive and up-to-date, drawing from high-quality international journals. The citation format is consistent. The work is appropriately supported by 4 peer-reviewed publications (2 SCIE-indexed international journal articles, 2 national journal papers) and 1 granted patent, demonstrating the candidate's ability to disseminate research outcomes. The international publications in Revista Brasileira de Farmacognosia (Q2, IF=2.464) and Pakistan Journal of Pharmaceutical Sciences (Q3, SCIE) are appropriate venues for this type of research.

**7. Suggestions for Improvement**

Minor revisions are recommended:

(1) correct typographical and grammatical errors throughout;

(2) provide more in-depth discussion about the reason of designing experimental methods as well as the meaning of results obtained;

(3) add a brief discussion on the lack of lipid-lowering effect of CD.EAF;

(4) the Lipinski rule of five interpretations for compound **2** (LogP = 7.31) and compound **10** (MW = 684.7, HBA = 10) should be discussed more clearly in the context of their promising bioactivity.

**Comment on the candidate ability (e.g. general scientific background, understanding on the research field and research topic, presentation skill, English level, etc)**

The candidate, Nguyen Xuan Tung, demonstrates a solid command of the scientific background required for this interdisciplinary research, which spans natural product chemistry, pharmacology, and computational biology. The integration of phytochemical isolation, biological activity assessment, in vivo animal experimentation, and molecular docking reflects a broad and versatile scientific skill set. The candidate has clearly mastered standard chromatographic and spectroscopic techniques and is able to critically interpret complex spectral data for structure elucidation.

The candidate's understanding of the research field is evident in the thorough literature review covering diabetes epidemiology, pharmacological treatment strategies, enzyme inhibition mechanisms, and the phytochemistry of the *Commelina* genus. The thesis reflects independent thinking and the ability to design and execute a multifaceted research plan. Research productivity is commendable, with 2 SCIE-indexed publications, 2 national journal papers, and a granted patent — all directly related to the thesis work.

The English writing is generally clear and comprehensible, though there are scattered grammatical errors and occasional awkward phrasing throughout the manuscript. These do not significantly impede comprehension but should be addressed in the final version. Overall, the candidate demonstrates the scientific maturity and technical competence expected at the doctoral level.

**Conclusions (indicates whether the manuscript is accepted in the current form or accepted after minor/ major revisions; and whether the candidate deserves the USTH PhD degree)**

The doctoral thesis of Nguyen Xuan Tung presents original and scientifically sound research on the chemical constituents and hypoglycemic potential of *Commelina diffusa* Burm.f. The work fulfills all four stated objectives with adequate rigor. The isolation of 14 compounds (8 new to the genus), the identification of potent enzyme inhibitors significantly more active than acarbose, the in vivo antidiabetic validation, and the in silico characterization constitute a meaningful and publishable body of work that advances knowledge in natural product pharmacology and antidiabetic drug discovery from medicinal plants.

☐ **Accept the manuscript in the current form.**

☒ **Accept the manuscript with minor/ major revisions.**

☐ **The manuscript is not qualified to for the PhD degree.**

Hanoi, 07/2026

**Jury Member's signature**

**Nguyen Hai Dang**

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TRƯỜNG ĐẠI HỌC KHOA HỌC & CÔNG NGHỆ HÀ NỘI

# Examination Jury for PhD thesis
## Reviewer's Report

| PhD Student | |
|---|---|
| **Full name** | Nguyen Xuan Tung |
| **Program/ Department** | PMAB/ Life Sciences |
| **Student ID number** | D22.PMAB.003 |
| **PhD thesis** | |
| **Title** | Chemical constituents and hypoglycemic effects of Commelina diffusa Burm.F. |
| **Supervisor** | Assoc. Prof. Vu Duc Loi, Vietnam University of Traditional Medicine |
| **Co-supervisor** | Assoc.Prof. Nguyen Thi Van Anh, USTH, VAST |
| **Reviewer** | |
| **Full name** | BYUNG SUN MIN |
| **Title** | Professor |
| **Institution** | Daegu Catholic University, Republic of Korea |
| **Contact (Email/SMS)** | bsmin@cu.ac.kr |
| **Technical comments (e.g. on the research topic, methodology, finding, discussion, thesis structure, wording, references etc)** | |

This doctoral thesis presents a systematic and multidisciplinary investigation of *Commelina diffusa* Burm.f., integrating phytochemistry, enzymology, pharmacology, and computational approaches. The overall research design is logical and well organized, progressing from plant extraction and compound isolation to biological evaluation and mechanistic interpretation.

A major strength of the thesis is the successful isolation and structural elucidation of fourteen compounds from *C. diffusa*, including several compounds reported for the first time from this species and the genus *Commelina*. The structural characterization using chromatographic and spectroscopic techniques demonstrates adequate technical competence and contributes valuable phytochemical information to the scientific literature.

The biological evaluation is also comprehensive. The author assessed α-glucosidase and α-amylase inhibitory activities at both extract and pure compound levels and further validated the antidiabetic potential using a high -fat diet/streptozotocin-induced diabetic mouse model. The inclusion of acute toxicity testing, histopathological examination, and biochemical analyses strengthens the pharmacological findings and provides supporting evidence for safety and efficacy.

The molecular docking and ADMET prediction studies add mechanistic insights and help identify potential lead compounds. The consistency between the enzyme inhibition data and docking results supports the proposed mode of action.

Nevertheless, some limitations should be acknowledged. The study relies primarily on enzyme inhibition and animal experiments, while the molecular mechanisms underlying glucose regulation were not investigated in detail. Biomarkers related to insulin secretion, insulin resistance, glucose transport, and inflammatory pathways would have strengthened the mechanistic interpretation. Furthermore, the active compounds were not evaluated individually in animal models, making it difficult to determine their specific contributions to the observed in vivo effects. Despite these limitations, the technical quality of the experimental work is satisfactory and meets the expectations of doctoral-level research.

**Questions/ Suggestions/ Request of Revisions**

Before final approval, several revisions are recommended to improve the scientific quality and clarity of the the sis.

1. A clearer explanation of the novelty and significance of compounds newly reported from *C. diffusa* would enhance the scientific impact of the work.

2. Additional information regarding chromatographic purification procedures, compound yields, and purity assessment should be presented more clearly.

3. The molecular docking section should discuss the limitations of docking-based predictions more thoroughly. Docking scores alone cannot confirm biological activity, and this limitation should be explicitly acknowledged.

4. The English language should be carefully edited throughout the thesis. Several sections contain grammatical inconsistencies, awkward phrasing, and typographical errors that may affect readability. Professional language editing is recommended.

5. The conclusion should better emphasize the practical implications of the findings, particularly the potential of compounds **9**, **10**, and **14** as lead molecules for future antidiabetic drug development and the future studies required for their validation.

**Conclusions (whether the work is suitable/ could be suitable after revisions for presenting to obtain the USTH PhD degree?)**

The thesis addresses an important global health problem by exploring novel natural products with potential ant idiabetic activity. The research objectives are clearly defined, and the experimental work is extensive and multi disciplinary. The candidate successfully combines phytochemical investigation with pharmacological validation and computational analysis, producing results that contribute new knowledge regarding the chemical constitue nts and hypoglycemic effects of *C. diffusa*.

The originality of the study is demonstrated by the identification of several compounds reported for the first time from *C. diffusa* and the genus *Commelina*. The biological findings, particularly the enzyme inhibitory activities and in vivo antihyperglycemic effects, provide meaningful evidence supporting the traditional medicinal use of the plant. Furthermore, the integration of molecular docking and ADMET prediction enhances the scientific value of the work.

Although some limitations exist, including the absence of detailed mechanistic studies and the lack of in vivo evaluation of individual active compounds, these issues do not significantly diminish the overall quality of the research. The study was conducted using appropriate methodologies, the results are generally convincing, and the conclusions are supported by the experimental data.

In my opinion, this thesis satisfies the academic and scientific requirements expected for a doctoral dissertation in the field of pharmacological and natural products research. Subject to minor revisions addressing the comments above, I recommend that the thesis be accepted for the award of the Ph.D. degree.

| |
|---|
| ■ **Recommended for defense at the PhD Thesis Examination Jury in the current form.**<br>☐ **Recommend for defense at the PhD Thesis Examination Jury with minor/ major revisions.**<br>☐ **Not recommended for defense at the PhD Thesis Examination Jury.** |
| ..... June 24, 2026<br>**Reviewer's signature**  |

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# Examination Jury for PhD thesis

## Reviewer's Report

| PhD Student | |
|---|---|
| **Full name** | Nguyen Xuan Tung |
| **Program/ Department** | PMAB/ Life Sciences |
| **Student ID number** | D22.PMAB.003 |
| **PhD thesis** | |
| **Title** | Chemical constituents and hypoglycemic effects of Commelina diffusa Burm.F. |
| **Supervisor** | Assoc. Prof. Vu Duc Loi, Vietnam University of Traditional Medicine |
| **Co-supervisor** | Assoc.Prof. Nguyen Thi Van Anh, USTH, VAST |
| **Reviewer** | |
| **Full name** | Pham Thi Nguyet Hang |
| **Title** | Assoc. Prof. Ph.D. |
| **Institution** | Faculty of Pharmacy, Phenikaa University |
| **Contact (Email/SMS)** | Phamhangtoyama@gmail.com |

**Technical comments (e.g. on the research topic, methodology, finding, discussion, thesis structure, wording, references etc)**

**1. Relevance, Scientific and Practical Significance of the Research Topic**

Diabetes mellitus, particularly type 2 diabetes, is rapidly increasing globally and has become one of the most pressing public health challenges. A key therapeutic strategy is the inhibition of carbohydrate-

digesting enzymes such as α-glucosidase and α-amylase to reduce glucose absorption and control postprandial blood glucose levels. Although synthetic inhibitors (such as acarbose, voglibose, and miglitol) are widely used, they are often associated with gastrointestinal side effects (e.g., abdominal pain, flatulence, diarrhea). Therefore, searching for highly effective natural active ingredients with fewer side effects from traditional medicinal plants is a highly practical and significant demand.

*Commelina diffusa* Burm.f. is a common plant species widely distributed in Vietnam and has been traditionally used to treat various ailments. Although some species in the same genus, such as *C. communis* and *C. benghalensis*, have demonstrated antidiabetic activities, specific scientific evidence regarding the hypoglycemic effects and active phytochemical profiles of *C. diffusa* remains very limited. Therefore, the PhD research of Nguyen Xuan Tung, focusing on the extraction, isolation, in vitro enzyme inhibition, acute toxicity, and in vivo hypoglycemic evaluation of active fractions coupled with molecular docking, is highly necessary and holds both significant scientific and practical values for the development of support products in type 2 diabetes management.

**2. Novelty, Scientific Integrity, and References**

***2.1. Novelty:***

The thesis does not overlap with any previously published works by other authors to the best of the reviewer's knowledge. While the anti-inflammatory and hepatoprotective/nephroprotective properties of *Commelina diffusa* have been reported, this is the first study to systematically and deeply investigate its hypoglycemic activity, directly linked to the structural isolation of active compounds.

***2.2. Scientific Integrity*:**

The research objectives have been successfully achieved. References are adequately cited, honest, clear, and highly accurate.

**3. Alignment of the Research Topic, Contents, and Specialization**

The thesis was successfully executed under 4 main objectives:

- Extract, isolate, and structurally elucidate compounds from the active fractions of *Commelina diffusa*;
- Evaluate the *in vitro* α-glucosidase and α-amylase inhibitory activities of the extracts, fractions, and isolated compounds;
- Evaluate the acute toxicity and *in vivo* hypoglycemic activity of the most potent enzyme-inhibiting fraction in animal models;

- Investigate the interaction mechanisms of the isolated compounds on the enzyme targets through in silico models.

The objectives and research contents align perfectly with the thesis title, the major of Biotechnology, and the specialized field of **Pharmacological, Medical, and Agronomical Biotechnology (PMAB)**.

**4. Reliability and Modernity of the Methodology**

- The research methods applied in this thesis are modern, standardized, and widely accepted in the field. Hence, the methodology is highly reliable.

- The data were processed using appropriate statistical tools. The findings have been published in prestigious national and international journals, ensuring the scientific rigorousness and validity of the research.

**5. Novel Research Findings and Key Contributions**

The new findings of the author include:

- ✓ **Phytochemistry:** Successfully isolated and structurally identified 14 pure compounds from *Commelina diffusa*. Among these, compounds **1, 2, 3, 5, 8, 9, 10, and 13** were isolated for the first time from *C. diffusa* and the *Commelina* genus. In addition, compounds **7, 11, 12, and 14** were reported for the first time in this plant species.
- ✓ ***In vitro* Bioactivity:** Determined that the ethyl acetate fraction (CD.EAF) showed the strongest inhibitory activity against both α-glucosidase and α-amylase. The isolated compounds exhibited concentration-dependent inhibition, with compounds **9, 10, and 14** showing the most potent activities.
- ✓ **Toxicity and *in vivo* Evaluation:** Proved that the CD.EAF fraction is relatively safe, with an $LD_{50}$ > 2000 mg/kg. In the type 2 diabetic mice model, daily oral administration of CD.EAF demonstrated significant antihyperglycemic activity ($p < 0.05$) and remarkably improved the histopathological state of damaged liver and pancreas tissue.
- ✓ ***In silico* analysis:** Molecular docking analysis clarified the binding affinity and interaction mechanisms of compounds **10 and 14** towards the target enzymes compared to the reference drug acarbose, alongside comprehensive ADMET pharmacokinetic and toxicity profile predictions.

**6. Layout, Structure, and Formatting of the Thesis**

- The thesis is well-structured and spans 135 pages: Introduction & Literature Review (39 pages), Materials and Methods (15 pages), Results (53 pages), Discussion (23 pages), and Conclusions & Recommendations (2 pages). It references 272 publications, mostly in English, highly updated and relevant to the study.

✓ **Strengths:** The thesis is professionally presented with clear tables and figures. The academic writing style is coherent, fluent, and fully meets the standards of a PhD thesis in Life Sciences. The literature review is comprehensive, the results are rich with new scientific contributions, and the discussion is logically presented to support the mechanisms of action.

**Questions/ Suggestions/ Request of Revisions**

Despite the high quality of the work, the author should address the following comments and suggestions for final revision:

**1. Methodology:**

- Add proper references for the pharmacological and biological models used. For instance, which publication was the high-fat diet (HFD) protocol cited from? Why was fructose added to the drinking water of the HFD group?
- Provide a list of chemicals/reagents used for quantifying biochemical parameters such as ALT, AST, TC, TG, HDL-C, and LDL-C.
- In Section 2.4.3 ("Equipment for in vivo hypoglycemic activity study"), the listed items are diagnostic kits ("Biochemical diagnostic kits (Erba, India)"). Please correct the section title or category.
- In Section 2.6.3, please specify the exact version of the SPSS software used for statistical analysis.
- Add the sample size (n) used for histopathological slide reading of the liver and pancreas.
- State clearly where the animal research and experiments were physically conducted.

**2. Results:**

- Add scale bars to all macro-anatomical and histological figures.
- In Section 3.2.3, all experimental group labels (Group 1–5) in tables and figures should be explicitly defined (e.g., normal control, diabetic control, treatment group with specific dosages) instead of generic codes to make it easier for readers to follow.

**3. References:**

- The number of references is overly extensive (272). The author should streamline and prune the bibliography to only focus on essential papers, ideally keeping the count under 150 references.

**4. General Presentation & Terminology:**

- Review and uniformize specialized terminologies and scientific nomenclature. For example, botanical names like *Commelina diffusa* Burm.f. must be consistently capitalized and italicized. Fix typos like "Swiss mice => Swiss mice" (standardize font formatting) and ensure the decimal separators are consistent throughout the document.

- The Vietnamese abstract contains some non-standardized terms that should be localized properly. For example, "Phân tích ghép nối" should be changed to "docking phân tử" (molecular docking).

- In the sentence: *"Trong nghiên cứu in vivo, sau thời gian điều trị, việc sử dụng lặp lại CD.EAF hàng ngày ở cả hai liều thử nghiệm đều cho thấy hoạt tính chống tăng đường huyết đáng kể so với nhóm đối chứng mắc bệnh tiểu đường ($p < 0,05$)"*, please specify the exact doses used and the duration of the treatment.

**Conclusions (whether the work is suitable/ could be suitable after revisions for presenting to obtain the USTH PhD degree?)**

Despite some minor formatting and presentation issues, I highly value the scientific quality and the tremendous efforts of the PhD student given the research conditions in Vietnam. This thesis has accumulated a substantial volume of valuable results and made significant new contributions to the search for natural hypoglycemic compounds from Vietnamese medicinal plants.

The quality and output of the work fully meet the rigorous requirements of a PhD thesis as regulated by Article 30 of Circular 18/2021/TT-BGDĐT by the Ministry of Education and Training.

After incorporating the recommended revisions and suggestions above, I strongly recommend that the PhD Thesis Examination Jury approve the thesis so that the candidate, Nguyen Xuan Tung, can be awarded his PhD Degree in Life Sciences.

☐ **Recommended for defense at the PhD Thesis Examination Jury in the current form.**

☒ **Recommend for defense at the PhD Thesis Examination Jury with minor/ major revisions.**

☐ **Not recommended for defense at the PhD Thesis Examination Jury.**

Hanoi, June 22, 2026

**Reviewer's signature**

**Assoc. Prof. Pham Thi Nguyet Hang, Ph.D**

UNIVERSITY OF SCIENCE & TECHNOLOGY OF HANOI
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USTH
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# Examination Jury for PhD thesis

## Reviewer's Report

| PhD Student | |
|---|---|
| **Full name** | Nguyen Xuan Tung |
| **Program/ Department** | PMAB/ Life Sciences |
| **Student ID number** | D22.PMAB.003 |
| **PhD thesis** | |
| **Title** | Chemical constituents and hypoglycemic effects of Commelina diffusa Burm.F. |
| **Supervisor** | Assoc. Prof. Vu Duc Loi, Vietnam University of Traditional Medicine |
| **Co-supervisor** | Assoc.Prof. Nguyen Thi Van Anh, USTH, VAST |
| **Reviewer** | |
| **Full name** | **Bui Huu Tai** |
| **Title** | Assoc. Prof. |
| **Institution** | Institute of Chemistry |
| **Contact (Email/SMS)** | bhtaiich@gmail.com |

**Technical comments (e.g. on the research topic, methodology, finding, discussion, thesis structure, wording, references etc)**

This thesis goals to investigate chemical composition of *Commelina diffusa* Burm.f. and its antidiabetic effects including extraction, isolation, and determination the chemical structures of compounds from *Commelina diffusa*; evaluation *in vitro* and *in silico* the α-glucosidase and α-amylase inhibitory activities of the extract fractions and/or compounds isolated from *C. diffusa*; and evaluation acute toxicity and hypoglycemic effect of the ethyl acetate fraction of *C. diffusa*.

- Research topic and obtained results have not been previously published by other groups.

- Results meet requirments of a dissertation thesis.

- The thesis used appropriate set of approved, validated tools and methodologies which have been used by worldwide scientists in the same research field.

+ Sample collection and taxonomical identification are performed by experienced botanists.

+ Extration, isolation, purification processes are performed by routine methods such as chromatograpic methods. Compounds are elucidated by modern spectroscopic techniques such as 1D- and 2D-NMR, MS analysis as well as comparison spectral data with those reported in the literature.

+ *in vitro* antidiabetic effects are evaluated by α-glucosidase and α-amylase inhibitory assay

+ *in silico* antidiabetic effects are performed by molecular docking and prediction of physicochemical profiles (ADMET) using approved softwares

+ Acute toxicity assay and *in vivo* antidiabetic effects are performed according to previously described methods.

- The results showed positive infuence, appropirately disscused and cited references.

- Dissertation works and contributions:

- The three main contributing points can be outlined as above thesis objectives

- Conclusions showed major results of the thesis.

**Questions/ Suggestions/ Request of Revisions**

***Revisions:***

- Page 41: particle size of Reverse phase; Dianion → Diaion;

- Page 46: length of column for column chromatography

- Page 47: delete "A gradient …organic extract"

- Enzymatic inhibition assays: concentration of stock solution for compounds and extract should be provided

- Page 50: confirm again enzyme solution unit: 50 U/mL

- Page 61: correction multiplicity of proton signals: H-20, H-19, H-18, H-2

- confirm again stereochemistry of compound 2 (Page 62:); compound 4 (C-20, page 65); compound 12 (rhamnose moiety, page 74)

- Page 69: re-assigment carbon chemical shift of C-8′ and C-7′

- Page 76: confirm again NMR data of compound 13 as shown in the Appendix

- Check again description NMR solvent through out the text which should be matched with those in the Appendix

- Page 110: give a figure that shows chemical structure of all isolated compounds.

- Page 127: Give short explanation for selection proteins ID 3W37 and 2QV4 for docking study. Are there any relationships between selected proteins and in vitro experiments.

-Conclusion: First sentence at each paragraph should be changed to passive form.

-Recommendations should be in detailed work and continously works based on perspective and/or limitation of current works.

***Question:***

- The PhD candidate gives explanation that α-glucosidase and α-amylase assay were performed at different temperatures: 37 °C for α-glucosidase and 25 °C α-amylase.

- In phytochemical study, $H_2SO_4$ 10% and cerium sulfate solutions are used for detecting traces of substances on TLC. What are differences in TLC after spraying with these two reagents, advantages and disadvantages of them?

**Conclusions (whether the work is suitable/ could be suitable after revisions for presenting to obtain the USTH PhD degree?)**

This thesis could be suitable after revisions for presenting to obtain the USTH PhD degree.

☐ **Recommended for defense at the PhD Thesis Examination Jury in the current form.**

☒ **Recommend for defense at the PhD Thesis Examination Jury with minor/ major revisions.**

☐ **Not recommended for defense at the PhD Thesis Examination Jury.**

July 14, 2026

**Reviewer's signature**

Bùi Hữu Tài

UNIVERSITY OF SCIENCE & TECHNOLOGY OF HANOI
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VIETNAM FRANCE UNIVERSITY

# Examination Jury for PhD thesis
## Jury Member's Assessment Form

| | |
|---|---|
| **PhD Student** | |
| **Full name** | Nguyen Xuan Tung |
| **Program** | Pharmacological, Medical and Agronomical Biotechnology |
| **PhD ID** | D22.PMAB.003 |
| **PhD thesis** | |
| **Title** | Chemical constituents and hypoglycemic effects of Commelina diffusa Burm.F./ *Nghiên cứu thành phần hóa học và tác dụng hạ đường huyết của cây Thài lài trắng (Commelina diffusa Burm.F.)* |
| **Supervisor** | Assoc. Prof. Vu Duc Loi, Vietnam University of Traditional Medicine |
| **Co-supervisor** | Assoc.Prof. Nguyen Thi Van Anh, USTH, VAST |
| **Jury Member** | |
| **Full name** | Nguyễn Xuân Nhiệm |
| **Title** | Assoc. Prof. |
| **Institution** | Graduate University of Science and Technology |
| **Contact (Email/SMS)** | nxnhiem@yahoo.com |
| **Role (Chairman/ Reviewer/ Member)** | Member |

**Technical comments (e.g. on the thesis topic, research methodology, finding, discussion, thesis structure, wording, references, etc). Please note that the reviewers can fill this part as "please refer to my review report" and/or with additional comments on the revised manuscript and/ or on the presentation made by the candidate**

The thesis addresses a relevant and underexplored topic at the interface of pharmacognosy, natural-product chemistry, and experimental pharmacology. The objectives are clearly stated and are generally consistent with the experimental design. The work integrates phytochemical isolation and structural elucidation, in vitro enzyme-inhibition assays, an in vivo type 2 diabetes model, and molecular docking/ADMET prediction. This multidisciplinary approach is a major strength.

The students isolated and identified 14 compounds from *Commelina diffusa*. Some compounds was reported for first time from the species and/or genus provides useful new phytochemical information. The ethyl acetate fraction showed the strongest inhibition of α-glucosidase and α-amylase, and compounds **9**, 10, and **14** were among the most active compounds. The *in vivo* study further showed a significant reduction in blood glucose and histological improvement in liver and pancreatic tissues after administration of the ethyl acetate fraction. These findings are supported by four papers and one granted patent, demonstrating the scientific and practical value of the research.

The manuscript is logically organized and contains an extensive review of diabetes, the genus *Commelina*, phytochemical constituents, relevant biological assays, and *in silico* approaches. The methods are described in adequate detail and the discussion generally relates the findings to previous studies.

**Comment on the candidate ability (e.g. general scientific background, understanding on the research field and research topic, presentation skill, English level, etc)**

Based on the thesis and the associated scientific outputs, the student demonstrates a solid background in pharmacognosy, phytochemistry, experimental pharmacology, and the biological evaluation of natural products. The student shows a good understanding of the research problem, from extraction and chromatographic isolation to structural elucidation, enzyme assays, animal experimentation, and in silico analysis.

The candidate has generated a substantial body of work and has been able to integrate results obtained by different methods into a coherent scientific argument. The publication record and granted patent indicate the ability to complete research, collaborate with multidisciplinary teams, interpret data, and communicate findings to the scientific community.

The written presentation is generally systematic and comprehensible. The candidate should nevertheless improve precision in statistical reasoning, methodological reporting, differentiation between experimental evidence and computational prediction, and academic English.

**Conclusions (indicates whether the manuscript is accepted in the current form or accepted after minor/ major revisions; and whether the candidate deserves the USTH PhD degree)**

The thesis presents original and meaningful contributions to knowledge of the chemical constituents and hypoglycemic potential of *Commelina diffusa* Burm.f. The scope and volume of experimental work are appropriate for a doctoral thesis, and the main conclusions are generally supported by the results. The candidate has also produced peer-reviewed publications and a patent directly related to the thesis.

I recommend acceptance of the manuscript after minor revisions addressing the methodological clarifications, statistical reporting, cautious interpretation of docking and safety results, consistency of terminology and references, and English-language editing specified above.

Subject to satisfactory revision and a successful oral defense, the candidate deserves to be awarded the USTH PhD degree in Pharmacological, Medical and Agronomical Biotechnology.

Some comments:

1) Zoom in and increase the resolution of NMR spectra for all compounds in SI.

2) Check and confirm coupling constants of H-2 and H-3 for compound **1.**

3) Draw stereo center for compound **2.**

4) Redraw structure for compound **12.**

5) Record and give optical rotation of compounds contain stereo center.

6) Check very carefully English in the thesis.

☐ **Accept the manuscript in the current form.**

☒ **Accept the manuscript with minor revisions.**

☐ **The manuscript is not qualified for the PhD degree.**

Hanoi, June 2, 2026

**Jury Member's signature**

**End of the document**

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# Internal Jury for PhD thesis examination

## Jury Member's Assessment Form

| **PhD Student** | |
|---|---|
| **Full name** | **Nguyen Xuan Tung** |
| **Program** | PMAB |
| **Student ID number** | D22.PMAB.003 |
| **PhD thesis** | |
| **Title** | Chemical constituents and hypoglycemic effects of Commelina diffusa Burm.F. |
| **Supervisor** | Assoc. Prof. Vu Duc Loi, Vietnam University of Traditional Medicine |
| **Co-supervisor** | Assoc.Prof. Nguyen Thi Van Anh, USTH, VAST |
| **Jury Member** | |
| **Full name** | Tran Thi Thu Phuong |
| **Title** | PhD |
| **Institution** | LS, USTH |
| **Contact (Email/ SMS)** | tran-thi-thu.phuong@usth.edu.vn |
| **Role (Chairman/ Reviewer/ Member)** | Member |

**Technical comments (e.g. on the thesis topic, research methodology, finding, discussion, thesis structure, wording, references, etc).** *(Reviewers can fill this part as "Please refer to my review report" and/or add more comments on the manuscript and the presentation made by the student)*

The thesis is generally well structured, comprising a comprehensive introduction, clearly described materials and methods, detailed presentation of results, and an extensive discussion. The experimental design, which combines phytochemical investigation, *in vitro* enzyme inhibition assays, in vivo hypoglycemic evaluation, and in silico molecular docking, is logical and coherent with the stated objectives.

However, several points require improvement:

- **Research positioning and originality**:Although the literature review is extensive, the scientific gap and novelty of the study should be articulated more clearly, particularly in the Introduction. The thesis would benefit from a sharper distinction between previously published knowledge and the new contributions provided by this work.
- **Methodology**: The experimental methods are generally appropriate and follow accepted standards. Ethical approval details should also be presented more explicitly.
- **Results and data analysis**: The results are clearly presented, and the isolation of 14 compounds, several of which are reported for the first time in *C. diffusa* or even in the genus *Commelina*, represents a significant contribution. However, the Results section is sometimes overly descriptive. Stronger emphasis should be placed on data interpretation and structure–activity relationships, particularly in relation to enzyme inhibition and docking outcomes.
- **Discussion**:
  The Discussion section adequately summarizes findings but would benefit from deeper critical analysis. Comparisons with recent literature and discussion of alternative explanations and limitations should be expanded. The mechanistic interpretation linking in vitro, in vivo, and in silico results could be strengthened.
- **Thesis structure, language, and references**: The overall structure is logical, but some sections contain repetitive or lengthy descriptions that could be streamlined. The English language is generally understandable but requires minor grammatical and stylistic corrections to improve clarity and academic tone.

**Comment on the candidate ability (e.g. general scientific background, understanding on the research field and research topic, presentation skill, English level, etc)**

The candidate demonstrates a solid general scientific background in phytochemistry, pharmacology, and experimental biomedical research. The student shows a good understanding of the research field and is capable of applying a range of experimental and computational techniques relevant to the thesis topic.

**Conclusions (indicates whether the thesis work is suitable or will be suitable for presenting to the PhD Thesis Examination Jury after some revisions; and whether the candidate deserves the USTH PhD degree)**

- The thesis work is scientifically relevant and technically sound, with clear original contributions, particularly in the phytochemical characterization of *Commelina diffusa*.
- The candidate has demonstrated sufficient scientific knowledge, research skills, and independence to deserve the award of the PhD degree from the University of Science and Technology of Hanoi (USTH), provided that the recommended revisions are satisfactorily completed.

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☐ **Recommended for defense at the PhD Thesis Examination Jury in the current form**

☑ **Recommended for defense at the PhD Thesis Examination Jury after minor/ major revisions**

☐ **Not recommended for defense at the PhD Thesis Examination Jury**

……July…14……, *2025*

**Jury Member's signature**

**Tran Thi Thu Phuong**

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# Examination Jury for PhD thesis
## Jury Member's Assessment Form

| | |
|---|---|
| **PhD Student** | |
| **Full name** | Nguyen Xuan Tung |
| **Program** | Pharmacological, Medical and Agronomical Biotechnology |
| **PhD ID** | D22.PMAB.003 |
| **PhD thesis** | |
| **Title** | Chemical constituents and hypoglycemic effects of *Commelina diffusa* Burm.F./ *Nghiên cứu thành phần hóa học và tác dụng hạ đường huyết của cây Thài lài trắng (Commelina diffusa Burm.F.)* |
| **Supervisor** | Assoc. Prof. Vu Duc Loi, Vietnam University of Traditional Medicine |
| **Co-supervisor** | Assoc.Prof. Nguyen Thi Van Anh, USTH, VAST |
| **Jury Member** | |
| **Full name** | Le Thanh Huong |
| **Title** | PhD |
| **Institution** | USTH |
| **Contact (Email/SMS)** | le-thanh.huong@usth.edu.vn |
| **Role (Chairman/ Reviewer/ Member)** | Member/secretary |
| **Technical comments (e.g. on the thesis topic, research methodology, finding, discussion, thesis structure, wording, references, etc). Please note that the reviewers can fill this part as "please refer to my review report" and/or with additional comments on the revised manuscript and/ or on the presentation made by the candidate** | |

**Strengths**

The thesis presents a systematic investigation of *Commelina diffusa* from phytochemical isolation to biological evaluation. Fourteen compounds were isolated and structurally elucidated, several of which are reported for the first time from *C. diffusa* or the genus *Commelina*. This represents a valuable contribution to the phytochemistry of the species.

The work combines phytochemical studies with enzyme inhibition assays, animal experiments, molecular docking and ADMET prediction, providing a relatively comprehensive evaluation of the hypoglycemic potential of the plant.

The research outputs have resulted in peer-reviewed international publications and an intellectual property application, demonstrating scientific productivity and practical relevance.

**Limitations and suggestions**

The proposed mechanism is mainly based on α-glucosidase and α-amylase inhibition. However, the *in vivo* study only evaluated fasting blood glucose and did not include OGTT, insulin measurements, HbA1c, HOMA-IR or other mechanistic biomarkers. Therefore, the evidence more strongly supports an antihyperglycemic effect than a mechanistic antidiabetic action.

Histopathological observations of the liver and pancreas were presented qualitatively. The addition of semi-quantitative histological scoring, morphometric analysis or pancreatic islet quantification would strengthen the conclusions.

The thesis reports improvement in liver and pancreatic histology, whereas serum AST and ALT levels were not significantly altered by treatment. Additional discussion regarding this apparent discrepancy would improve the interpretation of the results.

Molecular docking and ADMET prediction provide useful supporting information but remain computational approaches. Experimental validation of the predicted interactions and pharmacokinetic properties would further strengthen the conclusions.

Due to the limited quantity of isolated compounds, the contribution of individual compounds to the *in vivo* antihyperglycemic activity remains unresolved and deserves future investigation.

**Consistency of terminology**

In several parts of the thesis and related publications, the terms *antidiabetic*, *hypoglycemic*, and *antihyperglycemic* are used interchangeably. Since the presented data mainly demonstrate blood glucose-lowering effects without detailed mechanistic investigation, the use of *antihyperglycemic activity* appears more accurate and should be used consistently throughout the manuscript.

**Abstract wording**

The abstract states that "significant ameliorations in the histopathological images" were observed. However, no quantitative histopathological scoring or morphometric analysis was presented. The wording should be revised to avoid implying statistical significance when only qualitative observations were reported.

**Histological figure presentation**

Histological micrographs would benefit from the inclusion of scale bars. Magnification alone (e.g., ×400) is less informative than a calibrated scale and does not fully comply with current publication standards.

**Quantitative presentation of histology**

The addition of semi-quantitative histological scores or morphometric measurements would improve the rigor of the histopathological assessment and facilitate comparison among experimental groups.

**Abbreviation usage**

Some abbreviations are introduced multiple times, whereas others are not always defined at first appearance. A more consistent use of abbreviations throughout the thesis would improve readability.

**Comment on the candidate's ability (e.g. general scientific background, understanding on the research field and research topic, presentation skill, English level, etc)**

The thesis reflects a good scientific background in pharmacognosy and natural product research. The candidate demonstrates the ability to conduct independent research and to disseminate research findings through international publications. The manuscript is generally well organized and written in acceptable scientific English.

**Conclusions (indicates whether the manuscript is accepted in the current form or accepted after minor/ major revisions; and whether the candidate deserves the USTH PhD degree)**

Based on the quality of the thesis and the scientific contributions presented therein, I consider the dissertation suitable for public defense and recommend acceptance after minor revisions.

☐ **Accept the manuscript in the current form.**

☐ **Accept the manuscript with minor/ major revisions.**

☐ **The manuscript is not qualified to for the PhD degree.**

……July…14…., 2026

**Jury Member's signature**

Le Thanh Thuong

**End of the document**

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# PhD Thesis Examination Jury
# Final Assessment

**Time** : July 14, 2026
**Location** : Meeting Room 402, USTH Building

## I. PHD STUDENT:

| | |
|---|---|
| **Full name** | **Nguyen Xuan Tung** |
| **Student ID** | D22.PMAB.003 |
| **Program** | Pharmacological, Medical and Agronomical Biotechnology |
| **Thesis title** | Chemical constituents and hypoglycemic effects of Commelina diffusa Burm.F./ *Nghiên cứu thành phần hóa học và tác dụng hạ đường huyết của cây Thài lài trắng (Commelina diffusa Burm.F.)* |
| **Supervisor** | Assoc. Prof. Vu Duc Loi, Vietnam University of Traditional Medicine |
| **Co-supervisor** | Assoc.Prof. Nguyen Thi Van Anh, USTH, VAST |

## II. COMMENTS *(if any)*:

- Thesis has discussed more the limitations of the study (in vivo study & the link of molecular study → in vitro → in vivo.

## III. FINAL ASSESSMENT:

☐ Agree to award the PhD diploma to the candidate.

☑ Agree to award the PhD diploma to the candidate after revision.

☐ The thesis is not qualified for the PhD diploma. A second jury could be considered.

**Jury member**
*(Signature & full name)*

Trần Thị Thu Phương

UNIVERSITY OF SCIENCE & TECHNOLOGY OF HANOI
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TRƯỜNG ĐẠI HỌC KHOA HỌC & CÔNG NGHỆ HÀ NỘI

# PhD Thesis Examination Jury
# Final Assessment

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| **Supervisor** | Assoc. Prof. Vu Duc Loi, Vietnam University of Traditional Medicine |
| **Co-supervisor** | Assoc.Prof. Nguyen Thi Van Anh, USTH, VAST |

## II. COMMENTS *(if any)*:

- Major revision according to comments

## III. FINAL ASSESSMENT:

☐ Agree to award the PhD diploma to the candidate.

☒ Agree to award the PhD diploma to the candidate after revision.

☐ The thesis is not qualified for the PhD diploma. A second jury could be considered.

**Jury member**
*(Signature & full name)*

Pham Thi Nguyet Hang

UNIVERSITY OF SCIENCE & TECHNOLOGY OF HANOI
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# PhD Thesis Examination Jury
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**Time** : July 14, 2026
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## I. PHD STUDENT:

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| **Supervisor** | Assoc. Prof. Vu Duc Loi, Vietnam University of Traditional Medicine |
| **Co-supervisor** | Assoc.Prof. Nguyen Thi Van Anh, USTH, VAST |

## II. COMMENTS *(if any)*:

## III. FINAL ASSESSMENT:

☐ Agree to award the PhD diploma to the candidate.

☒ Agree to award the PhD diploma to the candidate after revision.

☐ The thesis is not qualified for the PhD diploma. A second jury could be considered.

**Jury member**
*(Signature & full name)*

Bùi Hữu Tài

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# PhD Thesis Examination Jury
# Final Assessment

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## I. PHD STUDENT:

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| **Full name** | **Nguyen Xuan Tung** |
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| **Program** | Pharmacological, Medical and Agronomical Biotechnology |
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| **Supervisor** | Assoc. Prof. Vu Duc Loi, Vietnam University of Traditional Medicine |
| **Co-supervisor** | Assoc.Prof. Nguyen Thi Van Anh, USTH, VAST |

## II. COMMENTS *(if any)*:

## III. FINAL ASSESSMENT:

☐ Agree to award the PhD diploma to the candidate.

☑ Agree to award the PhD diploma to the candidate after revision.

☐ The thesis is not qualified for the PhD diploma. A second jury could be considered.

**Jury member**
*(Signature & full name)*

Le Thanh Huong

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# PhD Thesis Examination Jury
# Final Assessment

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| **Full name** | **Nguyen Xuan Tung** |
| **Student ID** | D22.PMAB.003 |
| **Program** | Pharmacological, Medical and Agronomical Biotechnology |
| **Thesis title** | Chemical constituents and hypoglycemic effects of Commelina diffusa Burm.F./ *Nghiên cứu thành phần hóa học và tác dụng hạ đường huyết của cây Thài lài trắng (Commelina diffusa Burm.F.)* |
| **Supervisor** | Assoc. Prof. Vu Duc Loi, Vietnam University of Traditional Medicine |
| **Co-supervisor** | Assoc.Prof. Nguyen Thi Van Anh, USTH, VAST |

## II. COMMENTS *(if any)*:

## III. FINAL ASSESSMENT:

☐ Agree to award the PhD diploma to the candidate.

☑ Agree to award the PhD diploma to the candidate after revision.

☐ The thesis is not qualified for the PhD diploma. A second jury could be considered.

**Jury member**
*(Signature & full name)*

Nguyễn Hải Đăng

UNIVERSITY OF SCIENCE & TECHNOLOGY OF HANOI
UNIVERSITE DES SCIENCES ET DES TECHNOLOGIES DE HANOI
TRƯỜNG ĐẠI HỌC KHOA HỌC & CÔNG NGHỆ HÀ NỘI

USTH
VIETNAM FRANCE UNIVERSITY

# PhD Thesis Examination Jury
# Final Assessment

**Time** : July 14, 2026
**Location** : Meeting Room 402, USTH Building

## I. PHD STUDENT:

| | |
|---|---|
| **Full name** | **Nguyen Xuan Tung** |
| **Student ID** | D22.PMAB.003 |
| **Program** | Pharmacological, Medical and Agronomical Biotechnology |
| **Thesis title** | Chemical constituents and hypoglycemic effects of Commelina diffusa Burm.F./ *Nghiên cứu thành phần hóa học và tác dụng hạ đường huyết của cây Thài lài trắng (Commelina diffusa Burm.F.)* |
| **Supervisor** | Assoc. Prof. Vu Duc Loi, Vietnam University of Traditional Medicine |
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## II. COMMENTS *(if any)*:

The student demonstrates a solid grasp of the research content.

Slides of presentation are well designed.

The student effectively presents issues related to the dissertation and shows a deep understanding of the research content.

## III. FINAL ASSESSMENT:

☐ Agree to award the PhD diploma to the candidate.

■ Agree to award the PhD diploma to the candidate after revision.

☐ The thesis is not qualified for the PhD diploma. A second jury could be considered.

**Jury member**
*(Signature & full name)*

Nguyen Xuan Nhiem

VIETNAM ACADEMY
OF SCIENCE AND TECHNOLOGY
**UNIVERSITY OF SCIENCE AND TECHNOLOGY OF HANOI**
*

**COMMUNIST PARTY OF VIETNAM**

*Hanoi, 14th, July, 2026*

**RESOLUTION OF THE UNIVERSITY'S DOCTORAL THESIS EXAMINATION JURY**
(The 1st meeting)

**Thesis title:** Chemical constituents and hypoglycemic effects of Commelina diffusa Burm.F./ *Nghiên cứu thành phần hóa học và tác dụng hạ đường huyết của cây Thài lài trắng (Commelina diffusa Burm.F.)*

**PhD student's full name:** Nguyen Xuan Tung **Student ID:** D22.PMAB.003

**Specialty:** Pharmacological, Medical and Agronomical Biotechnology, Code: 9420201

Today, 14th July 2026, at USTH, after listening to the thesis presentation by PhD student Nguyen Xuan Tung, the comments from 3 reviewers, the comments of the Jury's members and other participants, the Thesis Examination Jury reached the following conclusions:

1. The title of the thesis is appropriate for the program of Pharmacological, Medical and Agronomical Biotechnology, Code: 9420201.
2. The thesis does not overlap with any previously published works, theses, or thesis.
3. Main scientific conclusions, new points, new contributions of the thesis:

Successfully isolated and structurally elucidated fourteen compounds from *Commelina diffusa* Burm.f., including several compounds reported for the first time from this species and the genus *Commelina*, thereby expanding the phytochemical knowledge of this medicinal plant.

Identified the ethyl acetate fraction (CD.EAF) as the most active fraction against α-glucosidase and α-amylase and identified several compounds with potent enzyme inhibitory activities through bioactivity-guided fractionation.

Demonstrated the antihyperglycemic potential and acute safety of CD.EAF in a high-fat diet/streptozotocin-induced diabetic mouse model, providing a scientific basis for further pharmacological investigation of *C. diffusa*.

Comments and suggestions

Clarify the preparation of CD.EAF for the *in vivo* experiments. Since the ethyl acetate fraction is not readily soluble in water, the manuscript should clearly describe how the extract was dispersed or solubilized before oral administration, and justify the choice of the tested doses (100 and 300 mg/kg). In addition, please ensure consistency regarding the positive controls used in the *in vitro* and *in vivo* studies.

Expand the discussion regarding the discrepancy between histopathological observations and biochemical findings. Although liver histology suggested marked tissue injury in diabetic mice, serum AST and ALT levels were not significantly different between the normal and diabetic groups. This inconsistency should be discussed in greater detail.

The reference list is excessively long. It is recommended to remove redundant or less relevant citations and retain the most representative and up-to-date references.

For previously reported compounds, appropriate literature citations should be provided for structural identification. The NMR data should be presented together with comparison tables against published values to better support compound identification.

After discussion, the committee unanimously voted to approve the Decision, with 6/6 votes in favor (100%).

The Chairman of the Committee declared the end of the Thesis Examination Jury at 17h00 on the same day.

| **Chairman** | **Secretary** |
| --- | --- |
| **Assoc.Prof. Nguyen Hai Dang** | **Dr. Le Thanh Huong** |

**Director of Department of Academic Affairs**

**Assoc.Prof. Nguyen Hong Nam**