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SECONDARY METABOLITES AND ESSENTIAL OIL ANALYSIS OF CLEMATIS SIMENSIS WITH ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES

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ABSTRACT. *Clematis simensis* is one of the most commonly used folk medicines in Ethiopia. This study was aimed to investigate the chemical constituents, antioxidant and antibacterial activities of the aerial part of *Clematis simensis*, and analysis of the composition of its essential oil. The phytochemical study led to the isolation of five compounds: 1-tricosanol (**1**), 1-hexacosanol (**2**), 2-deoxy-D-ribono-1,4-lactone (**3**), 5-hydroxylevulinic acid (**4**), and *β*-sitosterol-3-O-*β*-D-glucoside (**5**). Their chemical structures were determined by a comprehensive analysis of HR-ESI-MS, FT-IR, UV-Vis, and 1D/2D NMR experiments. The chemical compositions of the essential oils obtained by hydrodistillation from the aerial part of *Clematis semenses* were determined by GC-MS, which led to the detection of ten major compounds: (E)-2-nonen-1-ol (38.33%), 4-cyclopentene-1,3-dione (28.69%), cyclofenchene (5.65%), benzene acetaldehyde (4.74%), methyl salicylate (2.77%), salicylaldehyde (2.68%), benzaldehyde (2.60%), α-terpineol (2.10%), eucalyptol (1.21%), and 2,6-dimethylcyclohexanol (1.15%). Compound **4** showed high antioxidant activity (IC_{50} 14.89 µg/mL) but has no antibacterial activity against both Gram-positive and Gram-negative bacterial strains. According to the antimicrobial parameters, crude extract showed very weak activity at 500 mg/mL, 250 mg/mL, and 125 mg/mL. To our knowledge, this is the first report on the isolation of secondary metabolites from *Clematis simensis*.

KEY WORDS: *Clematis simensis*, Essential oil, Phytochemical investigation, Biological activities

INTRODUCTION

Clematis simensis is a tall, climbing shrubby plant that can grow up to 20 m, often behaving as a strong liane; younger stems are more or less hairy; leaves are unequally pinnate with five leaflets, and its flower buds have an ellipsoid shape [1]. The genus *Clematis* L. is widely spread in the northern hemisphere [2]. It is native and distributed in Eritrea, Ethiopia, Sudan, Malawi, Zimbabwe, Mozambique, Cameroon, Republic of the Congo, and Angola [1]. Locally it is known as 'Fiide' in Sidama [3], 'azo-hareg' [4] and 'Enderifa' [5] in Amaharic, and Feetii/Hidda [6] in Afaan Oromo. The leaves of this plant are traditionally used in Ethiopia to treat tinea capitis, dermatitis, tropical ulcers, and wounds. The seeds of this plant are also used to alleviate rheumatic pain, and the sap is used to treat animal bloat and as a febrifuge [7]. The stem bark of *Clematis simensis* is used for the treatment of toothache and cancer [8]. According to a recent study, the leaves of *Clematis simensis* have been used in combination with another plant belonging to the same family [9, 10].

Solvent extracts of the plant showed different biological activities. The aqueous and methanol extracts of *Clematis simensis* leaves showed inhibition against bacteria including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and fungus *Candida albicans* [11, 12]. Methanol and acetone extracts of *Clematis simensis* exhibited anti-inflammatory and antinociceptive activity at doses of 400 mg/kg and 800 mg/kg, respectively [13]. Recently, Birhan and coworkers reported that an

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80% methanol extract displayed that *Staphylococcus aureus* was the most susceptible bacteria, followed by *Pseudomonas aeruginosae* [8]. The chloroform fraction of *Clematis simensis* was cytotoxic against breast cancer cell lines (JIMT-1, MCF-7, and HCC1937) [14].

A preliminary phytochemical screening of the extracts of the stem bark [15], root [16] and leaves [17] revealed the presence of polyphenols, alkaloids, tannin cardiac glycosides, steroids, terpenoids, tannins, and flavonoids. However, no report on phytochemical investigation that leading to isolation of secondary metabolites of the plant. In our continued effort to investigate *Clematis* species, the aerial parts of *Clematis simensis* were investigated phytochemically leading to the isolation and identification of five compounds. Herein, are discussed the isolation and structure elucidation of these compounds as well as the essential oil constituents of the plant. The isolated compounds were tested for their free-radical scavenging antioxidant activity by employing a DPPH assay. The crude extract and the isolated compounds showed a wide range of DPPH scavenging activities from mild (IC_{50} value = 52.9 µg/mL) to strong (IC_{50} = 14.89 µg/mL). Furthermore, antibacterial activities of the compounds and crude extract were evaluated using the disk diffusion agar method against Gram-positive and Gram-negative bacterial strains of *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), Salmonella *typhi* (ATCC 6539) and *Pseudomonas aeruginosa* (ATCC 27853).

EXPERIMENTAL

General experimental procedures

NMR experiments (1D and 2D) were recorded by a 600 MHz Bruker Avance III spectrometer using DMSO, MeOD, $CDCl_3$ solvents, and TMS as internal references. Mass spectra were recorded on a high resolution mass spectrometer (Thermo Scientific, USA) equipped with an electrospray ionization (ESI) ion source. FT-IR (PerkinElmer) in the range 4000-400 cm^{-1} (resolution: 4 cm^{-1}, number of scans: 4) was used using KBr discs. Melting point was recorded using a METTLER TOLEDO FP82HT hot stage coupled with a METTLER TOLEDO FP90 central processor. Gas chromatography-mass spectrometry (GC-MS) experiments were conducted on Agilent Technologies 7820A GC system coupled with Agilent Technologies 5977E MSD, USA. Chromatographic peaks were identified by using NIST 2014 mass spectral library search and retention index (*I*) calculation. For column chromatography, silica gel 60 (70 – 230 mesh ASTM) and Sephadex LH−20 (18−111 µm, GE Healthcare Bio-Sciences AB, Sweden) were used. The isolation process was monitored by TLC (pre-coated sheets, ALUGRAM, Xtra SIL G/UV $_{254}$, 20 x 20 cm, coated with silica gel 60 fluorescent indicator, Germany), which was visualized under UV light and also sprayed with vanillin (1 g vanillin with 5% H_2SO_4 in MeOH) and cerium molybdate stain followed by heating gently for a few seconds.

Plant material

The aerial parts of *Clematis simensis* were collected from Debresina, Northern Shewa, Amhara region, 192 km from Addis Ababa, in June 2020. The plant specimen was identified by Mr. Melaku Wendafrash, Department of plant science, Addis Ababa University, Ethiopia. Voucher specimens were deposited at the National Herbarium, Addis Ababa University (No. AD001 *C. simenses*).

Extraction and isolation

The collected fresh aerial part of *Clematis simensis* was air dried at room temperature for two weeks under a shed until it became well dried. The dry plant materials were ground using an electric grinder to obtain a fine powder. The powder (0.5 kg) was soaked in 1:1 ratio of methanol to chloroform (2 L x 3, 24 h each) at room temperature. The mixture was filtered using a Buchner

funnel with Whatman filter paper. The combined filtrates were concentrated by a rotary evaporator under reduced pressure at a 45 °C. The concentrated crude extracts were allowed to dry to a constant weight at room temperature, and gave a total mass of 52.6 g. The methanol-chloroform crude extract (22.0 g) was dissolved in chloroform/methanol (1:1) solvent and adsorbed on 20 g silica gel, then stirred well and dried by rotary evaporator until a fine dry powder remains. The dried powder was applied to a glass column packed with silica gel and fractionated using a gradient solvent system containing petroleum ether/chloroform (from 50:50 to 0:100, v/v), chloroform/ethyl acetate (95:5 to 0:100, v/v), and chloroform/methanol (90:10 to 62.5:37.5, v/v) to furnish a total of 31 fractions. The fractions were combined based on their TLC profiles into twenty fractions (CSA-1C to CSA-20C). The major fraction CSA-7C (0.68 g) was dissolved in methanol and allowed to precipitate to give compound **1** (12.2 mg). Fraction CSA-17C (2.70 g) was subjected to column chromatography over silica gel (150 g) using a gradient of petroleum ether/chloroform (50:50 to 0:100, v/v) and chloroform/ethyl acetate (94:6 to 0:100, v/v) to give 64 sub-fraction, which were combined into seven groups (CSA-17C-1 to CSA-17C-7) based on their TLC profiles. Sub-fractions CSA-17C-3 and CSA-17C-5 from chloroform-ethyl acetate were recrystallized with diethyl ether to give compounds **3** (9.6 mg) and compound **4** (7.3 mg) as white solids, respectively. Fraction CSA-11C (1.2 g) was re-chromatographed over silica gel (100 g) eluted with petroleum ether/chloroform mixtures (70:30 to 0:100), followed by chloroform/ethyl acetate mixtures (99:1 to 50:50, v/v). Twelve fractions (11C-1 to 11C-12) were collected. Sub-fraction 11C-6 (87 mg) was concentrated and recrystallized from diethyl ether to afford compound **2** (6.1 mg). Fraction 20C (2.2 g) was further chromatographed on a glass column packed with silica gel (130 g) which was eluting with 40% ethyl acetate in hexane to obtain compound **5** (11.8 mg).

Antioxidant activity assays by 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The antioxidant activities of the extracts and isolated compounds were assessed using DPPH radical scavenging activity with ascorbic acid as the positive control, as per the previously described method [18]. All extracted compounds were separately dissolved in DMSO (1 mg/mL) and serially diluted with methanol, followed by the addition of 0.004% (4 mg/100 mL) methanolic solution of DPPH to get 3.12, 6.25, 12.5, 25, 50, and 100 µg/mL solutions, and the absorbance of each dilution, after 30 minutes, was measured at 517 nm. An ascorbic acid solution of the same concentration (3.12 µg/mL to 100 µg/mL) was prepared in similar fashion and measured. The DPPH radical scavenging activities of each of the tested compounds were reported as percentage inhibition using the following formula [19]:

$$\%\ \text{DPPH Inhibition} = \left[\frac{\text{A control} - \text{B sample}}{\text{A control}}\right] \times 100 \quad (1)$$

where A control is the absorbance of DPPH solution and B sample is the absorbance of the test sample (DPPH solution plus a compound or crude).

The relative half-maximal inhibitory concentration (IC_{50}) values were calculated by the linear regression equation between the isolated compounds/extract concentration and the corresponding scavenging effect. The final antioxidant activities of each compound and crude extract were expressed as the IC_{50} (µg/mL) values.

Antibacterial activity

The four common human bacterial strains: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), Salmonella *typhi* (ATCC 6539) and *Pseudomonas aeruginosa* (ATCC 27853), were collected from the Microbial, Cellular and Molecular Biology Department of Addis Ababa University. *In vitro* antibacterial susceptibility tests were determined by the disk diffusion method [20]. The medium was prepared by dissolving 38 g of Mueller Hinton agar (MHA) in

1000 mL distilled water, and autoclaved at 121 °C for 15 min. The autoclaved medium was poured into sterile petri dishes (20 mL/plate), and the plates were allowed to solidify under sterile conditions at room temperature. After solidification, the plates were seeded with an overnight grown culture approximately 1.5 x 10^8 CFU/mL by swabbing evenly on the surface of the medium with a sterile cotton swab. The crude extract and isolated compounds at concentrations of 125 mg/mL-500 mg/mL and 125-1000 µg/mL, respectively, were prepared by dissolving in 10% DMSO. Whatman filter paper No. 1 was used to prepare discs of 6 mm in diameter. The sterile discs were infused with the isolated compounds, placed on the surface of the medium with sterile forceps, and gently pressed down to ensure contact with the (MHA). Ciprofloxacin was used as a positive control, and then the plates were inverted and incubated at 37 °C for 24 hours. After incubation, the inhibition zone produced by the isolated compounds was evaluated by measuring the diameter (mm) of the clear zone around the disc and comparing it with 5 µg/mL positive controls.

Extraction of essential oil by hydrodistillation

The aerial part of *Clematis simensis* was crushed and prepared for hydrodistillation following previously published report [21]. 440 g of the plant material was placed into distillation flask (4 L) filled with 2 L distilled water, which was then attached to clevenger apparatus. On a heating mantle, the flask's contents were brought to boiling, and then hydrodistillation was carried out for eight hours. The essential oil was collected (~2.0 mL), dried over anhydrous Na_2SO_4, and stored in a sealed vial in the dark at 4 °C, until analysis by GC-MS. Then the essential oil obtained from aerial part of *Clematis simensis* was analyzed by using an Agilent Technology 7820A GC system coupled with an Agilent Technology 5977E MSD system equipped with an autosampler. The chromatographic separation was done on a column 30 m in length and 0.25 µm in thickness coated with HP-5MS, (5%-phenyl)-methylpolysiloxane, at a pressure of 8 psi and a flow rate of 0.97989 mL/min. Ultra-high pure helium (99.999%) was used as carrier gas at constant flow mode. An Agilent G4567A autosampler was used to inject 1 µL of the sample with a splitless injection mode into the inlet heated to 275 ºC with a total run time of 29.33 min. Oven temperature was programmed with the initial column temperature of 60 ºC and hold-time 2 min. The column temperature was increased at a rate of 10 ºC/min until the temperature reached 200 ºC and then heated again at the rate of 3 ºC/min until the temperature reached 240 ºC. No mass spectra were collected during the first 4 min of the solvent delay. The transfer line and the ion source temperatures were 280 ºC and 230 ºC, respectively. The detector voltage was 1600 V and the electron energy was 70 eV. Mass spectral data were collected from 40–650 *m*/*z*. The names, structures, and qualities of peaks were determined through National Institute of Standards and Technology (NIST) 2014 library search.

Spectroscopic data

Compound ***1***. A white powder; m.p. 73-75 °C; IRν (KBr, cm^{-1}): 3439, 2921, 2851, 1471, 1381, 1162, 1052; ^{1}H-NMR (600 MHz, $CDCl_3$) δ 3.67 (*t*, *J* = 6.7 Hz, 2H-1), 1.59 (*m*, 2H-2), 1.28 (*brs*, 34H-3 to 22), 0.90 (*t*, *J* = 6.9 Hz, 3H-23). ^{13}C-NMR (151 MHz, $CDCl_3$): Table 1; HR-ESI-MS (positive ion mode) m/z 364.4080 $[M+Na+H]^+$ (calcd. for $C_{23}H_{48}ONaH$, 364.6176).

Compound ***2***. A white powder; m.p. 80 to 82 °C; IRν (KBr, cm^{-1}): 3397, 2913, 2859, 1464, 1384, 1066, 720; ^{1}H-NMR (600 MHz, $CDCl_3$) δ 3.67 (*t*, *J* = 6.7 Hz, 2H-1), 1.59 (*m*, 2H-2), 1.27 (*brs*, 47H-3 to 25), 0.90 (*t*, *J* = 6.9 Hz, 3H-26). ^{13}C-NMR (151 MHz, $CDCl_3$): Table 1; HR-ESI-MS (positive ion mode) m/z 405.40669 $[M+Na]^+$ (calcd for $C_{26}H_{54}ONa$, 405.69710).

*Compound **3***. A white powder; m.p. 134-135 °C; IRν (KBr, cm^{-1}): 3408, 2915, 2847, 1634, 1539, 1470, 1059, 720; ^{1}H-NMR (600 MHz, DMSO) δ 5.50 (*s*, OH-3), 5.07 (*s*, OH-5), 4.34–4.18 (*m*, 1H-3, 4), 3.55 (*qd*, *J* = 12.3, 3.4 Hz, 2H-5), 2.82 (*dd*, *J* = 17.7, 6.5 Hz, $1H_1$-2), 2.24 (*dd*, *J* = 17.7, 2.3 Hz, $1H_2$-2). ^{13}C-NMR (151 MHz, DMSO): Table 2; HR-EI-MS (positive ion mode) m/z 132 (M^+, not observed); (calcd for $C_5H_8O_4$, 132.0427).

*Compound **4***. A white powder; m.p. 97-100 °C; IRν (KBr, cm^{-1}): 3443, 2926, 2855, 1739, 1634; ^{1}H-NMR (600 MHz, CH_3OH) δ 4.24 (*s*, 1H-5), 2.70 (*t*, *J* = 6.7 Hz, 1H-2), 2.59 (*t*, *J* = 6.7 Hz, 1H-3); ^{13}C-NMR (151 MHz, CH_3OH): Table 2; HR-EI-MS (positive ion mode) m/z 132 (M^+, not observed); (calcd for $C_5H_8O_4$, 132.0427).

*Compound **5***. A white powder; m.p. 285 - 287 °C; IRν (KBr, cm^{-1}): 3436, 2918, 2848, 1620; ^{1}H-NMR (601 MHz, DMSO) δ 5.30 (*m*, 1H-6), 4.22 (*d*, *J* = 7.8 Hz, 1H-1′), 3.65 (*ddd*, *J* = 11.7, 5.8, 2.0 Hz, 1H-6′), 3.42 (*m*, 1H-3), 3.41 (*dt*, *J* = 11.7, 5.9 Hz, 1H-6′), 3.12 (*td*, *J* = 8.8, 4.7 Hz, 1H-3′), 3.07 (*ddd*, *J* = 9.7, 5.9, 2.1 Hz, 1H-5′), 3.01 (*td*, *J* = 9.1, 5.1 Hz, 1H-4′), 2.88 (*m*, 1H-2′), 2.36 (*m*, 1H-4), 2.17 (*m*, 1H-4), 1.94 (*m*, 1H-12), 1.92 (*m*, 1H-7), 1.84 (*m*, 1H-16), 1.81(*m*, 1H-2), 1.79 (*m*, 1H-1), 1.63 (*m*, 1H-25), 1.57 (*m*, 1H-15), 1.52 (*m*, 1H-7), 1.42 (*m*, 1H-2), 1.38 (*m*, 1H-8), 1.35 (*m*, 1H-20), 1.34 (*m*, 1H-22), 1.24 (*m*, 1H-16), 1.22 (*m*, 1H-28), 1.21 (*d*, *J* = 5.7 Hz, 1H-11), 1.15 (*m*, 1H-23), 1.12 (*m*, 1H-17), 1.11 (*dd*, *J* = 11.9, 3.7 Hz, 1H-12), 1.08 (*m*, 1H-15), 1.04 (*m*, 1H),1.01 (*d*, *J* = 9.7 Hz, 1H-11), 1.00 (*d*, *J* = 6.6 Hz, 1H-1), 0.99 (*m*, 1H-14), 0.96 (*s*, 3H-19), 0.91 (*m*, 1H-24), 0.83 (*m*, 1H-9), 0.87 (*d*, *J* = 4.4 Hz, 1H), 0.82 (*t*, *J* = 7.2 Hz, 3H-29), 0.80 (*d*, *J* = 5.4 Hz, 3H-26), 0.78 (*d*, *J* = 7.7 Hz, 3H-21), 0.65 (*s*, 3H-18), ^{13}C-NMR (151 MHz, DMSO): Table 2*;* FAB-MS (positive ion mode) m/z 599.4236 $[M + Na]^+$ (calcd. for $C_{35}H_{60}O_6$, 599.8384).

RESULTS AND DISCUSSION

The chloroform-methanol extract of the air-dried aerial parts of *Clematis simensis* afforded five compounds namely 1-tricosanol (**1**), 1-hexacosanol (**2**), 2-deoxy-D-ribono-1,4-lactone (**3**), 5-hydroxylevulinic acid (**4**), and *β*-sitosterol-3-O-*β*-D-glucoside (**5**) (Figure 1). Their structures were elucidated by 1D- and 2D-NMR spectroscopy, HR-EI-MS and HR-ESI-MS.

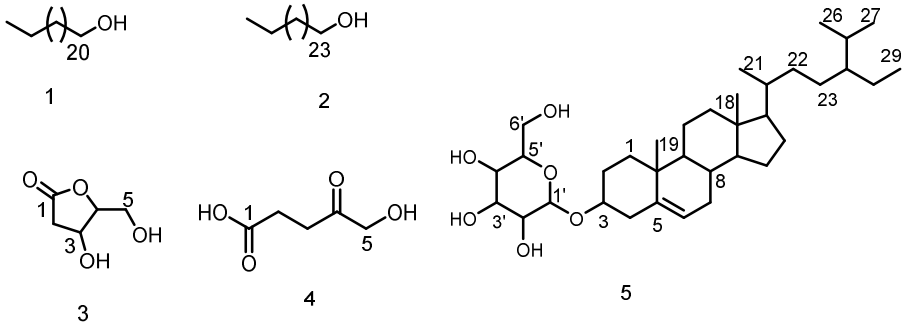

Figure 1. Chemical structures of compounds **1** to **5** isolated from *Clematis simensis*.

*Compound **1***. 1-Tricosanol was obtained as a white powder with a melting point of 73-75 °C. The FT-IR spectrum showed an absorption peak at 3439 cm^{-1} due to O-H stretching vibration, 2921 cm^{-1} and 2851 cm^{-1} due to C-H stretching vibrations, 1471 cm^{-1} due to C-H bending vibration, 1381 cm^{-1} due to CH_3 bending vibration, 1052 cm^{-1} due to C-O stretching vibration, and 725 cm^{-1} is due to methyl, C–H rocking in long chain alkane. The molecular formula was established as

$C_{23}H_{48}O$ by a quasi-molecular-ion peak in the HR-ESI-MS at *m/z* 364.4080 $[M+Na+H]^+$ (calcd. for $C_{23}H_{48}ONaH$, 364.6176). The fragment ion peak at *m/z* = 336.3743, consistent with the loss of C_2H_6 from a long chain alcohol. The ^{1}H-NMR spectrum of compound **1** showed a triplet signal at δ_H 0.90 (H-23) due to one terminal methyl protons, a big broad singlet signal at δ_H 1.28 (H-22) to 1.59 (H-2) because of protons of methylene units, a triplet signal at δ_H 3.67 (H-1) due to the protons of methylene unit attached with electronegative hydroxyl group, a multiplet signal at δ_H 1.59 (H-2) is assignable to the methylene beta to the hydroxyl group. ^{13}C-NMR and DEPT-135 spectra indicated one methyl group at δ_C 14.14 (C-23) and twenty-two methylene groups at δ_C 22.71 (C-22), 25.75 (C-3), 29.45 (C-20), 29.71 (C-4 to 19) and 31.94 (C-21). Compound **1** was identified as 1-tricosanol based on the description above (Table 1) and comparison with literature [22].

*Compound **2***. 1-Hexacosanol was obtained as a white powder with a melting point of 80 to 82 °C. The FT-IR showed an absorption peak at 3397 cm^{-1} due to O-H stretching vibration, 2913 cm^{-1} and 2859 cm^{-1} due to C-H stretching, 1464 cm^{-1} due to C-H bending vibration, 1384 cm^{-1} due to CH_3 bending vibration, 1066 cm^{-1} due to C-O stretching, and 720 cm^{-1} due to long aliphatic chain. The ^{1}H and ^{13}C-NMR spectrum of compound **2** is identical to those of **1** except the presence of additional three carbons atoms in **1**. The molecular formula was established to be $C_{26}H_{54}O$ from HR-ESI-MS at *m/z* 405.40669 $[M+Na]^+$ (calcd for $C_{26}H_{54}ONa$ 405.69710). In addition to HR-ESI-MS, its mass was further analyzed by GC-MS, one of the molecular ion peak was at m/z 364 and it was found to correspond with the molecular ion peak and molecular ion weight of compound **2**, suggested by the NIST (replib) library with chemical formula $C_{26}H_{54}O$. The fragment ion observed at m/z 43 indicating the loss of $C_3H_7^+$. The fragment ion peaks at *m/z* 336 consistent with the loss of H_2O and $[CH_2{=}CH_2]^+$ from the molecular ion. The intense peak at m/z 57 indicating the loss of $C_4H_9^+$, that it was a fragmentation of hydrocarbons after loss of hydroxyl group through water and ethylene. By comparing the spectroscopic data (Table 1) with literature, compound **2** was identified as 1-hexacosanol [23].

Table 1. ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) spectroscopic data for Compound **1** and **2** in DMSO-d_6.

Position	**1**		Position	**2**	
	δ_c	δ_H multiplet (J in Hz)		δ_c	δ_H multiplet (J in Hz)
1	63.13	3.67 *t* (6.9)	1	63.13	3.67 *t* (6.9)
2-22	22.71- 32.84	1.59-1.28 *brs*	2	32.84	1.59 *m*
23	14.14	0.9 *t* (6.9)	24	31.94	1.27 *brs*
			3-25	29.72- 22.71	1.27 *brs*
			26	14.14	0.9 *t* (6.9)

*Compound **3***. 2-Deoxy-D-ribono-1,4-lactone was obtained as white powder with a melting point of 134-135 °C. The FT-IR showed an absorption peak at 3408 cm^{-1} due to O-H stretching vibration, 2915cm^{-1} and 2847cm^{-1} due to C-H stretching, 1634 cm^{-1} due to C=O stretching vibration, 1470 cm^{-1} due to CH_2 bending vibration, and 1057 cm^{-1} due to C-O stretching. The HR-EI-MS spectrum of compound **3** exhibited fragment ion at *m/z* = 104.0515 due to the loss of [CO] and *m/z* 101.0279 due to loss of $[CH_2OH]^+$ from a compound which are characteristic of dihydroxylated γ-lactone with the molecular weight of 132.0427. The ^{1}H-NMR spectrums of compound **3** showed diastereotopic protons at δ_H 3.52 and 3.57 for H-5, indicated a methylene proton is attached to hydroxyl group. The other diastereotopic protons signals at δ_H 2.82 and 2.23 are due to the protons of methylene group alpha to the carbonyl carbon. Signals at δ_H 5.5 (*brs*, 1H) and 5.07 (*s*, 1H) are for OH peaks on C-5 and C-3, respectively. ^{13}C-NMR and DEPT spectra indicated two methine group at δ_C 67.85 (C-3) and 88.32 (C-4), one carbonyl group at δ_C 176.30 (C-1), and two methylene group at δ_C 60.84 (C-5) and at δ_C 38.06 (C-2). The HMBC spectrum

showed correlations from H_2-2 to C-1 and C-3, and from H-5 to C-3 and C-4. Based on these results and by comparing the spectral data (Table 2) with values published in the literature, compound **3** was determined to be 2-deoxy-D-ribono-1,4-lactone [24].

Table 2. ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) spectroscopic data for compounds **3, 4** and **5** in DMSO- d_6.

Position	**3**		**4**		**5**	
	δ_c	δ_H multiplet (*J* in Hz)	δ_c	δ_H multiplet (J in Hz)	δ_c	δ_H multiplet (*J* in Hz)
1	176.30	-	211.06	-	36.89	1.79 *m* 1.00 *d* (6.6)
2	38.05	2.82 *dd* (17.7, 6.5), 2.24 *dd* (17.7, 2.3)	33.91	2.70 *t* (6.7)	29.27	1.81 *m* 1.42 *m*
3	67.85	4.34 – 4.18 *m*	28.53	2.59 *t* (6.7)	76.88	3.42 *td* (11.2, 5.6)
4	88.32	4.34 – 4.18 *m*	176.44	-	38.36	2.36 *m*; 2.17 *m*
5	60.84	3.52 *dd* (2.3, 3.4) 3.57 *dd* (12.3, 3.4)	68.87	4.24 *s*	140.50	-
6					121.30	5.30 *m*
7					31.48	1.92 *m* ;1.52 *m*
8					31.40	1.38 *m*
9					49.66	0.83 *m*
10					36.27	-
11					20.46	1.21 *d* (5.7); 1.01 *d* (9.7)
12					39.30	1.94 *m*; 1.11 *dd* (11.9, 3.7)
13					41.9	-
14					56.23	0.99 *m*
15					23.92	1.57 *m*;1.08 *m*
16					27.90	1.84 m; 1.24 *m*
17					55.48	1.12 *m*
18					12.25	0.65 *s*
19					19.17	0.96 *s*
20					35.55	1.35 *m*
21					19.04	0.78 *d* (7.7)
22					33.4	1.34 *m*; 1.04 *m*
23					25.43	1.15 *m*
24					45.19	0.91 *m*
25					28.75	1.63 *m*
26					19.78	0.80 *d* (5.4)
27					18.99	0.87 *d* (4.4)
28					23.00	1.22 *m*
29					11.74	0.82 *t* (7.2)
1′					100.80	4.22 *d* (7.8)
2′					73.50	2.88 *m*
3′					76.76	3.12 *td* (8.8, 4.7)
4′					70.17	3.01 *td* (9.1, 5.1)
5′					76.76	3.07 *ddd* (9.7, 5.9, 2.1)
6′					61.10	3.65 *ddd* (11.7, 5.8, 2.0) 3.41 *dt* (11.7, 5.9)

*Compound **4***. 5-Hydroxylevulinic acid was isolated as a white powder with a melting point of 97-100 °C. The FT-IR spectra of compound **4** showed a broad absorption band at 3443 cm^{-1} due to O-H stretching vibration, 2926 cm^{-1} and 2855 cm^{-1} due to C-H stretching, 1736 cm^{-1} and 1634 cm^{-1} due to C=O stretching vibrations. The HR-EI-MS spectrum of compound **4** exhibited fragment ion peaks at *m/z* = 101 consistent with the loss of $[CH_2OH]^+$ from a 5-hydroxy-4-oxopentanoic acid with a molecular weight of 132.0427. The ^{1}H-NMR spectrums of compound **4** showed a triplet signal at δ_H 2.59 (*t*, *J* = 6.7 Hz, H-3) and δ_H 2. 70 (*t*, *J* = 6.7 Hz, H-2) due to the two linked methylene protons, a singlet signal at δ_H 4.24 (H-5), indicated the protons of methylene unit flanked between a hydroxyl group and a carbonyl carbon. The ^{13}C-NMR and DEPT revealed five carbon atoms which are due to a carboxylic acid group at δ_C 176.87 (C-4), one carbonyl carbon at δ_C 211.06 (C-1), a methylene carbon which is near to the hydroxyl at δ_C 68.80 (C-5) and two methylene carbon atoms which is next to the carboxylic acid at δ_C 33.91 (C-2) and δ_C 28.53 (C-3). The ^{1}H-^{1}H correlation spectroscopy (COSY) showed correlations between H-2 and H-3 which confirmed the two methylenes linked each other. HMBC showed a correlation from protons of H-2 and H-3 to a carbon of aliphatic acids at δ_C 176.8 (C-1). The second most intense signals were from protons at δ_H 4.24 (H-5), 2.69 (H-2), and 2.59 (H-3) correlated with carbonyl carbon at δ_C 211.06 (C-4). Accordingly, the chemical structure of compound **4** was elucidated as 5-hydroxylevulinic acid which was previously isolated from *Clematis delavayi var. spinescens* [25].

*Compound **5***. *β*-Sitosterol-3-O-*β*-D-glucoside was isolated as a white powder with a melting point of 285-287 °C. The FT-IR showed an absorption peak at 3436 cm^{-1} due to O-H stretching vibration, 2918 cm^{-1} due to C-H stretching, and 1620 cm^{-1} due to C=C stretching vibration. Its molecular formula was established as $C_{35}H_{60}O_6$ by FAB-MS at *m/z* 599.4236 $[M+Na]^+$ (calc. for $C_{35}H_{60}O_6$, 599.8384). Comparison of ^{1}H and ^{13}C-NMR data of compound **5** (Table 2) with literature value confirmed that compound **5** is *β*-sitosterol-3-O-*β*-D-glucoside [26].

Antioxidant activity

The antioxidant capacities of the isolated compounds, expressed as the DPPH free radical inhibition and the IC_{50} values, are presented in Table 3.

Table 3. Percent radical scavenging activity and IC_{50} values of the crude extract and isolated compounds.

Compounds/ crude extract	% DPPH inhibition at					IC_{50} µg/mL
	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.12 µg/mL	
CE	47.8	36.7	30.1	25.8	22.7	52.9
3	47.8	46.2	44.9	44.4	44.2	26.96
4	43.8	42.9	39.0	38.5	36.8	14.89
5	38.5	31.9	25.0	24.7	23.5	26.99
Ascorbic acid	97.32	97.27	97.25	97.19	96.96	0.36
Note: IC_{50} - half maximal inhibitory concentration; CE-crude extract						

In the current study, the antioxidant activities of the crude extract of the aerial part of *Clematis simensis* and the isolated compounds were evaluated using a DPPH assay. The DPPH method is the most common, cost-effective, and quick method to evaluate the antioxidant activities of natural products [27].

According to the parameters used, the IC_{50} value category is very strong if the IC_{50} value is < 10 µg/mL, strong if the IC_{50} value is between 10 and 50 µg/mL, mild if the IC_{50} value is between 50 and 100 µg/mL, weak if the IC_{50} value is between 100 and 250 µg/mL and not active if the IC_{50} is above 250 µg/mL [28]. The crude extract (52 µg/mL) showed mild antioxidant activity. The isolated compounds **3**, **4**, and **5** showed strong antioxidant activity with the IC_{50} values in the range of 14.89 to 26.99 µg/mL but were very low compared to the standard ascorbic acid (0.36 µg/mL).

Antimicrobial activity susceptibility tests

The antibacterial activity of the crude extract and isolated compounds were determined by the agar well diffusion method as recommended by the National Committee for Clinical Laboratory Standards against Gram-positive bacteria *Staphyloccocus aureus* (MTCC 1430), and Gram-negative bacteria *Escherichia coli* (MTCC 42), *Pseudomonas aeruginosa* (MTCC 1034), and *Salmonella typhi* (ATCC 6539) at 125-500 mg/mL concentrations, using 10% DMSO as a solvent (Table 4).

Table 4. Antibacterial activities of isolated compounds.

Organism	Conc.	Zones of inhibition in mm			
		Crude extract (mg/mL)	**3** (µg/mL)	**4** (µg/mL)	Ciprofloxacin
S. aureus	125	ND	ND	ND	18±0.3 (at 5 µg/mL)
	250	3.0±0.3	ND	ND	
	500	5.0±0.1	ND	ND	
P. aeruginosa	125	ND	ND	ND	15±0.8 (at 5 µg/mL)
	250	2.0±0.8	ND	ND	
	500	5.0±0.2	ND	ND	
E. coil	125	ND	ND	ND	32±1.0 (at 5 µg/mL)
	250	2.0±0.2	ND	ND	
	500	4.0±0.5	ND	ND	
S. *typhi*	125		ND	ND	27±0.6 (at 5 µg/mL)
	250	2.0±0.1	ND	ND	
	500	4.0±0.6	ND	ND	
Note: Conc: Concentration, ND: not detected					

The tested extract of *Clematis simensis* had antibacterial activity against both gram-positive and gram- negative bacteria at different concentrations. A higher inhibition zone (3.0±0.3 mm) was detected against *S. aureus,* followed by *P. aeruginosa* with a mean zone of inhibition of 2.0±0.8 mm at 250 mg/mL, which is one of the most common causes of a wide range of infections, such as skin infections, food poisoning, pneumonia, sepsis, and osteomyelitis [29]. The results showed that the chloroform:methanol (2:1) extracts of Clematis simensis leaves possessed relatively better antibacterial activity against the tested bacteria. Compounds **3** and **4** did not show any inhibition against the tested microorganisms. Ciprofloxacin, which was used as a positive control, produced a zone of inhibition of 15–32 mm. The antibacterial effects of the crude extract of *Clematis simensis* were far below the commercial antibiotic, ciprofloxacin (5 µg/mL**)**. This could attribute to the fact that the antibacterial principles found in the plant are less potent.

Extraction and characterization of compounds from the essential oil of aerial parts of Clematis simensis

The essential oil of the aerial parts of *Clematis simensis* was examined using gas chromatography-mass spectrometry (GC-MS) to determine the chemical composition. The GC-MS chromatogram of the essential oil shown in Figure 2, and Table 5 lists their peak area percentages, chemical structures, and biological activity. 31 compounds were identified using the GC-MS. The analyzed essential oil was composed of different compounds of different metabolite classes: phenols, aliphatic alcohols, hydrocarbons, and terpenes. The major volatile compounds were (E)-2-nonen-1-ol (38.33%), 4-cyclopentene-1,3-dione (28.69%), cyclofenchene (5.65%), benzene acetaldehyde (4.74%), methyl salicylate (2.77%), salicylaldehyde (2.68%), benzaldehyde (2.60%), α-terpineol (2.10%), eucalyptol (1.21%), and 2,6-dimethylcyclohexanol (1.15%).

Scientific reports revealed that some of the identified compounds have a considerable potential for therapeutic use. For example, (E)-2-nonen-1-ol (38.33%) is the major volatile organic compound that has antifungal activity [30], antimicrobial activity [31], sex pheromone [18], flavoring agent [32], antibacterial quorum sensing (anti-QS) [33], and deorphanize odorant receptors [34]. Whereas, 4-cyclopentene-1, 3-dione (28.69%), the second major component of in the essential oil has antifungal [35] and anticancer activity [36]. The other eight bioactive components of the essential oil, with their various pharmacological activities have been reported in many studies as antioxidant, anticancer, antiulcer, anticonvulsant, antihypertensive, and anti-nociceptive [37], favouring agent, analgesic, antiinflammatory, and rubefacient/counterirritant properties [38].

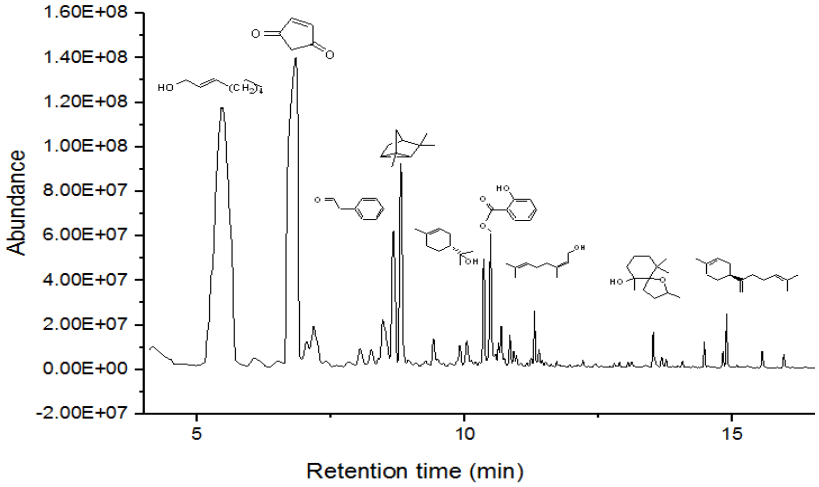

Figure 2. The GC-MS chromatogram of the essential oil of aerial parts of *Clematis simensis*.

Table 5. Compounds identified from the essential oil of the aerial parts of *Clematis simensis by* GC-MS analysis.

PK	RT	Area Pct	Compound names	Q	CF
1	5.45	38.32	(E)-2-Nonen-1-ol	78	$C_9H_{18}O$
2	6.06	0.81	5-Methyl-2(3H)-Furanone	83	$C_5H_6O_2$
3	6.84	28.69	4-Cyclopentene-1,3-dione	75.1	$C_5H_4O_2$
4	7.04	1.21	Eucalyptol	89	$C_{10}H_{18}O$
5	7.17	2.60	Benzaldehyde	97	C_6H_5CHO
6	8.04	0.84	(E,E)-2,4-Heptadienal	76	$C_7H_{10}O$
7	8.25	0.78	cis-Linalool oxide	70.3	$C_{10}H_{18}O_2$
8	8.47	2.68	Salicylaldehyde	97	$C_7H_6O_2$
9	8.66	4.74	Benzeneacetaldehyde	94	C_8H_8O
10	8.80	5.65	Cyclofenchene	83	$C_{10}H_{16}$
11	9.41	1.15	2,6-dimethyl Cyclohexanol, -	76	$C_8H_{16}O$
12	9.91	0.65	Terpinen-4-ol	83	$C_{10}H_{18}O$
13	10.04	0.79	Isopinocarveol	74.4	$C_{10}H_{16}O$
14	10.35	2.10	α-Terpineol	95	$C_{10}H_{18}O$
15	10.48	2.77	Methyl salicylate	94	$C_8H_8O_3$
16	10.57	0.26	α-Terpinen-7-al	78	$C_{10}H_{14}O$

17	10.63	0.36	p-Cymen-8-ol	86	$C_{10}H_{14}O$
18	10.68	0.78	m-Cymenene	95	$C_{10}H_{14}$
19	10.84	0.58	β-Cyclocitral	95	$C_{10}H_{16}O$
20	10.92	0.28	3-Carene	87	$C_{10}H_{16}$
21	10.96	0.19	(-)-cis-carveol	95	$C_{10}H_{16}O$
22	11.24	0.17	*β*-Homocyclocitral	74.8	$C_{11}H_{18}O$
23	11.30	0.76	cis-Geraniol	91	$C_{10}H_{18}O$
24	11.38	0.34	D-Carvone	94	$C_{10}H_{14}O$
25	13.52	0.59	6-Hydroxydihydrotheaspirane	67.8	$C_{13}H_{24}O_2$
26	13.68	0.13	cis-Jasmone	98	$C_{11}H_{16}O$
27	14.48	0.29	β-Ionene	98	$C_{13}H_{20}O$
28	14.82	0.22	β-Ionone epoxide	79.8	$C_{13}H_{20}O_2$
29	14.89	0.67	beta-Bisabolene	76	$C_{15}H_{24}$
30	15.56	0.25	5-pentyl-1,3-Benzenediol	78	$C_{11}H_{16}O_2$
31	15.96	0.20	2-Methyl-5-octyn-4-ol	83	$C_9H_{16}O$
Pk = peak number, Rt = retention time (min), Area Pct. = area percentage, Q = quality					

Chemotaxonomic significance

Previous phytochemical studies revealed that species of the *Clematis* genus are rich in pentacyclic triterpenoid, saponins, coumarins, flavonoids, and alkaloids [39]. This study led to the isolation of five compounds, including 1-tricosanol (**1**), 1-hexacosanol (**2**), 2-deoxy-D-ribono-1,4-lactone (**3**), 5-hydroxylevulinic acid (**4**), and *β*-sitosterol-3-O-*β*-D-glucoside (**5**). To our knowledge, 1-hexacosanol (**2**) is reported herein for the first time from the *Clematis* genus. 1-tricosanol (**1**), 2-deoxy-D-ribono-1,4-lactone (**3**), 5-hydroxylevulinic acid (**4**), and *β*-sitosterol-3-O-*β*-D-glucoside (**5**) are already reported from the *Clematis* genus [40]. 1-Tricosanol (**1**) has been isolated from *Clematis terniflora* [41]. *β*-Sitosterol-3-O-*β*-D-glucoside (**5**) has been previously isolated from many other plants, including flowers of *Viola odorata* [26], onion (*Allium cepa* L.) [42], and *Ocimum sanctum* L. [43]. The existence of these compounds may indicate a close relationship with the members of the genus *Clematis* in terms of their chemical composition.

CONCLUSION

The chemical components of *Clematis simensis* were investigated, which resulted in the separation of five compounds. Even though these compounds had been isolated before, this is the first time that they were isolated from this plant species. A total of ten major compounds have been identified from the current GC-MS analysis performed on the hydrodistilation essential oil of the aerial part of *Clematis simensis*. One of these major compounds, α-terpineol, is of particular importance since it has a variety of biological uses, including those for anti-inflammatory, antioxidant, anti-cancer, anticonvulsant, anti-ulcer, and anti-hypertensive qualities. Interestingly, none of the tested isolated compounds showed antibacterial activities, and the crude extract showed very weak activity against four common standard ATCC strains.

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REFERENCES

1. *Jstor.org*. Available: https://plants.jstor.org/compilation/*Clematis.simensis*. Accessed: 20-Dec-2023.

2. Hao, D.; Gu, X.; Xiao, P.; Peng, Y. Chemical and biological research of *Clematis* medicinal resources. *Chin. Sci. Bull.* **2013**, 58, 1120–1129.
3. Tuasha, N.; Petros, B.; Asfaw, Z. Medicinal plants used by traditional healers to treat malignancies and other human ailments in Dalle district, Sidama Zone, Ethiopia. *J. Ethnobiol. Ethnomed.* **2018**, 14, 1–21.
4. Tuasha, N.; Petros, B.; Asfaw, Z. Plants used as anticancer agents in the Ethiopian traditional medical practices: A systematic review. *Evid-Based Compl. Alt*. **2018**, 2018, 1–28.
5. Wubetu, M.; Sintayehu, M.; Aeta, M.A. Ethnobotany of medicinal plants used to treat various mental illnesses in Ethiopia: A systematic review. *Asian J. Plant Sci. Res*. **2018**, 8, 9–33.
6. Amare, D.; Egigu, M.C.; Sasikumar, J.M. Ethnobotanical study on medicinal plants used by ethnic people of Gechi district, South West Oromia, Ethiopia. *Nus. Biosci.* **2022**, 14, 104–116.
7. Tedila, H.; Assefa, A. In vitro antibacterial activity of *Rumex nervosus* and *Clematis simensis* plants against some bacterial human pathogens. *Afr. J. Microbiol. Res.* **2019**, 13, 14–22.
8. Birhan, M. Assessment of antimicrobial effects, antioxidant activity and phytochemical analysis of *Clematis simensis* leaves extract. *Asian J. Med. Pharm. Res*. **2018**, 8, 15–25.
9. Teka, A.; Asfaw, Z.; Demissew, S.; Van Damme, P. Traditional medicinal plant use of indigenous communities in Gurage Zone, Ethiopia. *Ethnobot. Res. Appl*. **2020**, 19, 1–31.
10. Gedif, T.; Hahn, H. Traditional treatment of skin disorders in Butajira, South-Central Ethiopia. *Ethiop. Pharm. J.* **2001**, 19, 48–56.
11. Desta, B. Ethiopian traditional herbal drugs. Part II: Antimicrobial activity of 63 medicinal plants. *J. Ethnopharmacol.* **1993**, 39, 129–139.
12. Costa, E.S.; Hiruma-Lima, C.A.; Lima, E.O.; Sucupira, G.C.; Bertolin, A.O.; Lolis, S.F. Antimicrobial activity of some medicinal plants of the Cerrado, Brazil. *Phytother. Res.* **2008**, 22, 705–707.
13. Tadele, A.; Asres, K.; Melaku, D.; Mekonnen, W. In vivo anti-inflammatory and antinociceptive activities of the leaf extracts of *Clematis simensis* fresen. *Ethiop. Pharm. J.* **2010**, 27, 33–41.
14. Tuasha, N. Cytotoxicity of selected Ethiopian medicinal plants used in traditional breast cancer treatment against breast-derived cell lines. *J. Med. Plant Res*. **2019**, 13, 188–198.
15. Ayana, M.T.; Mehari, Y.G.; Beyene, B.B. A phytochemical investigation and evaluation of the antioxidant and antibacterial activities of the stem bark extract of *Clematis simensis* (Yeazo hareg). *J. Chem. Res*. **2022**, 46, 17475198221127398.
16. Alemayehu, M.A.; Abdelwuhab, M.; Gelayee, D.A. In-vivo anti-malarial activity of crude extract and solvent fractions of the roots of *Clematis simensis Fresen*. (Ranunculaceae) in plasmodium berghei infected mice. *J. Pharm. Biol. Sci*. **2020**, 14, 52–62.
17. Teshome, N.; Degu, A.; Ashenafi, E.; Ayele, E.; Abebe, A. Evaluation of wound healing and anti-inflammatory activity of hydroalcoholic leaf extract of *Clematis simensis fresen* (Ranunculaceae). *Dermatol.* **2022**, 15, 1883–1897.
18. Isika, D.K.; Özkömeç, F.N.; Çeşme, M.; Sadik, O.A. Synthesis, biological and computational studies of flavonoid acetamide derivatives. *RSC Adv.* **2022**, 12, 10037–10050.
19. Yen, G.C.; Duh, P.D. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. *Agric. Food Chem*. **1994**, 42, 629–632.
20. Wayne, P.A. National committee for clinical laboratory standards. Performance standards for antimicrobial disc susceptibility testing. **2002**, 12, 01-53.
21. Elyemni, M.; Louaste, B.; Nechad, I.; Elkamli, T.; Bouia, A.; Taleb, M.; Chaouch, M.; Eloutassi, N. Extraction of essential oils of *Rosmarinus officinalis* L. By two different methods: hydrodistillation and microwave assisted hydrodistillation. *Sci. World J.* **2019**, 2019, 1–6.
22. Nagah, N.; Mostafa, I.; Osman, A.; Dora, G.; El-Sayed, Z.; Ateya, A.M. Bioguided isolation and in-silico analysis of Hep-G2 cytotoxic constituents from *Laurus nobilis* Linn, cultivated in Egypt. *Egypt. J. Chem.* **2021**, 64, 2731–2745.
23. Joshi, S.; Poudel, T.N. Isolation and characterization of the chemical constituents of *Sonchus wightianus* of Nepalese origin. *J. Nepal Chem. Soc.* **2013**, 28, 115–120.

24. Kitajima, J.; Ishikawa, T.; Tanaka, T.; Ida, Y. Water-soluble constituents of fennel. Glucides and nucleosides. *Chem. Pharm. Bull. (Tokyo)* **1999**, 47, 988–992.
25. Li, Y.; Wang, S.F.; Zhao, Y.L.; Liu, K.C.; Wang, X.M.; Yang, Y.P.; Li, X.L. Chemical constituents from *Clematis delavayi var. spinescens*. *Molecules* **2009**, 14, 4433–4439.
26. Peshin, T.; Ka, H. Isolation and characterization of *β*-sitosterol-3-O-*β*-D-glucoside from the extract of the flowers of *Viola odorata*. *Br. J. Pharm. Res.* **2017**, 16, 1–8.
27. Arulmozhi, S. In vitro antioxidant and free radical scavenging activity of *Alstonia scholaris* Linn. *Iran. J. Pharmacol. Ther.* **2007**, 6, 191–196.
28. Reviana, R.; Usman, A. N.; Raya, I.; Aliyah, Dirpan, A.; Arsyad, A.; Fendi, F. Analysis of antioxidant activity on cocktail honey products as female pre-conception supplements. *Gac. Sanit*. **2021**, 35, S202–S205.
29. Kakai, R.; Wamola, I.A. Minimising antibiotic resistance to *Staphylococcus aureus* in developing countries. *East Afr. Med. J*. **2002**, 79, 574–579.
30. She, J.; Mohottige, C.U.G.; King, M.; Jiang, Y.; Mlsna, M.; Clark, S.; Baird, R.; Mlsna, T. Estimation of total phenolic compounds and non-targeted volatile metabolomics in leaf tissues of American Chestnut and the backcross breeding generations. *J. Agric. Chem. Environ.* **2021**, 10, 222–256.
31. Nirubama, K.; Kanchana, G.; Rubalakshmi, G. Bioactive compounds in *Andrographis echiodes* (L.) leaves by GC-MS analysis. *Int. J. Curr. Res. Biosci. Plant Biol.* **2014**, 1, 92–97.
32. Smith, R.L.; Waddell, W.J.; Cohen, S.M.; Feron, V.J.; Marnett, L.J.; Portoghese, P.S.; Rietjens, I.M.; Adams, T.B.; Gavin, C.L.; McGowen, M.M.; Taylor, S.V. GRAS flavoring substances 24. *Food Technol*. **2009**, 63, 46–105.
33. Husain, F.M. Al-Shabib, N.A.; Noor, S.; Khan, R.A.; Khan, M.S.; Ansari, F.A.; Khan, M.S.; Khan, A; Ahmad, I. Current strategy to target bacterial quorum sensing and virulence by phytocompounds. In *New Look to Phytomedicine*. Edited by Khan, M.S; Ahmad, I and Chattopadhyay, D. Academic Press: New York; **2019**; pp. 301–329.
34. Pelletier, J.; Xu, P.; Yoon, K.S.; Clark, J.M.; Leal, W.S. Odorant receptor-based discovery of natural repellents of human lice. *Insect. Biochem. Mol. Biol*. **2015**, 66, 103–109.
35. Mokhtari, M. Bioactivity-guided metabolite profiling of *Feijoa* (*Acca sellowiana*) cultivars identifies 4-cyclopentene-1, 3-dione as a potent antifungal inhibitor of chitin synthesis. *J. Agric. Food Chem*. **2018**, 66, 5531–5539.
36. Kobayashi, E. European Patent Application. Googleapis.com. Available: https://patentimages.storage.googleapis.com/9b/c0/54/0c423d1f186e65/EP1018336A1.pdf. [Accessed: 20-Nov-2023].
37. Khaleel, C.; Tabanca, N.; Buchbauer, G. α-terpineol, a natural monoterpene: A review of its biological properties. *Open Chem*. **2018**, 16, 349–361.
38. Dasgupta, A.; Wahed, A. Common poisonings including heavy metal poisoning in clinical chemistry, immunology and laboratory quality control, *Elsevier*: Waltham, USA; **2014**, pp. 337–351.
39. Sun, F.; Yang, D. Advance in chemical constituents of genus *Clematis*. *Chinese Materia Medica*. **2009**, 34, 2660–2668.
40. Dong, C.X.; Shi, S.P.; Wu, K.S; Tu, P.F. Chemical constituents from the roots and rhizomes of *Clematis hexapetala* Pall. Z. Naturforsch. *Br. J. Chem. Sci*. **2007**, 62, 854–858.
41. Sun, F.; Zhang, L.; Tian, J. Studies on chemical constituents of *Clematis terniflora*. *Chin. Pharm. J*. **2007**, 42, 102.
42. Mizushina, Y.; Nakanishi, R.; Kuriyama, I.; Kamiya, K.; Satake, T.; Shimazaki, N.; Koiwai, O.; Uchiyama, Y.; Yonezawa, Y.; Takemura, M.; Sakaguchi, K.; Yoshida, H. *β*-Sitosterol-3-O-*β*-D-glucopyranoside: A eukaryotic DNA polymerase λ Inhibitor. *J. Steroid Biochem. Mol. Biol*. **2006**, 99, 100–107.
43. Njinga, N. Evaluation of antimicrobial and antioxidant activity of *β*-sitosterol-3-*O*-glucoside isolated from *Lannea kerstingii* eng and *K. krause* (Anacardiaceae). *Int. J. Glob. Sustain*. **2016**, 2, 9–9.