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PHYTOCHEMICAL PROFILING, ANTIDIABETIC, ANTIOBESITY, AND ANTIBACTERIAL ACTIVITIES OF *COMETES ABYSSINICA*

Ahmed A.M. Abdelgawad[1,2*]

[1]Department of Chemistry, College of Science, Jazan University, Jazan 45142, Saudi Arabia
[2]Medicinal and Aromatic Plants Department, Desert Research Center, Cairo, Egypt.

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ABSTRACT. This study aims to complete my chemical and biological studies on *Cometes abyssinica* (*CA*). The chemical profile was reported by LC-ESI-TOF-MS and GC-MS analyses. Antidiabetic (α-Glucosidase and α-amylase inhibitory activity assay), antiobesity (Lipase inhibitory activity assay), and antibacterial activity (agar well diffusion assay) were determined. The GC-MS analysis led to the identification of 27 hydrocarbons representing 93.4% of the total hydrocarbon content in hexane extract. 11-Octadecenoic acid, methyl ester (28.07%), citronellol (9.31%), hexadecanoic acid, methyl ester (7.64%), phytol (5.93%), and 10-epi-γ-eudesmol (4.91%) were the major components. The LC-ESI-TOF-MS analyses of aqueous-methanolic extract led to the identification of 99 secondary metabolites including phenolics, flavonoids, alkaloids, and vitamins. The percentages of inhibition of the n-hexane, chloroform, ethyl acetate, and methanol extracts of *CA* were evaluated against α-glucosidase, α-amylase, and pancreatic lipase enzymes at different concentrations. Hexane extract showed the most powerful α-glucosidase and α-amylase inhibitory activity with IC_{50} value of 0.22 and 0.27 mg/ml comparing with acarbose (IC_{50} 0.43 and 0.38), respectively. Among all *CA* extracts, only ethyl acetate extract revealed potent antiobesity as a lipase inhibitory activity with IC_{50} value of 0.079 mg/ml comparing with orlistat (IC_{50} 0.11 mg/ml). *CA* extracts showed mild-weak antibacterial effect against *Pseudomonas aeruginosa* and *Bacillus cereus*.

KEY WORDS: *Cometes abyssinica,* Chemical constituents, α-Amylase inhibitors, α-Glucosidase inhibitors, Lipase inhibitors

INTRODUCTION

Currently, one third of clinically used drugs come from nature. They are naturally isolated, synthesized, or semi-synthesized by modification of their phytochemical constituents [1]. In addition to the development of drugs, natural products are also involved in industry and agriculture. Stevioside and glycyrrhizin are used as sugars, they are isolated from and identified from candy leaf and licorice. Gardennin from *G. jasminoides*; tangerine from orange peel and shikonin from *L. erythrorhizon* are used as natural pigments. Recently, people have attempted to use natural medicines to avoid chemical side effects. These are further contributions of natural products chemistry to human health [2, 3].

Diabetes mellitus, one of the major public health problems worldwide, is a metabolic disorder of multiple etiologies distinguished by a failure of glucose homeostasis with disturbances of carbohydrate, fat, and protein metabolism because of defects in insulin secretion and/or insulin action [4, 5]. Before the discovery of insulin, traditional practices and folk medicines were the only ways for diabetes treatment [6]. α-Glucosidase and α-amylase enzyme break down complex carbohydrates like starch into simpler sugars like glucose or dextrin for absorption in the small intestine. Inhibiting these enzymes can be a safe and effective strategy for managing diabetes, as it slows down the rise in blood sugar levels after meals. α-Glucosidase and α-amylase enzymes inhibitors are a class of medications that work by blocking the enzyme α-glucosidase. Acarbose is an α-glucosidase and α-amylase inhibitor that slows the digestion of carbohydrates and can prevent sharp increases in blood sugar levels after meals [7, 8].

*Corresponding authors. E-mail: aawad@jazanu.edu.sa; ahmedawad26@hotmail.com

Obesity is a long-term problem that has persisted for many years. Obesity not only results in aesthetic problems but also causes abnormal physiological metabolism, which causes a series of physiological, psychological, and social problems. Obesity is an important risk factor for diseases such as cardiovascular disease [9], hypertension, hyperlipemia [10], diabetes [11] and even cancer [12], and it is closely related to the emergence of many chronic diseases [13]. Antiobesity candidates or drugs are expected to control or minimize weight with slight side effects. The increasing failure of antimicrobials and antibiotic resistance shown by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activities [14].

Cometes abyssinica (*CA*) belongs to the phylum Tracheophyta, is native to Africa and is mostly distributed in Palestine, Saudi Arabia, Egypt, Ethiopia, and other African deserts. *C. abyssinica*, commonly called Canxaxaf in Djibouti, is barely exists in the dry season, and used for treating Haemorrhage by heating crushed leaves and applied locally [15]. *CA* is a perennial plant, erect and widely branched leaves between 1.6-3.7 cm long, opposite, and lanceolate [16].

In our previous study on *CA* [17]; phytochemical investigation of methanol and ethyl acetate extracts resulted in the isolation of five known flavonoid compounds named formononetin, daidzein, eriodictyol, taxifolin, and kaempferitrin. The methanolic extract showed considerable inhibitory activity against colon (HCT-116) and hepatocellular (HepG2) carcinoma cell lines with IC_{50} 24.4-29.9 µg/mL compared to the drug reference Cisplatin. So, this study aims to complete the chemical screening of the aerial parts of *CA* as well as screen the antidiabetic, antiobesity, and antibacterial activities in continuation of our research [18-23] to find biological active natural constituents.

EXPERIMENTAL

Chemical and reagents

HPLC grade acetonitrile (MeCN) and methanol were purchased from Thermo-Fisher Scientific (Waltham, MA, USA). Formic acid 98%, ammonium hydroxide, and ammonium formate were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All the other chemicals were of analytical grades and purchased from Sigma Aldrich. All chemicals and reagents used for bioassay were purchased by BPTL-Alex team (Bioscreening and preclinical trial lab, biochemistry department, faculty of science, Alexandria University, Egypt).

α-Glucosidase and α-amylase inhibitory assay reagents. Glucose kit reagent: (1) Phosphate buffer (PB, 100 mM/L), (2) phenol (4 mM/L), (3) 4-amino-antipyrine (AAP, 1 mM/L), (4) glucose oxidase (GOD) > 20 KU/L), (5) peroxidase (POD) > 2 KU/L), (6) sodium azide (NaN_3, 8 mmol/L); for α-Glucosidase [bovine pancreatin enzyme solution: (5 mg/0.5 mL in 0.1 M phosphate buffer at pH = 7.4 then dilute 0.5 mL in 5 mL phosphate buffer); maltose substrate solution: (1% in distilled H_2O)]; for α-amylase assay [α-Amylase enzyme solution: (5 mg/0.5 mL in 0.1 M phosphate buffer at pH = 6.9 then dilute 0.5 mL in 5 mL phosphate buffer); dextrin substrate solution: (1% in distilled H_2O)].

Lipase inhibitory assay reagents. Copper reagent (270 mL of triethanolamine, 2 mM of 30 mL acetic acid, 1 M of 300 mL of cupric sulfate), sodium diethyldithiocarbamate (1 g/L in butanol), chloroform, standard orlistat in distilled water and plant extract in DMSO (5, 10, 50, 100, 200 and 500 µg/mL).

Plant material and extraction

The Aerial parts of *CA* were collected, identified, air-dried, and deposited in Desert Research Center Herbarium (CAIH) under the voucher specimen (CAIH-1021-R). Finely powder of *CA*

was extracted with methanol (95%), filtered, and lyophilized. The brown-sticky extract was suspended with distilled H_2O and successively fractionated with immiscible organic solvents such as n-hexane, chloroform, ethyl acetate, and methanol (MeOH). The resulting fractions were lyophilized, and their pharmacological properties were investigated. The aq. MeOH extract, and n-hexane fraction were applied for chromatographical analyses. This study is a continuation of my previous study on *CA* [17], so all the missing data in this section is reported in the first study.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis of the hexane fraction was performed at Regional Center for Mycology and Biotechnology at Al-Azhar University (RCMB-AZ) on Thermo Scientific Trace 1310 gas chromatograph attached with ISQ LT single quadrupole mass spectrometer, operating in the EI mode at 70 eV, equipped with a split/splitless injector (200 °C). Helium was used as carrier gas (1 mL/min) and the capillary columns used were DP5-MS (30 m x 0.25 mm; film thickness 0.25 mm). The transfer line temperature was kept at 290 °C and 300 °C, respectively, with electron multiplier voltage of 1 kV. Identification and interpretation of phytoconstituents was achieved based on their retention indices and fragment patterns using the National Institute of Standards and Technology (NSIT) and Willey 5 databases and mass finder, as well as data reported by Adams [24]. The relative percentages of the identified compounds were assayed and calculated based on their peak area.

LC-ESI-TOF-MS analysis of the methanol extract

LC-ESI-TOF-MS analyses of the aqueous-methanolic extract in the negative and positive modes were performed at Proteomics and Metabolomics Research Program (Children's Cancer Hospital-Cairo, Egypt) (PMRP-57357-Egypt) as described in detail in the literature [25, 26].

A complete solubility stock solution of lyophilized aqueous-methanolic extract was used for injection. The sample was injected in both positive and negative modes. Small molecules were separated on an ExionLC system connected with an autosampler system, an in-line filter disks pre-column, and an Xbridge C18 column was kept at 40 ºC, and at a flow rate of 300 µL/min was utilized.

The mass spectrometry was achieved on a Triple TOF 5600+ system equipped with a Duo-Spray source operating in the ESI mode (AB SCIEX, Concord, ON, Canada). The sprayer capillary and declustering potential voltages were 4500 and 80 eV in the positive mode, and -4500 and -80 V in the negative mode. The TripleTOF5600+ was operated using an information-dependent acquisition (IDA) protocol [27]. Data was processed using MS-DIAL 4.8, and the used reference databases were: ReSpect positive (2737 records) or ReSpect negative (1573 records). PeakView 2.2 with the MasterView 1.1 package were used for feature (peaks) extraction from Total ion chromatogram (TIC) [28].

In vitro biological activity

α-Glucosidase, α-amylase, and lipase inhibitory activities of *C. abyssinica* extracts were conducted at BPTL-Alex, Egypt.

α-Glucosidase and α-amylase inhibitory activity assay

α-Glucosidase [29] and α-amylase [30] inhibitory activities were assayed as briefly described here. 10 µL of plant extract (test and blank)/DMSO (negative control)/acarbose (standard) in microtiter plate wells were mixed with 110 µL of diluted pancreatin enzyme or diluted amylase enzyme. Microtiter plate wells were incubated at 37 °C for 30 min. After incubation, 60 µL of

maltose or dextrin was then incubated at 37 °C for 20 min and 100 µL of glucose kit reagent was added to all wells (add 100 µL of phosphate buffer in test blank only). The absorbance was measured at 490 nm using a spectrophotometer.

Lipase inhibitory activity assay

Lipase inhibitory activity [31] was assayed as briefly described here. In the test tube, 450 µL of plant extract/ standard/ chloroform (blank) was added to 50 µL 5 mg/mL porcine pancreatic lipase and incubated for 20 min at 37 °C. After that, 2 mL of glycerol triolein (80 mg dissolved in 0.1 M tris-HCL containing 15 mg bile salt) was added and incubated for 30 min at 37 °C. Then 2.5 mL copper reagent was added then shake vigorously with vortex. 5 mL chloroform was added to the solution and shake again vigorously for 1 min. The two layers were separated with centrifugation at 3000 rpm for 10 min. 3 mL of the lower layer was carefully transferred to another tube containing 500 µL of sodium diethyldithiocarbomate reagent. The absorbances were measured at 440 nm.

The α-glucosidase or α-amylase or lipase inhibition activity of plant extract was estimated from the following formula: inhibition (%) = $(\frac{Abs\ control - Abs\ fraction}{Abs\ control})$*100, where; Abs = absorbance of Blank and absorbance of the fraction. The α-glucosidase, α-amylase and lipase inhibition effect of plant extract was expressed as IC_{50}. IC_{50} value (mg/mL) is the inhibitory concentration at which 50% of α-glucosidase or α-amylase or lipase are repressed. Abs = absorbance of blank and absorbance of the fraction.

Antibacterial activity

Antibacterial activity was performed by agar well diffusion assay [32, 33] against two microbial species known to be pathogenic including gram (+) bacteria (*Pseudomonas aeruginosa* ATCC 27853), gram (-) bacteria (*Bacillus cereus* ATCC10876). The minimum inhibitory concentrations were evaluated by an agar well diffusion assay method and expressed as mg/ml. The bacteria were grown in nutrient broth at 37 °C for 24 h. After adjustment to standard turbidity, one hundred µL of the inoculums (1×108 cfu/mL) were inculcated on agar media and poured into the Petri plate. The sample was inoculated into the wells. All the tested bacterial plates were incubated at 37 °C for 24 h. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (mm), including the well diameter. The readings were taken in three different fixed directions in all triplicates and the average values were tabulated.

RESULTS AND DISCUSSION

The chemical makeup of the hexane extract of *C. abyssinica* aerial parts is gathered in Table 1 and the GC-MS chromatogram are presented in Figure 1. Twenty-seven hydrocarbon phytoconstituents were identified and represented about 93% of the total fraction content, including terpenes, fatty acid, and fatty acid esters. According to the results data, 11-octadecenoic acid, methyl ester (28.07%), citronellol (9.31%), hexadecanoic acid, methyl ester (7.64%), phytol (5.93%), and 10-epi-γ-eudesmol (4.91%) were the major components. Mono and sesquiterpenes were the predominated percentages around 17% and 15%, while phytol was the only diterpene identified from the hexane extract.

Monoterpenes such as citronellol and citronellyl acetate display biochemical and physiological effects in many plants, which make them able to act as insecticides for herbivorous insects [34]. Citronellol induces necroptosis of tumor cells via maximize-regulating TNF-α, RIP1/RIP3 activities, minimize-regulating caspase-3/caspase-8 activities and increase reactive oxygen species (ROS) accumulation. It is reported that citronellyl acetate has antinociceptive,

antimicrobial, larvicidal, and insecticidal properties [35]. 10-Epi-γ-eudesmol sesquiterpene, showed anti-inflammatory and antioxidant activity, and was suggested for characterizing the different cultivars and hybrids of pelargonium graveolens [36]. Fatty acids are vital phytoconstituents of the human body because of their biological and functional roles [37].

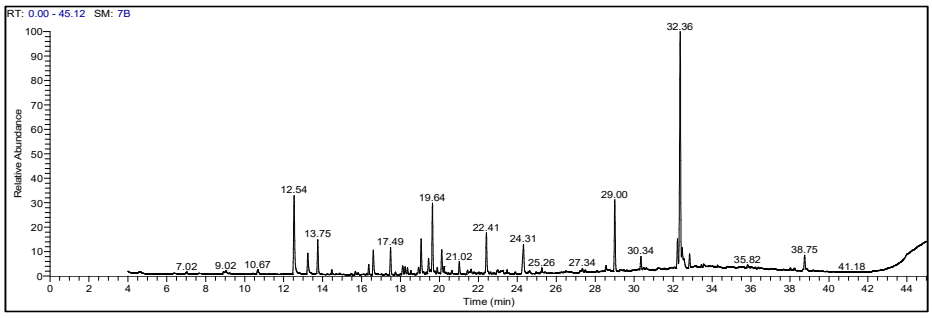


Figure 1. Gas chromatogram of the aerial parts of the hexane extract of *C. abyssinica*.

Table 1. List of the identified hydrocarbons from the hexane extract of *C. abyssinica* by GC–MS.

#	RT (min)	Compounds Identity **	%*	Molecular Formula (Precursor m/z)
1	12.54	Citronellol	9.31	$C_{10}H_{20}O$ (156.19)
2	13.24	Geraniol	2.47	$C_{10}H_{18}O$ (154.23)
3	13.75	Citronellol acetate	3.60	$C_{12}H_{22}O_2$ (198.31)
4	13.75	Citronellyl formate	3.60	$C_{11}H_{20}O_2$ (184.27)
5	16.37	α-Copaene	1.05	$C_{15}H_{24}$ (204.21)
6	16.60	(-)-α-Bourbonene	2.76	$C_{15}H_{24}$ (204.17)
7	17.49	Caryophyllene	2.88	$C_{15}H_{24}$ (204.34)
8	18.11	α-Muurolene	1.17	$C_{15}H_{24}$ (204.42)
9	18.24	Aromandendrene	0.88	$C_{15}H_{24}$ (204.49)
10	18.36	Humulene	0.70	$C_{15}H_{24}$ (204.33)
11	18.93	Geranyl propionate	0.71	$C_{13}H_{22}O_2$ (210.18)
12	19.06	Germacrene D	3.69	$C_{15}H_{24}$ (204.09)
13	19.44	α-Elemene	1.54	$C_{15}H_{24}$ (204.55)
14	20.24	Citronellyl butyrate	0.66	$C_{14}H_{26}O_2$ (226.24)
15	22.41	10-Epi-γ-eudesmol	4.91	$C_{15}H_{26}O$ (222.32)
16	23.85	*cis*-Lanceol	0.31	$C_{15}H_{24}O$ (220.12)
17	28.55	Palmitoleic acid	0.73	$C_{16}H_{30}O_2$ (254.47)
18	29.00	Hexadecanoic acid, methyl ester	7.64	$C_{17}H_{34}O_2$ (270.41)
19	30.34	Hexadecanoic acid, ethyl ester	1.47	$C_{18}H_{36}O_2$ (284.08)
20	32.22	Methyl (Z,Z)-9,12-octadienoate	2.66	$C_{19}H_{34}O_2$ (294.03)
21	32.36	11-Octadecenoic acid, methyl ester	28.07	$C_{19}H_{36}O_2$ (296.15)
22	32.46	Methyl (Z)-9-octadecenoate	2.09	$C_{19}H_{36}O_2$ (296.15)
23	32.58	Phytol	5.93	$C_{20}H_{40}O$ (296.33)
24	32.84	Methyl stearate	1.52	$C_{19}H_{38}O_2$ (298.05)
25	35.82	Glycidyl palmitate	0.52	$C_{19}H_{36}O_3$ (312.16)
26	38.02	(Z)-9-Octadecenoic acid	0.39	$C_{18}H_{34}O_2$ (282.25)
27	38.75	Glycidyl oleate	2.10	$C_{21}H_{38}O_3$ (338.33)
	Total		93.36	

RT = Retention time; *relative percentages of the identified compounds in the hexane extract were calculated based on the total peak area in the chromatogram; **tentative identity.

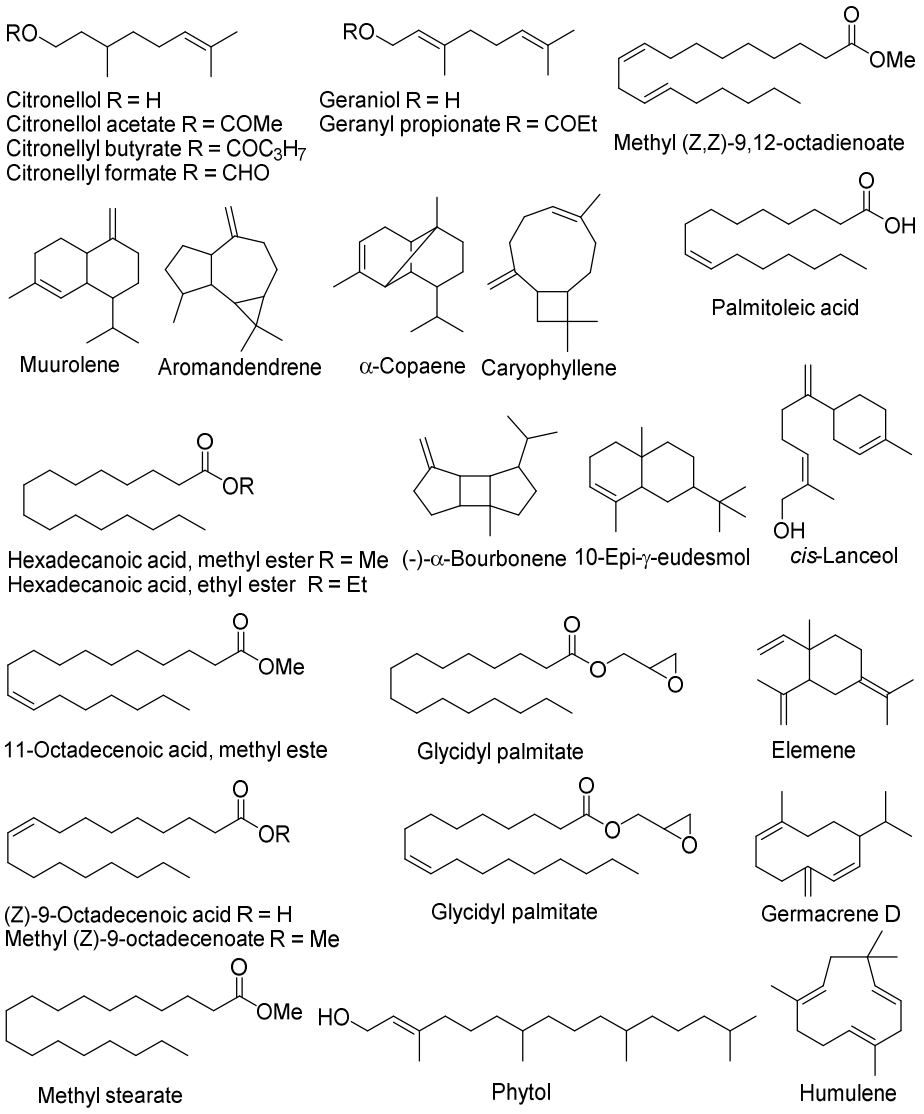


Figure 2. The chemical structure of the identified hydrocarbons from *C. abyssinica*.

The LC–ESI-TOF–MS analyses in both positive and negative modes were applied for secondary metabolites profiling of the aqueous-methanolic extract of *C. abyssinica*. As portrayed in Table 2, the analysis identified 99 phytoconstituents belonging to chemically diverse classes of phytoconstituents, notably phenolic, flavonoid, amino acids, alkaloids, and vitamins.

The MS-DIAL 4.8 open-source software [28] was used to identify products while ReSpect databases were applied as the reference database. Identifying each single compound was mainly based on mass spectrum, fragmentation patterns, and previous reports on the phytoconstituents of *C. abyssinica* and other plants of the genus *Cometes* were referenced in identifying the

compounds. Additionally, the relative percentages of the identified compounds in the plant extracts were calculated based on the total peak area in the chromatogram.

In the LC-ESI-TOF-MS positive ion mode, the metabolites **79** and **88** (RT = 1.41 and 5.88 min), were identified as glycosides, showing the ion $[M+H]^+$ at m/z 449.10 and 611.18 as the base beak with another ions $[M+H-H_2O]^+$ at m/z 431.18 and 303.05, another main ions [aglycone + H]$^+$ at m/z 287.09 and 303.05 corresponding to maritimetin and hesperetin aglycones respectively. These metabolites **79** and **88** were identified as maritimetin-6-O-glucoside and hesperetin-7-O-neohesperidoside (Figure 3).

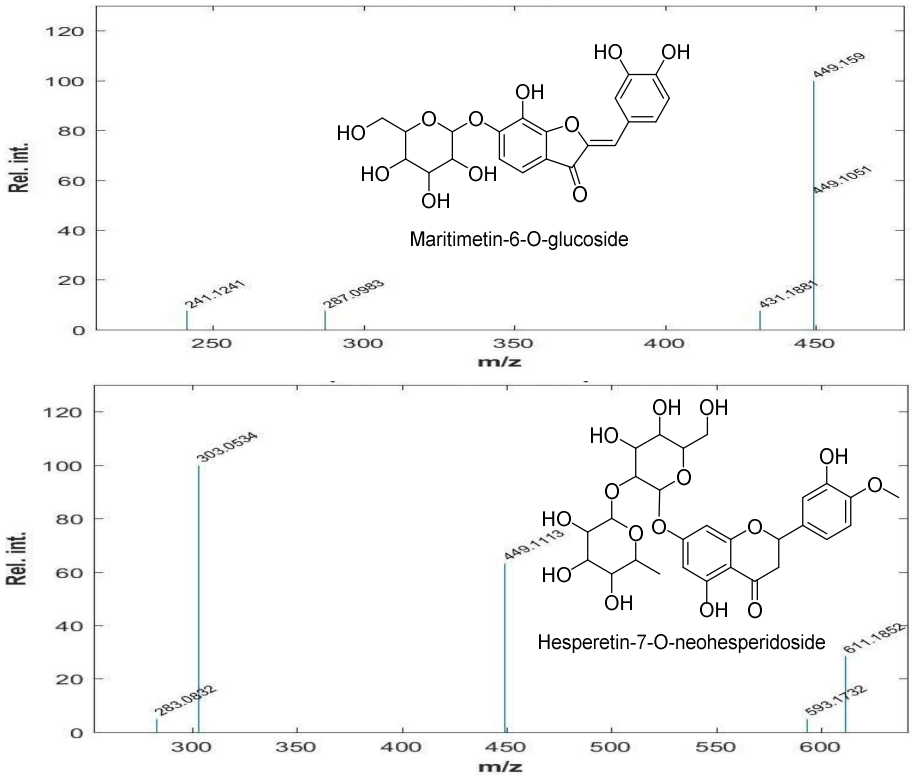


Figure 3. Mass spectra of maritimetin-6-O-glucoside **79** and mesperetin-7-O-neohesperidoside **88** identified from *C. abyssinica* by LC–ESI-TOF–MS in positive modes.

In the LC-ESI-TOF-MS negative ion mode, the metabolite **103**, was identified as aglycone, showing the ion $[M-H]^-$ at m/z 299.05 as the base beak with another ions at m/z 298.08, 284.03 and 256.03 corresponding to the losing of another proton (1 amu), methyl (15 amu), and carbon monoxide (28 amu) fragments. This metabolite **103** was identified as Kaempferide. Compound **92** (RT = 6.17 min) gave an $[M-H]^-$ ion at m/z 577.15. In its tandem MS, the observation of the characteristic fragment ions at m/z 457.10 and 413.08 corresponded to the loss of $C_4H_8O_4$ (120 amu) and a rhamnose moiety (164 amu) $[A]^-$, respectively. The fragmentation ions $[A-C_4H_8O_4]^-$ at m/z 293.04 were identical to the presence of 6-C substituent. Based on these results, compound **92** was tentatively identified as vitexin-2"-O-rhamnoside (Figure 4).

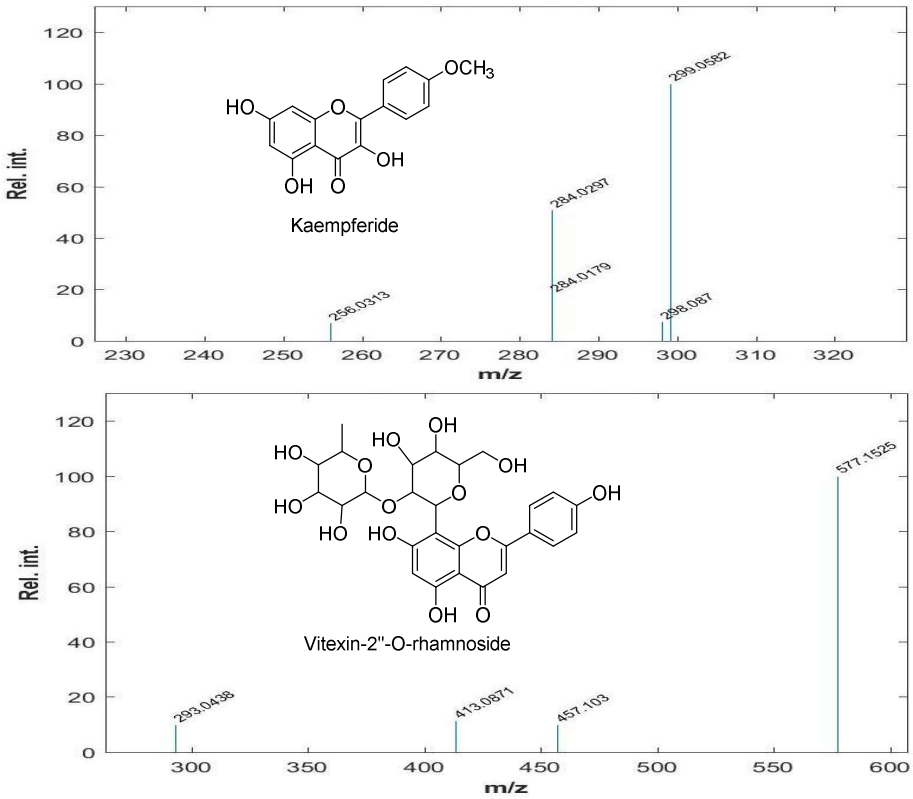


Figure 4. Mass spectra of vitexin-2"-O-rhamnoside **92** and kaempferide **103** identified *C. abyssinica* by LC–ESI-TOF–MS in negative mode.

N-Compounds represent about 45% of the total content of the aqueous-methanolic extract including alkaloids, amino acids, purine and pyrimidine nucleotides, vitamins, xanthines, and other related compounds. Amino acids were the major class representing about 34% of the total content. Among the identified N-compounds, *N,N*-dimethylglycine, L-proline and L-*β*-homotryptophan accounted as the major constituents tentatively identified in *C. abyssinica*.

Table 2. List of tentatively identified N-metabolites, phenolic and flavonoid from the aqueous-methanolic extract of *C. abyssinica* by LC–ESI-TOF–MS in both positive and negative modes with their retention times, %, molecular formula.

No	RT (min)	%*	Adduct ion	Molecular formula (precursor m/z)	Mass error ppm	Compounds identity**
			alkaloids			
28	1.26	0.15	$[M+H]^+$	$C_{13}H_{14}N_2O$ (215.12)	0.6	Harmaline
29	1.32	1.97	$[M+H]^+$	$C_7H_7NO_2$ (138.05)	2.8	Trigonelline
			Amino Acids			
30	1.18	17.75	$[M+H]^+$	$C_4H_9NO_2$ (104.07)	0.7	*N,N*-Dimethylglycine
31	1.27	3.39	$[M+H]^+$	$C_{12}H_{14}N_2O_2$ (219.11)	2.5	L-*β*-homotryptophan-HCl
32	1.31	0.20	$[M+H]^+$	$C_6H_9N_3O_2$ (156.07)	-0.1	His

33	1.32	1.95	$[M+H]^+$	$C_5H_{11}NO_2$ (118.08)	5.5	Glycine-Betaine
34	1.36	4.45	$[M+H]^+$	$C_5H_9NO_2$ (116.07)	3.1	L-Proline
35	1.38	0.07	$[M-H]^-$	$C_5H_6N_2O_4$ (157.02)	4.8	Dihydroorotate
36	1.41	0.23	$[M+H]^+$	$C_6H_{11}NO_2$ (130.08)	0.1	Pipecolate
37	1.44	0.04	$[M+H]^+$	$C_9H_{11}NO_3$ (182.08)	2.7	Tyr
38	1.92	1.48	$[M+H]^+$	$C_5H_7NO_3$ (130.04)	-0.3	L-5-Oxoproline
39	4.32	0.09	$[M+H]^+$	$C_6H_{13}N_3O_3$ (176.10)	1.2	Citrulline
40	5.48	0.16	$[M+H]^+$	$C_6H_{12}N_2O_4S_2$ (241.03)	-0.8	L-Cystine
41	8.14	0.07	$[M-H]^-$	$C_7H_{15}NO_2$ (144.10)	-0.6	L-*β*-Homoisoleucine
			Purine nucleotides			
42	1.11	0.40	$[M-H]^-$	$C_{10}H_{12}N_4O_5$ (267.07)	2.9	Inosine
43	1.124	0.09	$[M+H]^+$	$C_{10}H_{14}N_5O_8P$ (364.06)	2.9	Guanosine 5'-monophosphate
44	1.30	2.73	$[M+H]^+$	$C_{10}H_{13}N_4O_9P$ (365.04)	1.2	Xanthosine-5'-monophosphate
45	1.89	0.07	$[M-H]^-$	$C_5H_5N_5$ (134.04)	7.4	Adenine
46	4.52	0.02	$[M-H]^-$	$C_{10}H_{15}N_5O_{10}P_2$ (426.02)	-1.9	Adenosine 5'-diphosphate
47	5.07	0.06	$[M+H]^+$	$C_{10}H_{13}N_5O_4$ (268.10)	0	Adenosine
48	6.73	0.04	$[M+H]^+$	$C_{10}H_{12}N_5O_6P$ (330.05)	-0.1	Adenosine 3':5'-cyclicmonophosphate
49	7.56	0.07	$[M+H]^+$	$C_{10}H_{13}N_5O$ (220.11)	1.6	*trans*-Zeatin
50	8.43	0.10	$[M+H]^+$	$C_{10}H_{14}N_5O_7P$ (348.07)	6.6	Adenosine 5'-monophosphate
51	10.34	0.06	$[M+H]^+$	$C_{10}H_{14}N_5O_6P$ (332.07)	0.1	2'-Deoxyadenosine 5'-monophosphate
			Pyrimidine nucleotides			
52	1.39	0.32	$[M+H]^+$	$C_9H_{14}N_3O_8P$ (324.05)	4.9	Cytidine-5'-monophosphate
53	1.39	0.28	$[M-H]^-$	$C_9H_{12}N_2O_6$ (243.06)	-3.4	Uridine
54	2.14	0.14	$[M-H]^-$	$C_{10}H_{14}N_2O_5$ (241.08)	0.9	Thymidine
55	5.50	0.09	$[M+H]^+$	$C_5H_6N_2O_2$ (127.05)	-0.5	Thymine
56	5.61	0.08	$[M+H]^+$	$C_9H_{13}N_3O_5$ (244.09)	5.8	Cytidine
57	5.66	0.14	$[M-H]^-$	$C_{15}H_{22}N_2O_{18}P_2$ (579.02)	0.2	Uridine 5'-diphosphoglucuronic acid
58	6.67	0.07	$[M+H]^+$	$C_{14}H_{26}N_4O_{11}P_2$ (489.11)	0.1	Cytidine 5'-diphosphocholine
59	14.32	1.09	$[M+H]^+$	$C_9H_{15}N_3O_{11}P_2$ (404.02)	-0.4	Cytidine-5'-diphosphate
60	23.52	0.58	$[M-H]^-$	$C_9H_{15}N_2O_{14}P_3$ (466.96)	0.6	2'-Deoxyuridine-5'-triphosphate sodium salt
			Vitamins			
61	1.08	0.27	$[M-H]^-$	$C_{17}H_{21}N_4O_9P$ (455.09)	4	Riboflavin-5'-monophosphate sodium salt hydrate
62	16.46	0.78	$[M+H]^+$	$C_{11}H_{15}N_2O_8P$ (335.06)	-0.8	β-Nicotinamide mononucleotide
63	16.86	0.47	$[M+H]^+$	$C_{17}H_{20}N_4O_6$ (377.14)	3.5	(-)-Riboflavin
				Xanthines		
64	5.56	0.06	$[M+H]^+$	$C_5H_4N_4O_3$ (169.03)	4.2	Uric acid
Others						
65	1.41	0.62	$[M+H]^+$	$C_2H_4N_4$ (85.05)	-0.8	3-Amino-1,2,4-triazole
66	2.06	1.04	$[M-H]^-$	$C_2H_8NO_3P$ (124.01)	2.1	2-Aminoethylphosphonate
67	20.03	0.76	$[M+H]^+$	$C_{15}H_{22}N_6O_5S$ (399.14)	0.5	S-Adenosyl-L-methionine
			Anthocyanidines			
68	7.08	0.08	$[M]^+$	$C_{21}H_{21}O_{11}$ (449.10)	6.6	Cyanidin-3-glucoside
69	7.79	0.09	$[M]^+$	$C_{22}H_{23}O_{12}$ (479.11)	0.2	Petunidin-3-*O*-beta-glucopyranoside
70	8.10	0.36	$[M]^+$	$C_{22}H_{23}O_{11}$ (463.12)	-0.2	Peonidine-3-*O*-glucoside chloride
71	8.26	0.06	$[M]^+$	$C_{23}H_{25}O_{12}$ (493.13)	5.1	Malvidin-3-galactoside
			Coumarin			
72	1.44	0.08	$[M-H]^-$	$C_{10}H_8O_3$ (175.04)	3.2	7-Hydroxy-4-methylcoumarin
73	5.12	0.19	$[M-H]^-$	$C_{15}H_{16}O_9$ (339.07)	-3.9	Esculin
74	8.04	0.09	$[M+H]^+$	$C_9H_6O_4$ (179.03)	1.1	Daphnetin

			Flavonoids and related metabolites			
75	1.08	0.05	$[M-H]^-$	$C_{21}H_{22}O_{11}$ (449.10)	1.5	Okanin-4'-*O*-glucoside
76	1.11	2.06	$[M+H]^+$	$C_{15}H_{12}O_6$ (289.07)	0.3	Eriodictyol
77	1.33	0.48	$[M-H]^-$	$C_{16}H_{12}O_7$ (315.05)	-1.5	3'-Methoxy-4',5,7-trihydroxyflavonol
78	1.33	0.23	$[M-H]^-$	$C_{21}H_{18}O_{12}$ (461.07)	0.4	Kaempferol-3-Glucuronide
79	1.41	0.10	$[M+H]^+$	$C_{21}H_{20}O_{11}$(449.10)	0	Maritimetin-6-O-glucoside
80	1.69	3.13	$[M+H]^+$	$C_{15}H_{12}O_7$ (305.08)	0.2	Taxifolin
81	1.87	0.14	$[M+H]^+$	$C_{15}H_{10}O_7$ (303.04)	4.6	Quercetin
82	3.33	3.18	$[M+H]^+$	$C_{16}H_{12}O_4$ (269.08)	0.4	Formononetin
83	4.11	2.41	$[M+H]^+$	$C_{15}H_{10}O$ (255.06)$_4$	1.1	Daidzein
84	4.14	0.15	$[M+H]^+$	$C_{21}H_{18}O_{11}$ (447.09)	7.3	Baicalein-7-*O*-glucuronide
85	5.02	0.05	$[M+H]^+$	$C_{21}H_{20}O_{13}$ (481.09)	-1.9	Gossypin
86	5.13	0.09	$[M-H]^-$	$C_{28}H_{32}O_{14}$ (591.17)	0.7	Acacetin-7-*O*-rutinoside
87	5.59	0.08	$[M+H]^+$	$C_{30}H_{26}O_{12}$ (579.14)	-1.9	Procyanidin B2
88	5.88	0.10	$[M+H]^+$	$C_{28}H_{34}O_{15}$ (611.19)	-0.3	Hesperetin-7-*O*-neohesperidoside
89	5.94	0.13	$[M-H]^-$	$C_{27}H_{30}O_{16}$ (609.14)	-0.6	Luteolin-3', 7-di-*O*-glucoside
90	5.94	0.14	$[M+H]^+$	$C_{16}H_{12}O_5$ (285.07)	-2	Acacetin
91	6.10	1.60	$[M+H]^+$	$C_{21}H_{20}O_{11}$ (449.10)	-0.7	Luteolin-6-*C*-glucoside
92	6.17	3.04	$[M-H]^-$	$C_{27}H_{30}O_{14}$ (577.15)	0.2	Vitexin-2"-*O*-rhamnoside
93	6.35	1.87	$[M-H]^-$	$C_{27}H_{30}O_{15}$ (593.15)	0.7	Kaempferol-7-neohesperidoside
94	6.41	0.10	$[M-H]^-$	$C_{28}H_{32}O_{16}$ (623.16)	4.6	Isorhamnetin-3-*O*-rutinoside
95	6.84	0.20	$[M+H]^+$	$C_{21}H_{20}O_{10}$ (433.11)	0.6	Apigenin 8-*C*-glucoside
96	6.85	0.55	$[M+H]^+$	$C_{28}H_{32}O_{15}$ (609.18)	1.7	Diosmin
97	6.96	0.54	$[M-H]^-$	$C_{21}H_{20}O_{11}$ (447.09)	-0.8	Luteolin-7-*O*-glucoside
98	7.031	2.57	$[M-H]^-$	$C_{27}H_{30}O_{14}$ (577.15)	-0.2	Kaempferitrin
99	9.97	0.07	$[M+H]^+$	$C_{15}H_{10}O_6$ (287.05)	0.6	Luteolin
100	10.10	0.03	$[M-H]^-$	$C_{15}H_{12}O_5$ (271.06)	9.6	Naringenin
101	10.53	0.25	$[M-H]^-$	$C_{15}H_{10}O_5$ (269.04)	-6	Apigenin
102	10.57	0.04	$[M+H]^+$	$C_{15}H_{10}O_8$ (319.04)	-2.1	Myricetin
103	10.80	0.83	$[M-H]^-$	$C_{16}H_{12}O_6$ (299.05)	-0.1	Kaempferide
104	14.18	0.23	$[M-H]^-$	$C_{21}H_{20}O_{10}$ (431.09)	-0.9	Kaempferol-3-*O*-alpha-L-rhamnoside
105	15.82	0.03	$[M+H]^+$	$C_{21}H_{20}O_{11}$ (449.10)	4.6	Luteolin-8-*C*-glucoside
106	20.11	2.45	$[M+H]^+$	$C_{16}H_{12}O_7$ (317.06)	0.1	3,3',4',5-tetrahydroxy-7-methoxyflavone
107	23.48	2.19	$[M-H]^-$	$C_{21}H_{20}O_{12}$ (463.08)	2.6	Myricitrin
108	23.81	0.05	$[M+H]^+$	$C_{21}H_{20}O_9$ (417.11)	0.6	Puerarin
			Phenolic and carboxylic acid derivatives			
109	1.12	1.44	$[M-H]^-$	$C_6H_{12}O_7$ (195.05)	-0.4	Gluconate
110	1.13	2.36	$[M-H]^-$	$C_7H_{12}O_6$ (191.05)	4.2	D-(-)-Quinic acid
111	1.13	0.37	$[M-H]^-$	$C_4H_4O_4$ (115.00)	7.3	Maleic acid
112	1.173	0.69	$[M-H]^-$	$C_4H_6O_5$ (133.01)	0	D-(+)-Malic acid
113	1.18	4.23	$[M-H]^-$	$C_5H_8O_4$ (131.03)	-8.9	Glutaric acid
114	1.23	0.02	$[M-H]^-$	$C_4H_6O_4$ (117.01)	0.2	Succinic acid
115	1.24	0.20	$[M-H]^-$	$C_4H_6O_6$ (149.00)	1.3	L-(+)-Tartrate
116	1.31	0.21	$[M-H]^-$	$C_7H_6O_3$ (137.02)	0.1	Salicylic acid
117	1.33	0.09	$[M-H]^-$	$C_8H_8O_4$ (167.03)	-4.4	3,4-Dihydroxyphenylacetic acid
118	1.84	0.33	$[M-H]^-$	$C_9H_8O_3$ (163.04)	0.7	3-(4-Hydroxyphenyl)prop-2-enoic acid
119	2.00	0.09	$[M+H]^+$	$C_{14}H_{12}O_3$ (229.08)	0	Resveratrol
120	4.62	0.26	$[M+H]^+$	$C_{11}H_{12}O_5$ (225.07)	0.5	3-(4-Hydroxy-3,5-dimethoxy-phenyl)-2-propenoic acid
121	5.10	2.11	$[M+H]^+$	$C_9H_{10}O_5$ (198.17)		Syringic acid
122	5.20	0.09	$[M+H]^+$	$C_{16}H_{18}O_9$ (355.10)	-0.5	Chlorogenic acid

123	5.86	0.18	$[M-H]^-$	$C_{20}H_{22}O_9$ (405.11)	6	E-3,4,5'-Trihydroxy-3'-glucopyranosylstilbene
124	12.96	3.56	$[M+H]^+$	$C_{14}H_6O_8$ (302.34)	1.2	Ellagic acid
125	14.35	1.22	$[M+H]^+$	$C_{17}H_{22}O_{10}$ (387.12)	-0.2	1-*O*-b-D-glucopyranosyl sinapate
126	15.6	4.07	$[M+H]^+$	$C_7H_6O_4$ (154.26)	0.2	Protocatechuic acid

*Relative percentages of the identified compounds in the hexane extract were calculated based on the total peak area in the chromatogram; **tentative identity.

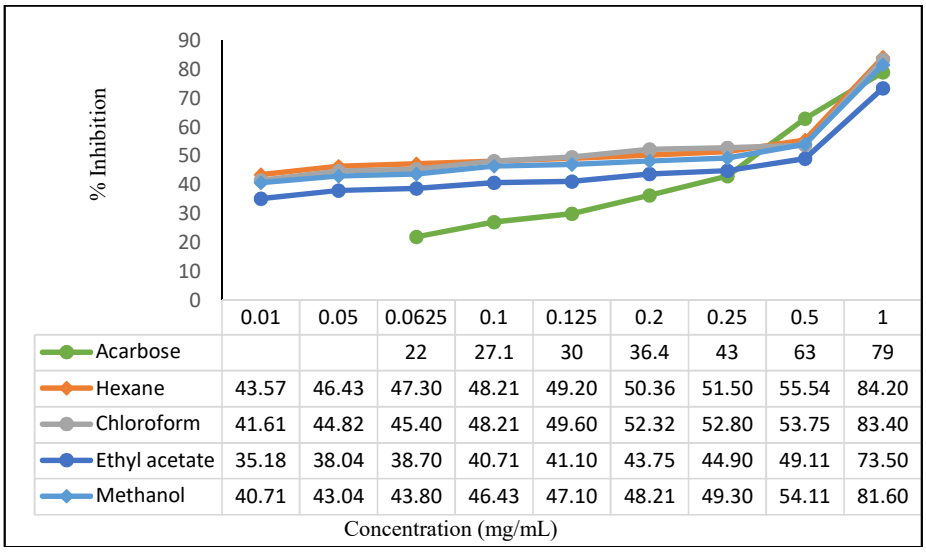
The phenolic and carboxylic acid derivatives represent about 21.5%. Among the identified phenolic and carboxylic acid, glutaric acid, protocatechuic acid and ellagic acid accounted as the major constituents tentatively identified in *C. abyssinica*. While the flavonoids and related metabolites represent about 30%. Among the identified flavonoids and related metabolites, formononetin, taxifolin, and vitexin-2''-*O*-rhamnoside accounted as the major constituents tentatively identified. The total phenolic and flavonoid content represents about 51.6%.

Identified flavonoids such as formononetin are highly applicable because of their biological properties, as antioxidant, anticancer, and anti-inflammatory activities, among others [38]. Taxifolin, is reviewed as a potent therapeutic agent for the treatment of inflammatory and cancer diseases. A semi-synthetic taxifolin is available in markets as Venoruton [39]. Kaempferitrin, a naturally potent glycoside, exhibited antioxidant, antimicrobial, anti-inflammatory and anti-diabetic activities [40]. Daidzein [41] and naringenin [42] exerted anti-cancer effects by reducing ROS generation and enhancing the activity of superoxide dismutase, catalase, glutathione.

Antidiabetic activity

An *in-vitro* antidiabetic activity of the different extracts of *C. abyssinica* was determined by α-glucosidase and α-amylase enzymes inhibitory assay at concentrations of 0.01, 0.05, 0.0625, 0.125, 0.2, 0.25, 0.5, and 1.0 mg/mL (Figure 5 and 6). The hexane, chloroform, ethyl acetate and methanol extracts of *C. abyssinica* exhibited a remarkable α-glucosidase inhibitory activity with an IC_{50} value of 0.22, 0.26, 0.50, and 0.31 mg/mL, respectively. Also, they showed a remarkable α-amylase inhibitory activity with an IC_{50} value of 0.27, 0.25, 0.34, and 0.52 mg/mL, respectively. Acarbose was used as a standard reference drug, which showed α-glucosidase inhibitory activity with an IC_{50} value of 0.43 mg/mL and α-amylase inhibitory activity with an IC_{50} value of 0.38 mg/mL. From our data, all tested extracts showed potent antidiabetic activity, except ethyl acetate extract as α-glucosidase and methanol as α-amylase inhibitory activity. Among all, hexane extract has shown the best enzyme inhibitory activity with an IC_{50} value of 0.22 and 0.27 mg/mL (α-glucosidase and α-amylase) which were comparable with that of acarbose.

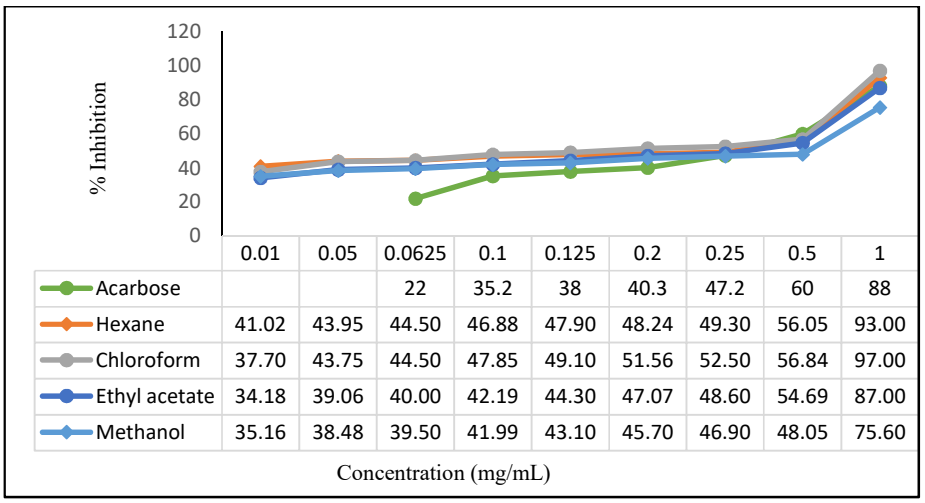
It is reported that monoterpenes have antidiabetic activities [43]. The antihyperglycemic potential of acyclic monoterpene alcohols including citronellol and geraniol was verified in STZ-induced diabetic rats [44, 45]. Geraniol was effective in changing activities of enzymes involved with the carbohydrate metabolism, while citronellol showed similar effects with a 4-fold lower dose, showing that both monoterpenoids could be considered to develop nutraceuticals or functional formulations for diabetes management.



	0.01	0.05	0.0625	0.1	0.125	0.2	0.25	0.5	1
Acarbose			22	27.1	30	36.4	43	63	79
Hexane	43.57	46.43	47.30	48.21	49.20	50.36	51.50	55.54	84.20
Chloroform	41.61	44.82	45.40	48.21	49.60	52.32	52.80	53.75	83.40
Ethyl acetate	35.18	38.04	38.70	40.71	41.10	43.75	44.90	49.11	73.50
Methanol	40.71	43.04	43.80	46.43	47.10	48.21	49.30	54.11	81.60

Concentration (mg/mL)

Figure 5. α-Glucosidase inhibitory effects of *C. abyssinica* extracts and acarbose.



	0.01	0.05	0.0625	0.1	0.125	0.2	0.25	0.5	1
Acarbose			22	35.2	38	40.3	47.2	60	88
Hexane	41.02	43.95	44.50	46.88	47.90	48.24	49.30	56.05	93.00
Chloroform	37.70	43.75	44.50	47.85	49.10	51.56	52.50	56.84	97.00
Ethyl acetate	34.18	39.06	40.00	42.19	44.30	47.07	48.60	54.69	87.00
Methanol	35.16	38.48	39.50	41.99	43.10	45.70	46.90	48.05	75.60

Concentration (mg/mL)

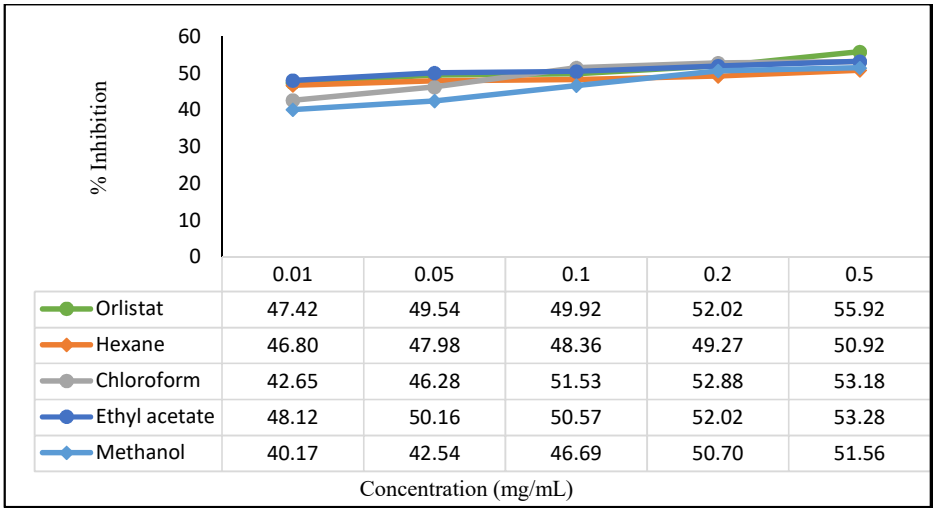
Figure 6. α-Amylase, and lipase inhibitory effects of *C. abyssinica* extracts and acarbose.

Antiobesity activity

Lipases are digestive enzymes responsible for breaking down fats (triglycerides) into smaller molecules (fatty acids and glycerol) for absorption by the intestines. Lipase inhibitory activity refers to the ability of a substance to decrease the activity of the lipase enzyme. By reducing fat absorption, lipase inhibitors can potentially contribute to weight loss or obesity management.

Orlistat, a pancreatic lipase inhibitor, acts by blocking lipase activity in the small intestine, thereby reducing dietary fat absorption. Weight loss using orlistat is relatively modest, but effective in lowering low-density lipoprotein cholesterol, total cholesterol, and triglyceride levels [46].

The inhibition of lipase enzyme of *C. abyssinica* extracts was *in vitro* determined at concentrations of 0.01, 0.05, 0.1, 0.2, and 0.5 mg/mL (Figure 7). The hexane, chloroform, ethyl acetate and methanol extracts of *C. abyssinica* exhibited a lipase inhibitory activity with an IC_{50} value of 0.35, 0.21, 0.079, and 0.34 mg/mL, respectively. Orlistat was applied as a standard drug, which revealed lipase inhibitory activity with an IC_{50} value of 0.11 mg/mL. Among all the tested extracts, ethyl acetate extract has shown potent enzyme inhibitory activity with an IC_{50} value of 0.079 mg/mL comparing with that of orlistat.



	0.01	0.05	0.1	0.2	0.5
Orlistat	47.42	49.54	49.92	52.02	55.92
Hexane	46.80	47.98	48.36	49.27	50.92
Chloroform	42.65	46.28	51.53	52.88	53.18
Ethyl acetate	48.12	50.16	50.57	52.02	53.28
Methanol	40.17	42.54	46.69	50.70	51.56

Figure 7. Lipase inhibitory effects of *C. abyssinica* extracts and orlistat.

Antibacterial activity

The antibacterial effect of n-hexane, chloroform, ethyl acetate, and MeOH extracts of *C. abyssinica* was evaluated using the agar diffusion method at concentrations 50, 75, and 100 mg/mL against gram-positive bacteria (*Bacillus cereus*) and gram-negative bacteria (*Pseudomonas aeruginosa*). Among all test extracts, hexane extract only showed an antibacterial effect at all concentrations against *Bacillus cereus* (Table 3). All tested extracts showed mild antibacterial effect against *Pseudomonas aeruginosa* at all tested concentrations except methanol extract showed no activity at all concentrations. The antibacterial effect of hexane against the tested bacteria may be due to the presence of antibacterial, antifungal, insecticidal agents citronellol and citronellyl acetate [33].

Table 3. Antibacterial activity of *C. abyssinica* extracts at different concentrations.

Sample Concentration	Inhibition zone diameter (mm)**			MIC
	100 mg/ml	75 mg/ml	50 mg/ml	
Gram-positive bacteria (*Bacillus cereus ATCC10876*)				
Hexane	15	14	12	50
Chloroform	-	-	-	-
Ethyl acetate	-	-	-	-
Methanol	13	-	-	100
Gentamycin (0.1%)	34			
Gram-negative bacteria (*Pseudomonas aeruginosa ATCC 27853*)				
Hexane	18	16	15	50
Chloroform	17	15	12	50
Ethyl acetate	18	17	12	50
Methanol	-	-	-	100
Gentamycin (0.1%)	30			

**Diameter include 5 mm well diameter; (-); Not detected; MIC; Minimum inhibition concentration (mg/mL).

CONCLUSION

The present study identified 126 phytoconstituents from the aerial parts of *C. abyssinica* by GC-MS and LC-ESI-TOF-MS analyses belongs to terpenes, hydrocarbons, phenolics, flavonoids, amino acids, alkaloids, and vitamins. Terpenes and hydrocarbons showed strong antidiabetic. Among hydrocarbon, 11-Octadecenoic acid, methyl ester, citronellol, hexadecanoic acid, methyl ester, phytol, and 10-epi-γ-eudesmol were the major components. It is suggested that these compounds may exert a synergistic effect towards anti-diabetic properties. On the other hand, flavonoids and phenolic revealed strong antiobesity activity. In summary, the outcomes of this study suggest further *in vivo* validation of *C. abyssinica* in ameliorating diabetes and other related metabolic illnesses.

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