Azhar Int J Pharm Med Sci 2025; Vol 5 (1):128-140
Research Article

Open Acces

Print ISSN: 2735-4598
Online ISSN: 2735-4601

Antidiabetic Activity and GC-MS Analysis of *n*-Hexane Leaf Extract of *Codiaeum variegatum* (Euphorbiaceae)

Samar S. Tolba[1*], Hala Sh. Mohammed[2], Mosad A. Ghareeb[3], Abd El-Salam Mohamed[4]

[1] National Food Safety Authority food safety inspector.
[2] Department of Pharmacognosy and Medicinal Plants, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo 11311, Egypt.
[3] Department of Medicinal Chemistry, Theodor Bilharz Research Institute, Giza 12411, Egypt.
[4] Department of Pharmacognosy and Medicinal Plants, Faculty of Pharmacy (Boys), Al-Azhar University.

***** Correspondence: E-mail: sammaarsaad61@gmail.com

Article history: Received: 20-01-2024 **Revised:**01-04-2024 **Accepted:** 11-07-2024

Abstract: This study aimed to discover bioactive fatty esters in the n-hexane leaf extract of *Codiaeum variegatum* (L.) [*C. variegatum* (L.)] through GC-MS analysis. The extract, known for its antidiabetic properties, was subjected to *in vitro* antidiabetic activity tests using α-glucosidase and α-amylase assays. The GC-MS analysis identified 55 potential therapeutic compounds. The main phytoconstituents comprised;, Linoleic acid, methyl ester (20.67%), 9-Octadecenoic acid, 12-hydroxy-methyl ester, [R-(Z)] (10.89%), Cyclopropane- octanoic acid, 2-[[2-[(2-Ethyl cyclopropyl) methyl]cyclopropyl]methyl], methyl ester (7.91%), Linoleic acid, hydroxy methyl ester (7.01%), Hexadecanoic acid, methyl ester (6.12%), and Methyl stearate (4.65%). The extract showed weak to moderate inhibition on α-glucosidase and α-amylase with IC_{50} values of 27.40 and 24.43 µg/ml, respectively, compared to acarbose. More n-hexane extract is required for similar inhibition, indicating acarbose higher potency. This suggests *C. variegatum* (L.) has diverse bioactive chemicals. While these findings are significant for therapy development, further research is needed to understand potential applications and risks. This study opens avenues for future research into bioactive compounds and their therapeutic potential.

Keywords: *C. variegatum* (L.); *n*-hexane extract; GC-MS; Fatty esters; Antidiabetic

This is an open access article distributed under the CC BY-NC-ND license https://creativecommons.org/licenses/by/4.0/

1. INTRODUCTION

Codiaeum variegatum (L.) Rumph. ex A. Juss. (Euphorbiaceae family), native to the Moluccan Islands, Indonesia, is also found in tropical regions like the Philippines, Papua New Guinea, and Australia. This species, commonly known as "garden croton", is widely cultivated with many varieties developed from it [1]. Traditionally, various parts of the plant have been used in folk medicine to treat ailments such as amoebic dysentery, stomachache, gastric ulcers, skin infections, fever, cough, and cold[1]. Various chemical classes have been identified in different parts of the plant, including flavonoids[2,3], phenolic acids [2,4], diterpenoids[5], triterpenoids [5,6], sterols[5], alkaloids[5], and volatiles[7,8]. The plant has demonstrated a wide range of biological activities, including anti-amoebic[9], wound healing[10], antiviral[11], anti-inflammatory[12], cytotoxic[5,13], antifungal[4], anticonvulsant[14], antibacterial [15], and anti-diarrheal properties [16].

Fatty acids are known to display antidiabetic properties. For example, a study investigating the nutritional attributes and *in vitro* antidiabetic effects of blue and yellow corn extracts revealed that, despite the established biological activity of polyphenolic compounds, the fat component demonstrated the most potent *in vitro* antidiabetic activity[17]. Dietary free fatty acids (FFAs), including ω-3 fatty acids, are known to control metabolic and anti-inflammatory processes. Many of these effects are due to FFAs' interaction with a group of G protein-coupled receptors. This evidence implies that fatty acids could have a substantial impact on diabetes management[18]. *C. variegatum* (L.) is recognized for its diverse bioactive compounds,

Cite this article: Tolba S.S., Mohammed H. Sh., Ghareeb M.A., and Mohamed A. Antidiabetic Activity and GC-MS Analysis of *n*-Hexane Leaf Extract of *Codiaeum variegatum* (Euphorbiaceae). Azhar International Journal of Pharmaceutical and Medical Sciences, 2025; 5(1):xx-xx. doi: 10.21608/aijpms.2024.264259.1250

DOI : 10.21608/aijpms.2024.264259.1250

including fatty acid esters[19]. Although specific research on the antidiabetic effects of these fatty acid esters in *C. variegatum* (L.) is sparse, studies have indicated that fatty acids can display antidiabetic properties[20]. This study aims to analyze the potential bioactive components in the *n*-hexane leaf extract of *C. variegatum* (L.), identify the compounds using GC-MS analysis, and evaluate the *in vitro* antidiabetic activity.

2. METHODS

2.1. Plant material

Fresh leaves of *Codiaeum variegatum* (L.) Rumph. ex A. Juss. were collected in May 2020 from the Experimental Plants Station at the Faculty of Pharmacy, Cairo University, Giza, Egypt. The plant was identified and authenticated by experts at the Orman Botanical Garden, Giza, Egypt. A voucher specimen (No. C.v/l/2020) was stored in the herbarium of the Department of Pharmacognosy and Medicinal Plants at the Faculty of Pharmacy, Al-Azhar University, Egypt.

2.2. Extraction

In a Soxhlet extractor, 250 g of powdered C. *variegatum* (L.) leaves were individually extracted with n-hexane (3 x 500 mL) at a temperature between 60 and 65°C for 24 hrs. The solvent was then evaporated using a rotating vacuum evaporator to produce viscous semi-solid masses (200mg). This semi-dry n-hexane crude extract was then subjected to GC-MS analysis[21].

2.3. Gas chromatography-Mass Spectrometry (GC-MS) analysis

The GC-MS analysis was carried out following the established protocols[21, 22].

2.4. In vitro antidiabetic activity

The extract under investigation was assessed for its in vitro antidiabetic activity through α-glucosidase and α-amylase enzyme inhibition tests[23].

2.5. Statistical analysis

Mean was calculated using a Microsoft Excel worksheet. References and citations were compiled using the EndNote software.

3. RESULTS

3.1. GC-MS analysis of the *n*-hexane extract of *C. variegatum* (L.)

Gas Chromatography-Mass Spectrometry (GC-MS) is an analytical method used for analyzing mixtures of organic chemicals[24, 25]. The NIST/NBS spectral database is a key tool for compound identification via mass spectra. It houses reference spectra for GC/MS and LC-MS/MS, and gas phase retention indices for GC. The library's main function is to identify compounds by matching ion fragmentation "fingerprints". It offers a more sensitive and robust identification method than alternatives, enhancing the success rate of confident chemical identification by providing high-quality spectra[21]. The study identified 58 compounds in the n-hexane leaf extract of C. variegatum (L.), constituting 94.36% of the total composition of the extract **(Table 1, Figure 1).** The primary chemical classes identified included fatty esters (51.91%), fatty ester derivatives (21.13%), fatty acids (5.64%), phthalate ester (5.63%), sesquiterpenoids (2.33%), acyclic hydrogenated diterpene alcohols (2.06%), and valero lactones (1.90%). The main compounds identified were Hexadecanoic acid, methyl ester (6.12%), n-Hexadecanoic acid (3.42%), Hexadecanoic acid, trimethylsilyl ester (2.11%), Linoleic acid, hydroxy-methyl ester (7.01%), Linoleic acid, methyl ester (20.67%), Phytol (1.63%), Methyl stearate (4.65%), Cyclopropane-octanoic acid, 2-[[2-[(2-Ethyl cyclopropyl) methyl]cyclopropyl]methyl], methyl ester (7.91%), Octadecanoic acid (1.54%), 9-Octadecenoic acid, 12-hydroxy-methyl ester, [R-(Z)] (10.89%), 7,10-Octadecadienoic acid, methyl ester (1.98%), cis-5,8,11-Eicosatrienoic acid, methyl ester (3.71%), Delta cuprenene (1.37%), and 2H-Pyran-2-one, tetrahydro-6-tridecyl-(1.37%). This suggests a diverse range of bioactive chemicals present in C. variegatum (L.) **(Figure 2).**

3.2. α-glucosidase and α-amylase enzymes inhibition activities of *C. variegatum* (L.) *n*-hexane extract

The data presented in **Figure 3**, show the inhibitory effects of the *n*-hexane extract and acarbose on α-glucosidase and α-amylase activities at different concentrations. As expected, the percentage inhibition decreases as the concentration of the substances decreases, indicating less availability of the inhibitor to impede the enzyme. The IC_{50} value, which represents the concentration of the inhibitor required to suppress 50% of the enzyme's activity, is used to gauge the potency of an inhibitor. A lower IC_{50} value signifies a more potent inhibitor. According to the current finding, acarbose appears to be a more potent inhibitor of both α-glucosidase (IC_{50} = 2.11 µg/ml) and α-amylase (IC_{50} = 12.29 µg/ml) compared to the *n*-hexane extract (IC_{50} = 27.40 µg/ml for α-glucosidase and IC_{50} = 24.43 µg/ml for α-amylase). This suggests that the *n*-hexane extract,

while still acting as an inhibitor, exhibits a moderate to weak inhibitory effect in comparison to acarbose. Therefore, a higher concentration of the *n*-hexane extract would be needed to achieve the same level of enzyme inhibition as acarbose.

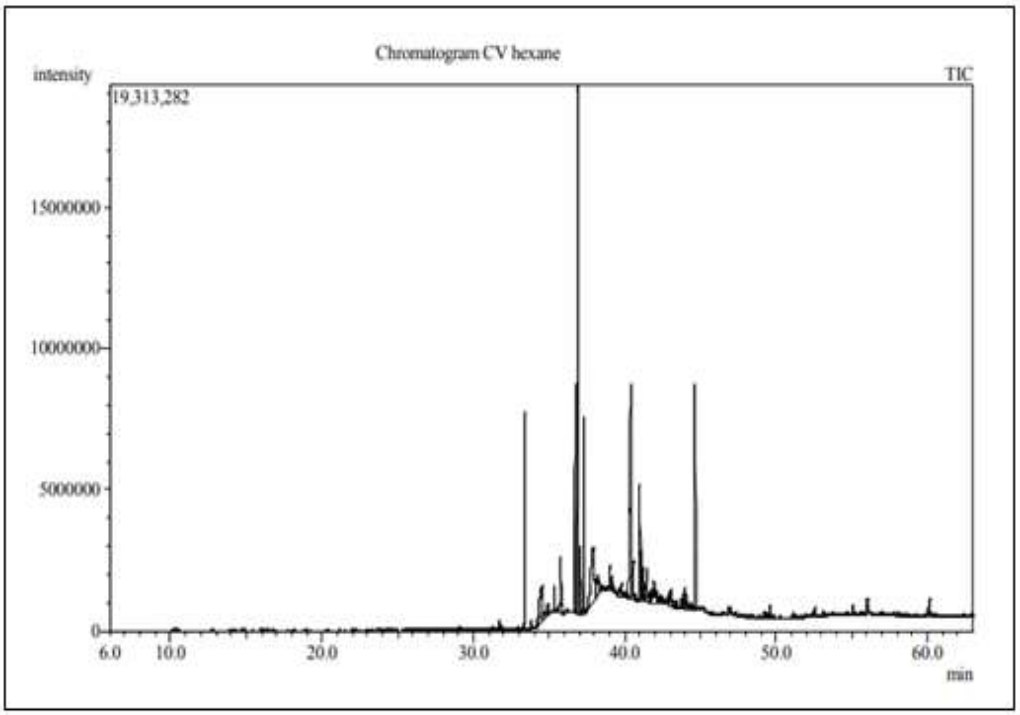


Figure 1. TIC chromatogram of the *n*-hexane extract of *C. variegatum* (L.)

4. DISCUSSION

Croton species are known for their wide range of biological activities, including potential anti-diabetic effects[57]. For example, the dichloromethane extract of *Croton bonplandianus* has been found to inhibit α-glucosidase activity, with an IC_{50} value of 14.93 mg/ml[56]. In a similar vein, *n*-Hexane and ethyl acetate extracts of *Croton krabas* have shown inhibitory activity against α-glucosidase, with inhibition percentages of 73.58% and 44.55% at a concentration of 150 µg/mL[57], respectively. Additionally, the ethyl acetate extract of the leaf part of *Croton thurifer* has been reported to inhibit α-glucosidase activity, with an IC_{50} value equal to 1.77 mg/mL[58]. These findings suggest that Croton species could potentially be utilized in diabetes management. This provides motivation to further explore the inhibitory effects of an *n*-hexane extract from *C. variegatum* (L.) and acarbose on two key enzymes: α-glucosidase and α-amylase. These enzymes play a crucial role in the breakdown of carbohydrates into glucose. By inhibiting these enzymes, the digestion of carbohydrates is slowed, which subsequently reduces the rise in blood sugar levels post-meal. This mechanism is particularly beneficial in managing certain types of diabetes. The *n*-hexane extract and acarbose inhibit α-glucosidase and α-amylase activities. As their concentrations decrease, so does their inhibitory effect due to less availability of the inhibitor. The IC_{50} value measures an inhibitor's potency, with a lower value indicating a stronger

inhibitor. acarbose has lower IC_{50} values (2.11 µg/ml for α-glucosidase and 12.29 µg/ml for α-amylase) than the *n*-hexane extract (27.40 µg/ml for α-glucosidase and 24.43 µg/ml for α-amylase), making it a stronger inhibitor. Thus, more n-hexane extract is needed to achieve the same inhibition level as acarbose. This comparison clearly indicates the superior inhibitory potency of acarbose over the *n*-hexane extract. These findings provide valuable insights into the potential use of these substances in managing blood sugar levels, particularly in the context of diabetes. However, it's important to note that further studies would be needed to fully understand their therapeutic effects, safety profiles, and potential side effects.

Table 1. Reported activity and chemical composition of the *n*-hexane extract of *C. variegatum* (L.)

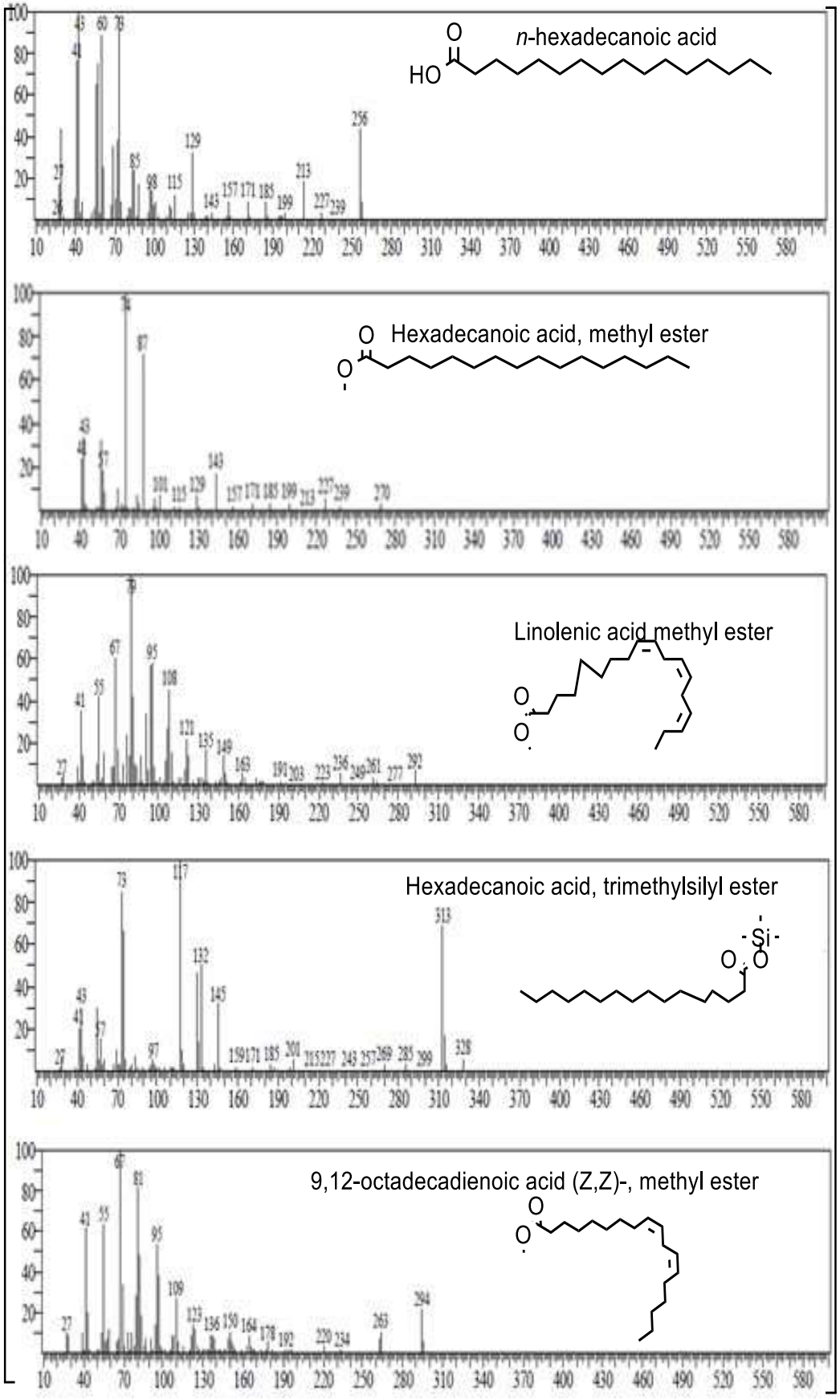
No.	Compound	R_t(min)	M.wt.	M.F.	Area %	Chemical class	Reported bioactivity	Reference
1	2-Undecanone, 6,10-dimethyl	31.67	198	$C_{13}H_{26}O$	0.29	Long chain ketone	Antimicrobial	26
2	Tetradecanoic acid, trimethylsilyl ester	31.79	300	$C_{17}H_{36}O_2Si$	0.11	Fatty esters	Antibacterial	27
3	Hexadecanoic acid, methyl ester	33.43	270	$C_{17}H_{34}O_2$	6.12	Fatty esters	Antibacterial, Antidiabetic	27, 28
4	*n*-Pentadecanoic acid, trimethylsilyl ester	33.82	314	$C_{18}H_{38}O_2Si$	0.22	Fatty esters	Antibacterial	27
5	*n*-Hexadecanoic acid	34.46	256	$C_{16}H_{32}O_2$	3.42	Fatty acids	Antimicrobial, Antidiabetic	29, 30
6	Hexadecanoic acid, methyl ester	34.64	270	$C_{17}H_{34}O_2$	0.68	Fatty acids	Antimicrobial	31
7	α-Methyl linolenate	34.89	292	$C_{19}H_{32}O_2$	0.39	Fatty esters	Antibacterial	27
8	Heptadecanoic acid, methyl ester	35.37	284	$C_{18}H_{36}O_2$	0.54	Fatty esters	Antibacterial	27
9	Hexadecanoic acid, trimethylsilyl ester	35.79	328	$C_{19}H_{40}O_2Si$	2.11	Fatty esters	Antibacterial	27
10	l-(+)-Ascorbic acid 2,6-dihexadecanoate	36.18	652	$C_{38}H_{68}O_8$	0.11	Fatty ester derivatives	Antibacterial	32
11	Linoleic acid, hydroxy-, methyl ester	36.74	294	$C_{19}H_{34}O_2$	7.01	Fatty esters	Antimicrobial	33
12	Linolenic acid methyl ester	36.92	292	$C_{19}H_{32}O_2$	20.67	Fatty esters	Analgesic,Antipyretic,Anticonvulsant, Antidiabetic	34
13	Phytol	37.09	296	$C_{20}H_{40}O$	1.63	Acyclic hydrogenated diterpene alcohol	Antioxidant, Antidiabetic	35, 36
14	Methyl stearate	37.32	298	$C_{19}H_{38}O_2$	4.65	Fatty esters	Antifoaming	37
15	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl) methyl] cyclopropyl] methyl], methyl ester	37.89	334	$C_{22}H_{38}O_2$	7.91	Fatty ester derivatives	Antioxidant	38
16	Octadecanoic acid	38.19	284	$C_{18}H_{36}O_2$	1.54	Fatty acids	Antibacterial	39
17	9-Octadecenal, (Z)-	38.50	266	$C_{18}H_{34}O$	0.34	Fatty aldehyde	Antibacterial	40
18	Ethyl 3-hydroxyhexadecanoate	38.89	300	$C_{18}H_{36}O_3$	0.13	Fatty ester derivatives	Antibacterial	41
19	Unknown	39.02	-	-	0.83	-	-	-
20	Linolenic acid methyl ester	39.16	292	$C_{19}H_{32}O_2$	0.44	Fatty esters	Anti-inflammatory	42
21	Hexadecanoic acid, 2-hydroxy-, methyl ester	39.60	286	$C_{17}H_{34}O_3$	0.52	Fatty ester derivatives	Antibacterial	41

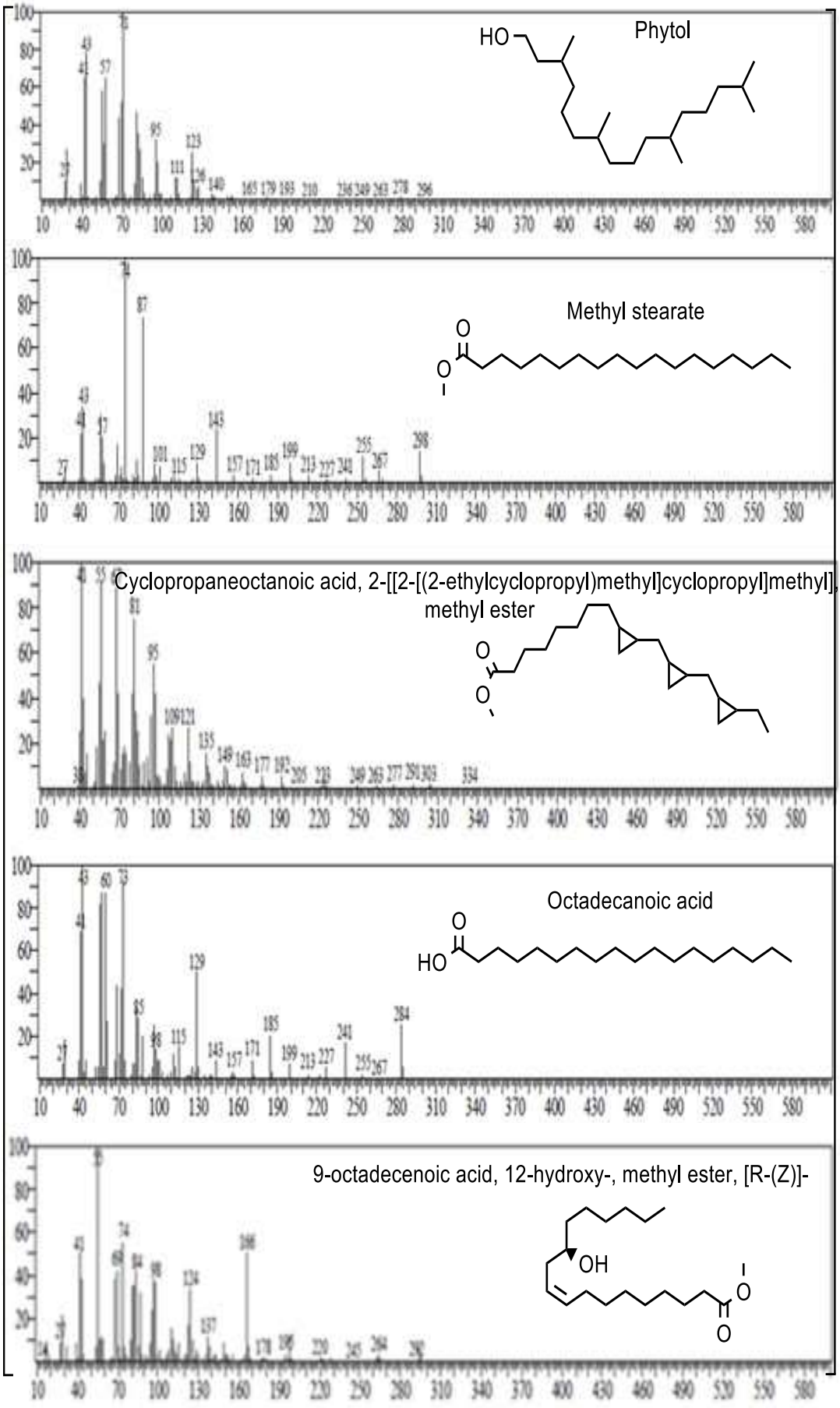
Table 1: Cont.

No.	Compound	R_t (min)	M.wt.	M.F.	Area %	Chemical class	Reported bioactivity	Reference
22	Phytol	39.81	296	$C_{20}H_{40}O$	0.43	Acyclic hydrogenated diterpene alcohol	Antioxidant	43, 44
23	Cyclohexene, 1-acetyl-2-(1-hydroxyethyl)-	40.05	168	$C_{10}H_{16}O_2$	0.27	Cycloalkene derivatives	-	-
24	9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]-	40.38	312	$C_{19}H_{36}O_3$	10.89	Fatty ester derivatives	Hepatoprotective, antihistaminic	45
25	7,10-Octadecadienoic acid, methyl ester	40.55	294	$C_{19}H_{34}O_2$	1.98	Fatty esters	Anti-inflammatory	42
26	1-Heptatriacotanol	40.67	537	$C_{37}H_{76}O$	0.31	Long chain alcohol	Anti-hypercholesterolemic effects	42
27	cis-5,8,11-Eicosatrienoic acid, methyl ester	41.0	320	$C_{21}H_{36}O_2$	3.71	Fatty esters	-	-
28	Delta cuprenene	41.09	204	$C_{15}H_{24}$	1.37	Sesquiterpenoids	-	-
29	Linolenic acid methyl ester	41.23	292	$C_{19}H_{32}O_2$	0.94	Fatty esters	Anti-inflammatory	42
30	2H-Pyran-2-one, tetrahydro-6-tridecyl-	41.45	282	$C_{18}H_{34}O_2$	1.90	Delta valerolactones	Bioactive compounds suitable for human use	46
31	Unknown	41.71	-	-	0.72	-	-	-
32	Cis-5,8,11-Eicosatrienoic acid, methyl ester	41.92	320	$C_{21}H_{36}O_2$	1.64	Fatty esters	-	-
33	Unknown	42.22	-	-	0.30	-	-	-
34	5-hydroxy-7-methoxyflavanone	42.43	270	$C_{16}H_{14}O_4$	0.56	Flavonoids	-	-
35	Naphthalene, decahydro-1,6-dimethyl-	42.94	166	$C_{12}H_{22}$	0.84	Polycyclic hydrocarbons	-	-
36	2,6-Difluorobenzoic acid, tridec-2-ynyl ester	43.04	336	$C_{20}H_{26}F_2O_2$	0.79	Unsaturated esters	Antioxidant,	47
37	6,9,12,15-Docosatetraenoic acid, methyl ester	43.18	346	$C_{23}H_{38}O_2$	0.32	Fatty esters	Antibacterial and antibiofilm	48
38	Unknown	43.44	-	-	0.23	-	-	-
39	Doconexent	43.76	328	$C_{22}H_{32}O_2$	0.65	A polyunsaturated long-chain fatty acid	Antineoplastic	49
40	6,7-Epoxyoctadecanoic acid methyl ester	43.87	312	$C_{19}H_{36}O_3$	0.67	Fatty esters	-	-
41	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	44.02	330	$C_{19}H_{38}O_4$	0.83	Fatty ester derivatives		-
42	Unknown	44.33	-	-	0.35	-	-	-
43	Unknown	44.47	-	-	0.10	-	-	-
44	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z, Z, Z)-	46.93	352	$C_{27}H_{44}O_3$	0.74	Fatty ester derivatives	Urine acidifier, increase zinc bioavailability	50

Table 1: cont.

No.	Compound	R_t (min)	M.wt.	M.F.	Area %	Chemical class	Reported bioactivity	Reference
45	Tetracosanoic acid, methyl ester	47.32	382	$C_{25}H_{50}O_2$	0.16	Fatty esters	-	-
46	α-Tocospiro A	49.23	462	$C_{29}H_{50}O_4$	0.38	Sesterterpenoids	Antioxidant	51
47	α-Tocospiro A	49.55	462	$C_{29}H_{50}O_4$	0.58	Sesterterpenoids	Antioxidant	51
48	Nonanoic acid, 9-(nonyloxy)-, methyl ester	51.16	314	$C_{19}H_{38}O_3$	0.13	Fatty esters	Antibacterial	52
49	Unknown	51.89	-	-	0.08	-	-	-
50	Unknown	52.50	-	-	0.38	-	-	-
51	Vitamin E	53.08			0.15		Antioxidant, Antidiabetic	35, 53
52	Stigmasterol	55.05	412	$C_{29}H_{48}O$	0.29	Steroid derivative	Antifungal	54
53	Unknown	55.57	-	-	0.23	-	-	-
54	ɤ-Sitosterol	56.0	414	$C_{29}H_{50}O$	0.55	Steroids	Decreases in serum cholesterol, Antidiabetic	55
55	Unknown	56.92	-	-	0.20	-	-	-
56	Unknown	58.0	-	-	0.18	-	-	-
57	Phytol decanoate	60.10	450	$C_{30}H_{58}O_2$	0.85	Fatty acid phytyl ester	Antioxidant	56
	• Fatty esters and derivatives				73.04			
	• Fatty acids				5.64			
	• Sesquiterpenoids				2.33			
	• Acyclic hydrogenated diterpene alcohols				2.06			
	• Valerolactones				1.90			
	• Others				9.39			
	Total				**94.36 %**			


n-hexadecanoic acid
Hexadecanoic acid, methyl ester
Linolenic acid methyl ester
Hexadecanoic acid, trimethylsilyl ester
9,12-octadecadienoic acid (Z,Z)-, methyl ester


Phytol
Methyl stearate
Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl], methyl ester
Octadecanoic acid
9-octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]-

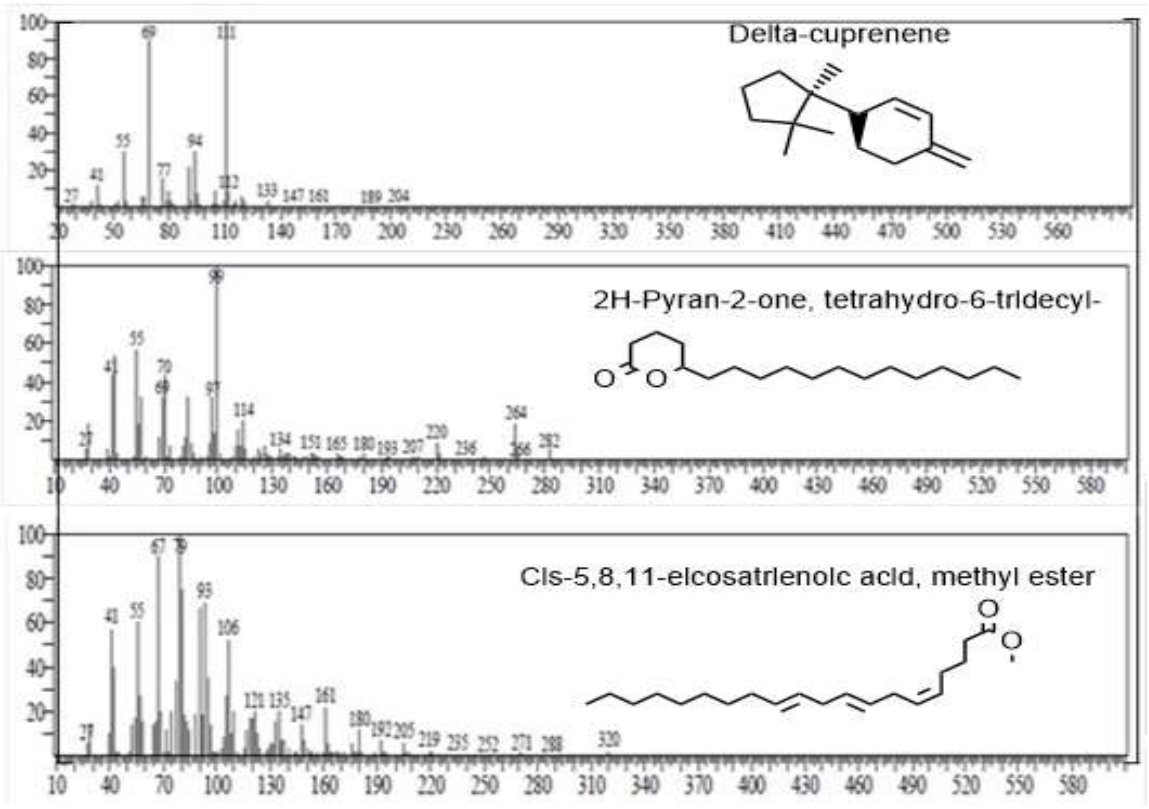


Figure 2. Mass spectra of major C. variegatum (L.) n-hexane leaf extract compounds

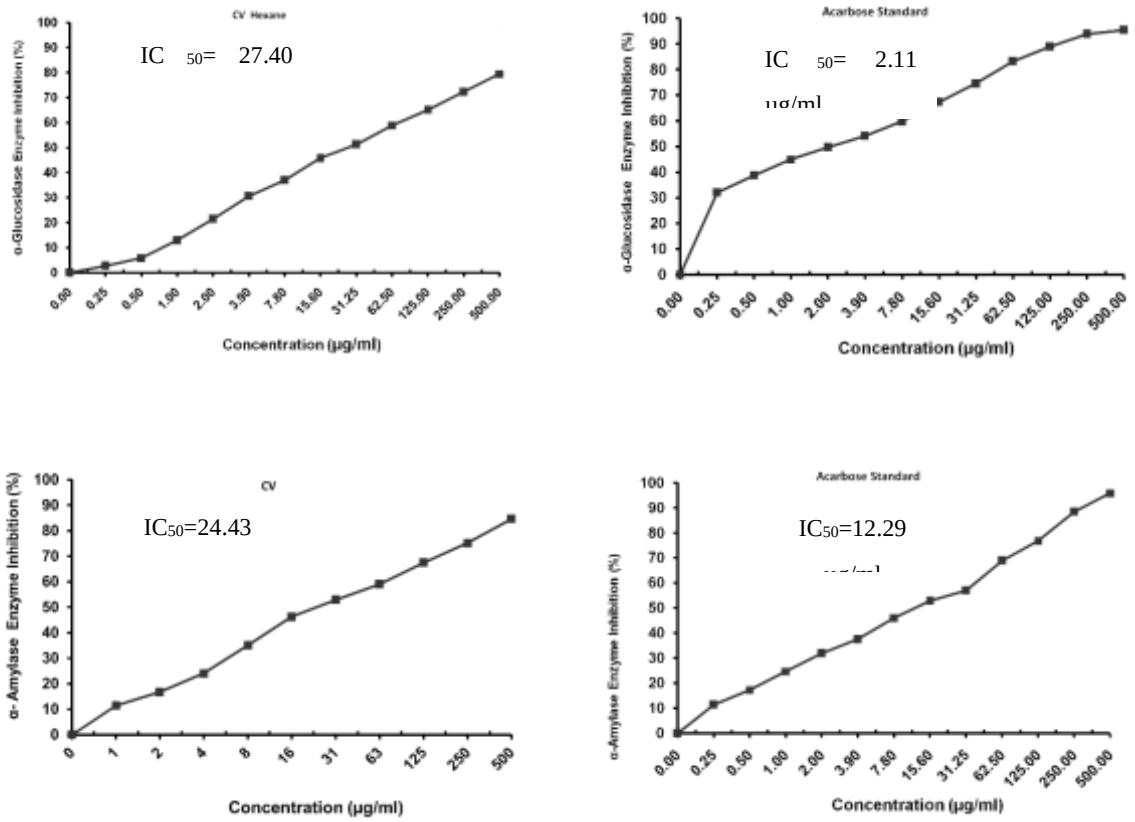


Figure 3. Inhibitory effects of *C. variegatum* (L.) leaf *n*-hexane extract and acarbose on α-glucosidase and α-amylase enzymes activity

5. CONCLUSIONS

In conclusion, the study reveals a significant link between antidiabetic inhibitor enzymes and the inhibitory effects of both acarbose and the n-hexane extract from Codiaeum variegatum (L.) leaves. The IC50 values indicate that acarbose has a stronger inhibitory effect on α-glucosidase and α-amylase activities than the n-hexane extract. This suggests that a higher concentration of the n-hexane extract is needed to match acarbose's enzyme inhibition level. These findings have important implications for developing treatments and therapies. However, further research is needed to fully explore potential applications and understand any associated risks or side effects. The identification of bioactive compounds and their potential therapeutic applications provides a promising avenue for future research.

Funding statement: NA.

Acknowledgment: The authors very grateful to Al-Azhar University, Faculty of Pharmacy (Girls Cairo, Egypt for offering the facilities for this study.

Conflict of interest: There are no conflicts of interest declared by the authors.

Author Contribution: All listed authors have contributed significantly to the manuscript.

List of Abbreviations: GC-MS: Gas Chromatography-Mass Spectrometry; Rt**:** Retention time; M.wt.: Molecular weight; M.F. Molecular formula.

REFERENCES

1. Njoya EM, Fewou PM, Niedermeyer TH. *Codiaeum variegatum* (L.) Rumph. ex A. Juss.(Euphorbiaceae): An overview of its botanical diversity, traditional uses, phytochemistry, pharmacological effects and perspectives towards developing its plant-based products. Journal of Ethnopharmacology. 2021;277:114244.

2. Hassan EM, Hassan RA, El-Toumy SA, Mohamed SM, Omer EA. Phenolic metaboloites and antioxidant activity of *Codiaeum variegatum* CV. Spirale. Journal of Pharmacy Research. 2014;8(5):619-23.

3. Saffoon N, Uddin R, Subhan N, Hossain H, Reza HM, Alam MA. In vitro anti-oxidant activity and HPLC-DAD system based phenolic content analysis of *Codiaeum variegatum* found in Bangladesh. Advanced Pharmaceutical Bulletin. 2014;4(Suppl 2):533.

4. Naidu GP. Antifungal activity in *Codiaeum variegatum* leaf extract. Current Science. 1988;57(9):502-4.

5. Hassan E, Hassan R, Salib J, Mohamed S, El-Toumy S. Chemical constituents and cytotoxic activity of *Codiaeum variegatum* CV. petra. Journal of Applied Sciences Research. 2013;9(8):4884-8.

6. Chauhan S, Singh A. Impact of Taraxerol in combination with extract of Euphorbia tirucalli plant on biological parameters of Lymnaea acuminata. Revista do Instituto de Medicina Tropical de São Paulo. 2011;53:265-70.

7. Cepeda G, Lisangan MM, Roreng MK. Ekstraksi, Karakterisasi dan Identifikasi Senyawa Bioaktif Daun Rumput Kebar (*Biophytum petersianum* Klotszch):- Characterization and Identification of Bioactive Compounds of Kebar Grass (Biophytum petersianum Klotszch) Leaves Extracts. Jurnal Tumbuhan Obat Indonesia. 2023;16(2):44-53.

8. Lawal OA, Ogunwande IA, Gbetoyon FS, Kasali AA, Opoku AR. Chemical composition and insecticidal activity of essential oils of four varieties of *Codiaeum variegatum* (L.) from Nigeria. Journal of Essential Oil Bearing Plants. 2018;21(3):840-7.

9. Moundipa PF, Flore KGM, Bilong CF, Bruchhaus I. I*n vitro* amoebicidal activity of some medicinal plants of the Bamun region (Cameroon). African Journal of Traditional, Complementary and Alternative Medicines. 2005;2(2):113–21-–21.

10. Sangeetha G, Krishna LM, Aruna G, Babu MS, Balammal G. Study on wound healing activity of root of *Codiaeum variegatum*. Int J Innova Drug Dis. 2011;1(1):19-23.

11. Forero JE, Avila L, Taborda N, Tabares P, López A, Torres F, et al. In vitro anti-influenza screening of several Euphorbiaceae species: Structure of a bioactive Cyanoglucoside from *Codiaeum variegat*um. Phytochemistry. 2008;69(16):2815-9.

12. Bijekar SR, Gayatri M, Rajanna L. Evaluation of anti-inflammatory activity of flavonoid fractions from Euphorbiaceae members on raw 264.7 cell lines. J Cytol genet. 2015;16(1):39-46.

13. Anim MT, Larbie C, Appiah-Opong R, Tuffour I, Owusu KB-A, Aning A. Extracts of *Codiaeum variegatum* (L.) A. Juss is cytotoxic on human leukemic, breast and prostate cancer cell lines. Journal of Applied Pharmaceutical Science. 2016;6(11):087-93.

14. Moshi M, Kagashe G. A study of the effect of extracts of *Codiaeum variegatum* (L.) A. Juss on Picrotoxin-induced convulsions in mice. Tanzania Medical Journal. 2004;19(1):12-4.

15. Bhot M, Saha M, Phatak A, Chandra N. Antimicrobial activity of leaf extracts of *Codiaeum variegatum* (l.) Blume. International Journal of Pharmacology and Biological Sciences. 2010;4(1):17.

16. Labu ZK, Laboni FR, Al Mamun MMA, Howlader MSI. Antidiarrhoeal activity and total tannin content of ethanolic leaf extract of *Codiaeum variegatum*. Dhaka University Journal of Pharmaceutical Sciences. 2015;14(1):87-90.

17. Smorowska AJ, Żołnierczyk AK, Nawirska-Olszańska A, Sowiński J, Szumny A. Nutritional properties and *in vitro* antidiabetic activities of blue and yellow corn extracts: A comparative study. Journal of Food Quality. 2021;2021:1-10.

18. Watterson KR, Hudson BD, Ulven T, Milligan G. Treatment of type 2 diabetes by free fatty acid receptor agonists. Frontiers in endocrinology. 2014;5:137.

19. Fahmy MA, Farghaly AA, Hassan EE, Hassan ZM, Abd-Alla HI. Protective role of *Codiaeum variegatum* against genotoxicity induced by carmustine in somatic and germ cells of male mice. Molecular Biology Reports. 2022;49(10):9543-53.

20. Nie T, Cooper GJ. Mechanisms underlying the antidiabetic activities of polyphenolic compounds: A review. Frontiers in Pharmacology. 2021;12:798329.

21. Abdallah HM, Jaleel GAA, Mohammed HS, Mahmoud SS, Yassin NA, el Din AG, et al. Phytochemical screening, gas chromatography-mass spectrometry analysis, and antidiabetic effects of *Corchorus olitorius* leaves in rats. Open Access Macedonian Journal of Medical Sciences. 2020;8(A):385-94.

22. Madkour HM, Ghareeb MA, Abdel-Aziz MS, Khalaf OM, Saad AM, El-Ziaty AK, et al. Gas chromatography-mass spectrometry analysis, antimicrobial, anticancer and antioxidant activities of n-hexane and methylene chloride extracts of *Senna italica*. Journal of Applied Pharmaceutical Science. 2017;7(6):023-32.

23. Epps DE, Marschke CK. Fluorescence-based high throughput sereening assays for protein kinases and phosphatases. Google Patents; 2001.

24. Garcia A, Barbas C. Gas chromatography-mass spectrometry (GC-MS)-based metabolomics. Metabolic profiling: Methods and protocols. 2011:191-204.

25. Karasek FW, Clement RE. Basic gas chromatography-mass spectrometry: principles and techniques: Elsevier; 2012.

26. Aati HY, Perveen S, Orfali R, Al-Taweel AM, Aati S, Wanner J, et al. Chemical composition and antimicrobial activity of the essential oils of *Artemisia absinthium*, *Artemisia scoparia*, and *Artemisia sieberi* grown in Saudi Arabia. Arabian Journal of Chemistry. 2020;13(11):8209-17.

27. El-Din SMM, El-Ahwany AM. Bioactivity and phytochemical constituents of marine red seaweeds (*Jania rubens*, *Corallina mediterranea* and *Pterocladia capillacea*). Journal of Taibah University for Science. 2016;10(4):471-84.

28. Kumar P, Pravinkumar P, Iqbal SS, Pillai KS, Michael M. Phytochemical profile, heavy metals contents and antioxidant activities of an antidiabetic polyherbal formulation. Journal of Pharmacognosy and Phytochemistry. 2015;3(6):08-16.

29. Abubakar MN, Majinda RR. GC-MS analysis and preliminary antimicrobial

activity of *Albizia adianthifolia* (Schumach) and P*terocarpus angolensis* (DC). Medicines. 2016;3(1):3.

30. Tan DC, Kassim NK, Ismail IS, Hamid M, Ahamad Bustamam MS. Identification of antidiabetic metabolites from *Paederia foetida* L. twigs by gas chromatography-mass spectrometry-based metabolomics and molecular docking study. BioMed research international. 2019;2019.

31. Chandrasekaran M, Kannathasan K, Venkatesalu V. Antimicrobial activity of fatty acid methyl esters of some members of Chenopodiaceae. Zeitschrift für Naturforschung C. 2008;63(5-6):331-6.

32. Shaheed KA, Alsirraj MA, Allaith SA, Noori NA, Obaid MH, Mouhsan ZM. The biological activities of seeds extracts for fenugreek and black cumin and its inhibitory influences towards some pathogens. Iraq Medical Journal. 2018;2(2).

33. Krishnaveni M, Dhanalakshmi R, Nandhini N. GC-MS analysis of phytochemicals, fatty acid profile, antimicrobial activity of Gossypium seeds. Int J Pharm Sci Rev Res. 2014;27(1):273-6.

34. Srivastava R, Mukerjee A, Verma A. GC-MS analysis of Phytocomponents in, pet ether fraction of wrightia tinctoria seed. Pharmacognosy Journal. 2015;7(4).

35. Zhang J, Dai Y, Yang Y, Xu J. Calcitriol alleviates hyperosmotic stress-induced corneal epithelial cell damage via inhibiting the NLRP3–ASC–caspase-1–GSDMD pyroptosis pathway in dry eye disease. Journal of Inflammation Research. 2021;14:2955.

36. Fatima N, Anwar F, Saleem U, Khan A, Ahmad B, Shahzadi I, et al. Antidiabetic effects of *Brugmansia aurea* leaf extract by modulating the glucose levels, insulin resistance, and oxidative stress mechanism. Frontiers in Nutrition. 2022;9:1005341.

37. Elshafie HS, Racioppi R, Bufo SA, Camele I. In vitro study of biological activity of four strains of *Burkholderia gladioli* pv. agaricicola and identification of their bioactive metabolites using GC–MS. Saudi journal of biological sciences. 2017;24(2):295-301.

38. Yao XT, Ling PX, Jiang S, Lai PX, Zhu CG. Analysis of the essential oil from *Gaillardia pulchella* Foug. and its antioxidant activity. Journal of oleo science. 2013;62(5):329-33.

39. Pu Z-H, ZHANG Y-q, YIN Z-q, Jiao X, JIA R-y, Yang L, et al. Antibacterial activity of 9-octadecanoic acid-hexadecanoic acid-tetrahydrofuran-3, 4-diyl ester from neem oil. Agricultural Sciences in China. 2010;9(8):1236-40.

40. Doughari JH, Saa-Aondo M. Phytochemical analysis of crude methanol extracts and antimicrobial activity of n-hexane fractions of methanol seed and pod extracts of P*rosopis africana* on some selected microrganisms. Archives. 2021;2:121-37.

41. Igwe OU, Okwu DE. GC-MS evaluation of bioactive compounds and antibacterial activity of the oil fraction from the seeds of *Brachystegia eurycoma* (HARMS). Asian Journal of Plant Science and Research. 2013;3(2):47-54.

42. Dong M, Oda Y, Hirota M. (10E, 12Z, 15Z)-9-hydroxy-10, 12, 15-octadecatrienoic acid methyl ester as an anti-inflammatory compound from *Ehretia dicksonii*. Bioscience, biotechnology, and biochemistry. 2000;64(4):882-6.

43. Zhang X, Lu X, Li H. Isolation and identification of a novel allelochemical from *Ruppia maritima* extract against the cyanobacteria *Microcystis aeruginosa*. Environmental Technology & Innovation. 2021;21:101301.

44. Sipahutar H, Gaol AY, Prasetya E. Antidiabetic Potentials of Ethanol Extract of *Timonius flavescens* (Jacq.) Baker Leaf. Tropical Journal of Natural Product Research. 2023;7(1).

45. Momodu I, Okungbowa E, Agoreyo B, Maliki M. Gas Chromatography–Mass Spectrometry Identification of Bioactive Compounds in Methanol and Aqueous Seed Extracts of *Azanza garckeana* Fruits. Nigerian Journal of Biotechnology. 2022;38(1):25-38.

46. Negri G, Salatino MLF, Salatino A. Unusual chemical composition of a sample of Brazilian propolis, as assessed by

analysis of a chloroform extract. Journal of apicultural research. 2003;42(4):53-6.

47. Santhiya N, Ramasamy M. GC-MS analysis of bioactive compounds from Freshwater mussels of *Parreysia corrugata* (Muller 1774) and their pharmacological activities. Journal of Drug Delivery and Therapeutics. 2019;9(4-A):155-8.

48. Fathi M, Ghane M, Pishkar L. Phytochemical composition, antibacterial, and antibiofilm activity of *Malva sylvestris* against human pathogenic bacteria. Jundishapur Journal of Natural Pharmaceutical Products. 2022;17(1).

49. Islam MT, Ali ES, Uddin SJ, Shaw S, Islam MA, Ahmed MI, et al. Phytol: A review of biomedical activities. Food and chemical toxicology. 2018;121:82-94.

50. Javaid A, Khan IH, Ferdosi MF. Bioactive constituents of wild *Cannabis sativa* roots from Pakistan. Pakistan Journal of Weed Science Research. 2021;27(3):359.

51. Bano I, Deora G. Preliminary phytochemical screening and GC-MS analysis of methanolic leaf extract of *Abutilon pannosum* (Forst. F.) Schlect. from Indian Thar desert. Journal of Pharmacognosy and Phytochemistry. 2019;8(1):894-9.

52. Sahin N, Kula I, Erdogan Y. Investigation of antimicrobial activities of nonanoic acid derivatives. Fresenius Environmental Bulletin. 2006;15(2):141-3.

53. Izu GO, Adeyi AO, Erukainure OL, Islam MS. Gamma-sitosterol–rich fraction from the methanolic extract of *Ficus exasperata* restores diabetes associated pathophysiological alterations in an alloxan-induced diabetic rats. Biokemistri. 2022;33(1).

54. Gabay O, Sanchez C, Salvat C, Chevy F, Breton M, Nourissat G, et al. Stigmasterol: a phytosterol with potential anti-osteoarthritic properties. Osteoarthritis and cartilage. 2010;18(1):106-16.

55. Farquhar JW, Smith RE, Dempsey ME. The effect of beta sitosterol on the serum lipids of young men with arteriosclerotic heart disease. Circulation. 1956;14(1):77-82.

56. Qaisar MN, Chaudhary BA, Sajid MU, Hussain N. Evaluation of α-glucosidase inhibitory activity of dichloromethane and methanol extracts of *Croton bonplandianum* Baill. Tropical Journal of Pharmaceutical Research. 2014;13(11):1833-6.