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CHEMICAL COMPOSITION, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF ESSENTIAL OILS FROM *CYPHOSTEMMA ADENOCAULE* AND *ZIZIPHUS SPINACHRISTI*

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ABSTRACT. In the present work, the chemical composition, antibacterial and antioxidant potencies of essential oils (EOs) extracted from the leaves and roots of *Cyphostemma adenocaule* and *Ziziphus spinachristi* were evaluated. Hydrodistillation was used to extract the EOs and the chemical compositions were analyzed by GC-MS. The antibacterial activity of the EOs were evaluated against four bacterial strains by agar disc diffusion method. Moreover, the antioxidant properties were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay method. Perillyl alcohol (13.08%) and phytone (12.64%) were the major compounds detected in the leaves and roots of *Cyphostemma adenocaule* respectively. Whereas, nootkatone was the principal compound detected in the leaves (30.12%) and roots (26.52%) of *Ziziphus spinachristi*. The antibacterial activity results showed that, the EOs of *Cyphostemma adenocaule* and *Ziziphus spinachristi* displayed the highest inhibition zones against *Streptococcus pyogenes* (13.67 ± 0.34 and 12.67 ± 0.10 mm respectively) at 10 mg/mL. The antioxidant activity of the EOs were also promising, and the strongest IC_{50} value (4.15 µg/mL) was calculated for *Ziziphus spinachristi* (leaves). Thus, the antibacterial and antioxidant properties of the EOs enlighten the use of these plants for the aforementioned activities and as a common ingredients in cosmetic applications.

KEY WORDS: Essential oils, Antibacterial activity, Antioxidant activity, *Cyphostemma adenocaule*, *Ziziphus spinachristi*

INTRODUCTION

Essential oils (EOs) are volatile mixtures of organic and natural bioactive compounds in plants and possess as many as 10-70 compounds in different concentrations [1]. They are characterized by their contents of 1-3 major compounds in relative high concentrations compared to the other components of the essential oil present in minimal amounts, and are mainly comprised of terpenoids, fatty acid methyl esters and phenyl propanes with different functional groups (i.e, aldehydes, ketones, alkanes, alcohols, acids and esters) [1]. EOs are colorless liquids naturally present in all parts of plants including flowers, barks, roots, stem, seeds and leaves. As a result of their good aroma and flavor, EOs have been widely used in many parts of the world as cosmetics, perfumes, medicines and foods [2]. In addition to aromatic qualities their biological activities against a wide range of microorganisms have also gave a valuable evidence which can be used as proper candidates of natural food preservatives [3], and are also frequently used by manufacturers and consumers replacing the function of synthetic preservatives in many food industries as the later can lead to some allergic effects, cancer, intoxications and other degenerative diseases [2].

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EOs are secondary metabolites and plays a great role in defense mechanism, hence possess many medicinal properties including antioxidant [3], antihelminthic [4], antibacterial [5], insecticidal [6], antiviral [7], anti-inflammatory [8], antidepressant [9], antimalarial [10], and antifungal [11] activities. For instance, EOs extracted from different plants which contains terpenes and oxygenated derivatives show remarkable inhibitory effects against pathogenic bacteria and are used as antioxidant and flavoring agents. Mostly, the medicinal plants used in the traditional Chinese medicine (TCM), Ayurveda and Siddha have long been used as the major sources of many volatile components, which are responsible for different biological and pharmacological properties [2].

The genus *Cyphostemma* (Vitaceae family) [12] and *Ziziphus* (Rhamnaceae family) [13] have a wide array of bioactive compounds which are responsible for their medicinal activities. Scores of research studies showed that plant EOs from the genus of the two plants possess chemical compounds with broad pharmacological and biological properties [14]. In Ethiopia, the genus *Cyphostemma* have long been used for the treatment of rabies, snake bites and skull wounds, and also possess antimalarial, anticancer, antitumor and antihelminthic properties [15]. EOs of the genus *Ziziphus* have also been used in folk medicine against some diseases, such as diabetes, skin infections, diarrhea, fever, obesity and digestive disorders [16]. Moreover, various extracts and compounds isolated from the genus *Ziziphus* were reported to have antimicrobial, antioxidant, analgesic, anti-inflammatory, sedative, antidiabetic and antipyretic properties [17].

Cyphostemma adenocaule (Figure 1A) which belongs to the Vitaceae family, is locally called Aserkuh Aserkush (Amharic), Hareg Temen (Tigrinya) and Hida Bofa (Afan Oromo), and is traditionally used to treat urinary tract infections, syphilis, bloody diarrhea, rabies and snake bite [12], helminthic infections and anthrax [15]. Previous *in vitro* biological studies also revealed that, the plant possess anticancer [18], antioxidant [19], antibacterial [20], antitumor [18], antiplasmodial [12] and deworming [21] activities. Reports of the phytochemistry and essential oil composition of *C. adenocaule* are very limited, thereby similar species of the plant were used for comparison. Earlier GC-MS analysis of the EOs of a related species, i.e., leaves of *Cyphostemma juttae* revealed a total of 39 compounds among which phytol (29.6%), neophytadiene (6.6%), hexadecanoic acid (5.5%), 3-(2,6,6,-trimethyl-1-cyclohex-1-yl)-2-propenal (5.5%) and isophytol (4.6%), accounting 56.4% of the total oil composition were the most abundant compounds reported from the plant [22]. According to the report, most of the identified compounds were terpenoids contributing to 60% of the essential oil, and this class of compounds possess multiple ecological functions, such as; defense against bacteria, fungi and herbivores, attraction of pollinator insects and birds, allelopathy and protection from abiotic factors [22].

Ziziphus spinachristi (Figure 1B), one of the species of the genus *Ziziphus*, is widely distributed in various parts of Africa and India with prominent nutritious and medicinal properties [23]. In Ethiopia, it has different vernacular names, such as, Gaba (Amharic and Tigrinya), Qurqura (Afan Oromo), and is used to treat wounds [24], dandruff [25], hair loss [25], constipation [26], diarrhea and malaria [27]. Previous GC-MS analysis of the leaves EO of the plant revealed *trans*-caryophyllene (17.31%), α-pinene (15.50%), β-phellandrene (10.86%), β-pinene (7.32%), β-myrcene (6.26%), L-menthone (4.90%), carane (4.75%) and bicyclogermacrene (4.62%) as the major constituents [28]. A related study on the EOs of the leaves and flowers of the plant also revealed the presence of carotol (42.20%), hexadecanoic acid (13.75%), linoleic acid (11.76%), vetivenic acid (9.56%) and valeranone (7.06%) [24]. GC-MS analysis of EO from the leaves of *Z. spinachristi* collected from Iran also revealed 34 components comprising 92.2% of the total oil. Geranyl acetone (14.0%), methyl palmitate (10.0%), farnesyl acetone (9.9%), methyl stearate (9.9%), cetyl alcohol (9.7%) and ethyl stearate (8.0%) were the major constituents identified in the report [29].

A) B)

Figure 1. Aerial parts of *C. adenocaule* (A) and *Z. spinachristi* (B).

As a result of the adverse problems of the conventional antioxidant and antibacterial drugs, the increased pathogenic resistance and the high cost of drugs, efforts are always active to get alternative sources of medicine from plants which are health friendly and with few side effects [30]. In Ethiopia, the two plant species possess numerous traditional applications in both folk and livestock medicines and thus further investigations are desirable. Reports of the chemical composition, antibacterial and antioxidant activities of EOs from *C. adenocaule*, and roots of *Z. spinachristi* are also lacking. Therefore, the present study aimed to extract EOs from the leaves and roots of *C. adenocaule* and *Z. spinachristi* to analyze the constituents by GC-MS and evaluate their antibacterial and antioxidant potency. We believe that, the findings of the study offer remarkable data to confirm the ethno medicinal potential of the two plants.

EXPERIMENTAL

Collection and identification of the plant materials

The fresh leaves and roots of the two plants were collected from the mountains of Adama city, in October, 2021 (*C. adenocaule* and leaves of *Z. spina-christi*) and June, 2022 (roots of *Z. spina-christi*). The plant materials were authenticated by Mr. Melaku Wendafrash, a taxonomist at the National Herbarium, Department of Biology, Addis Ababa University, Ethiopia. A voucher specimen numbers HCA003 and HZS007 for *C. adenocaule* and *Z. spinachristi* respectively, were deposited at the Herbarium of Ethiopia, Department of Biology, Addis Ababa University, Ethiopia. After collection the plant materials were washed repeatedly with tap water and with sterilized distilled water and allowed to air dry at room temperature without direct exposure to sunlight. The dried plant materials were ground in to fine powder using a blender and stored in polyethylene bag.

Extraction of essential oils via hydrodistillation

EOs were extracted from different parts (leaves and roots) of the two plant materials *via* hydro-distillation method. The ground leaves of *C. adenocaule* and *Z. spinachristi* (40 g each) and roots (30 g each) were hydro-distilled separately by Clevenger's apparatus (Aarson Scientific Works, India) at atmospheric pressure for 3 hrs. The EOs were separated from the aqueous layer by adding 100 mL of chloroform (Loba Chemie, India) in separatory funnel. Small amount of aqueous form left with the chloroform was dried by adding 5 g of anhydrous sodium sulphate and filtered using

Whattman No 1 filter paper [22, 24]. Finally, the mixture was concentrated using rotary evaporator (DW-RE-3000, China) and the oil was kept in refrigerator until required for analysis. The extraction yield of the EOs was determined based on the following equation (equation 1).

$$\text{Extraction yield (\%)} = \frac{\text{Mass of concentrated oil}}{\text{Dry mass of plant powder used}} \text{x } 100 \quad (1)$$

Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical compositions of the EOs extracted from the leaves and roots of *C. adenocaule* and *Z. spinachristi* were analyzed *via* gas chromatography-mass spectrometry (GC-MS) using the previously reported method [31]. A GC–MS instrument from Agilent Technologies (Santa Clara, CA, USA) equipped with a 6890 N network GC system, 5975 inert mass selective detector, 7683B series auto sampler injector (10 µL in size), HP5MS column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness), coated with 5% phenyl 95% methyl poly siloxane, G1701DA GC/MSD Chem Station was used for analyzing the samples. 2 µL EO solutions in chloroform were injected through auto sampler and analyzed with HP5MS column. Column temperature was programed as follows: 55–120 °C at 20 °C/min, 120–150 °C at 1.5 °C/min, 150–250 °C at 20 °C/min, 250 °C (10 min) and 3 min solvent delay. The temperature of mass spectra transfer line was 280 °C. The carrier gas used was helium (1 mL/min) with a split ratio of 100:1. The mass spectra were recorded in electron ionization mode at 70 eV with scanning from 50 to 500 amu (atomic mass unit) at 0.5 s with the mass source being set at 230 °C [31].

Identification of the components

The components of the EOs were identified from the generated chromatograms based on their elution time, retention indexes, mass fragmentation patterns and by comparison with the spectral data available in the literature and NIST library. The relative percentage amount of each compound was calculated from the electronic integrations by comparing its average peak area to the total area and the integration of peaks were performed using Hewlett Packard Chem-Station software (G1701BA Version B.01.00) for quantification of the peaks.

Antibacterial activity tests

The bacterial strains that were used in this study were American type culture collections (ATCCs). The ATCC bacterial strains of *Eschericia coli* (ATCC-25922)*, Staphylococcus aureus* (ATCC-25923)*, Pseudomonas aeruginosa* (ATCC-27853) and *Streptococcus pyogenes* (ATCC-19615) were collected from the Ethiopian Public Health Institute (EPHI). The microorganisms were handled and transported aseptically. The strains were grown and preserved in test tubes containing nutrient broths at 4 °C in a refrigerator (Samsung electronics, South Korea) until required for the bioassay.

Preparation of inoculum, culture media and plates

The Mueller Hinton broth (MHB) and Mueller Hinton Agar (MHA) which were manufactured by HiMedia Laboratories (India) were organized as per the directions of the manufacturers. The media were cooled and sterilized at 45 °C and 20 mL of it was poured in to a sterilized petri-dishes to form the Mueller Hinton media with uniform thickness of about 3 mm. The MHA plates were of six equal segments to confirm that the disks were not very closer than 24 mm on the MHA. Each bacterial strain was cultured over night at 37 °C in petri-dishes containing the MHA. The bacterial inoculums were prepared by direct colony suspension method [9].

Agar disc diffusion assay

The mode of action of the EOs against the strains was evaluated by agar disc-diffusion method as per the standard protocols of the clinical and laboratory standards institute (CLSI) [32]. About five colonies of each bacterial strains were transferred in to a saline solution using an incubating loop and grown in brain heart infusion (BHI) broth. The turbidity of the bacterial strains was attuned in reference to about 0.5 MCFarland standard solution (1.5 x 10^8 CFU/mL). The bacterial suspension was transferred by swapping with a sterilized cotton swap on to the petridishs containing the media (MHA) (solidified 20-25 mL). A Whatman No.1 filter paper discs (6 mm in diameter) was then prepared using a puncher for the impregnation of the test samples and sterilized in an oven at 180 ^oC for 1 h. A stock solution of the leaves and roots EOs (10 mg/mL) was prepared in 4% DMSO followed by the preparation of different concentrations by serial dilution. Accordingly, two different concentrations (5 and 2.5 mg/mL) of EOs were prepared from the pre-prepared stock solution. A standard antibiotic (ciprofloxacin, 0.5 mg/mL) and DMSO were used as positive and negative controls, respectively. Each solution (1 mL) was loaded over a separate petri dishes containing sterilized paper discs of about 6 mm in diameter and left for 30 min for a complete absorption of the solutions by the paper discs. The paper discs were then transferred on to the petri-dishes containing the bacterial culture inoculated MHA, left for 30 min for diffusion and incubated at 37 ^oC for 24 h [32]. The antibacterial activities were evaluated by measuring the zone of inhibition (mm) of the extracts using the digital zone of inhibition measuring caliper against the test organism. The entire tests were performed in triplicate and the results were recorded as mean ± standard deviation using statistical analysis software.

After the completion of the assay, the used bacterial inoculums, petri dishes, swabs and other disposable materials were autoclaved and discarded in to trashes. The experimental tests were done at the research laboratory of the Department of Biology, Adama Science and Technology University, Adama, Ethiopia in collaboration with microbiologists.

Antioxidant activity tests

In this study, the antioxidant activities of the leaves and roots EOs of *C. adenocaule* and *Z. spinachristi* were investigated using *in vitro* 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay method. Thus, the antioxidant activities of the EOs along with the standard (ascorbic acid) was tested against DPPH radical (Calbiochem, Germany) using the procedure described by Khorasani Esmaeili *et al.* [33]. Four different concentrations (500, 250, 125 and 62.5 µg/mL) of the EOs of the two plants were prepared from the corresponding stock solution (1000 µg/mL in MeOH). Similar concentrations of ascorbic acid (AA) were also prepared which served as the standard antioxidant agent. To each of the above prepared concentrations, freshly prepared DPPH solution (2 mL, 0.04% w/v in MeOH) was added followed by incubating for 30 min at room temperature. After incubation, the absorbance of each concentration was measured at 517 nm using the UV-Vis spectrophotometer (Cecil CE4001 UV/ VIS, Cambridge, England) in triplicate. Sample free DPPH solution in methanol (Loba Chemie, India) was used as negative control. The anti-DPPH free radical potential of each tested sample was expressed in terms of percentage scavenging activity using equation 2 [33].

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{A}{A_o}\right) \text{x } 100 \qquad (2)$$

where; A = the absorbance of DPPH radical in methanol + sample in methanol, A_0 = the absorbance of DPPH radical in methanol.

The results were expressed as mean ± SEM, after the calculations using equation 2. Then, the antioxidant power was expressed as a value of concentration of *C. adenocaule* and *Z. spinachristi*

EOs which were responsible to scavenge 50% of the DPPH radical (IC_{50}) and compared with ascorbic acid (AA). The IC_{50} values of the EOs and the standard were calculated from the relationship curves (logarithmic regressions) of the radical scavenging activity versus concentrations of the tested samples. The antioxidant potency levels of the EOs were rated based on their IC_{50} values as very strong ($IC_{50} < 50$ µg/mL), strong ($50 < IC_{50} < 100$ µg/mL), medium ($100 < IC_{50} < 150$ µg/mL), weak ($150 < IC_{50} < 200$ µg/mL) and very weak (>200 µg/mL) [34].

Statistical data analysis

The antibacterial and antioxidant data were tabulated in a Microsoft excel spread sheet and the values were conveyed as mean ± standard error of the mean (SEM). The antibacterial activities were investigated by comparing the inhibition zones of the target samples with the inhibition zones of the control drug and the solvent. The one-way ANOVA with Tukey's *posthoc* test was applied for data comparisons. SPSS version 21.0 was used and the data were assumed significant for $p < 0.05$. Origin 8 software were also used to draw the graphical sketch of the antioxidant activities of the samples and analyze the data. For GC-MS data, the compounds were identified by a means of their elution time, retention indexes, mass spectral fragmentation patterns and in comparison with the NIST 2005 library of mass spectra. For identification of the compounds, greater than > 60% identity match with the library of the compounds was required. The molecular formula, retention time (t_R), names of the compounds and chemical structures of the compounds were established.

RESULTS AND DISCUSSION

Yield and composition of the essential oils

The powdered leaves (40 g) and roots (30 g) of *C. adenocaule* yielded a colorless and pleasant odor EOs of about 20 mg (0.05% w/w) and 15.04 mg (0.05% w/w) respectively. Moreover, the hydrodistilation of the leaves (40 g) and roots (30 g) EOs of *Z. spinachristi* yielded a colorless and sweet floral aroma EOs of 34.53 mg (0.12% w/w) and 17.64 mg (0.06% w/w), respectively. The results showed that *Z. spinachristi* generated better yields than *C. adenocaule.* The EOs were dissolved in chloroform (0.5 mg/mL) and 2 µL of the solution was injected and chromatographed in GC-MS and displayed plenty of compounds. The GC-MS analysis (Figure 2A) of the leaves EO of *C. adenocaule* showed a total of 70 components of which 43 compounds accounting for 94.30% of the total composition were reported based on their areal records (compounds with peak area of 0.20% and above were reported). Perillyl alcohol (13.08%), geranyl isovalerate (10.29%), germacra-4(15),5,10(14)-trien-1α-ol (9.65%), (+)-spathulenol (7.45%), piperitenone (7.06%), τ-cadinol (5.76 %) and caryophyllene oxide (4.83%) were among the major compounds identified from the leaves of the plant. The chemical classes of the compounds along with their composition were also determined, and sesquiterpenes and derivatives (43.45%) comprised the majority of the leaves EO of *C. adenocaule* followed by monoterpenes and derivatives (41.76%), fatty acids (3.91%), aromatics (2.18%), diterpenes (1.53%), others (1.18%) and hydrocarbons (0.29%) (Table 1).

Table 1. Essential oil composition of the leaves of *C. adenocaule*.

No.	Compound name	Molecular formula	RT	RI	Relative percentage (%)
Monoterpenes and derivatives					
1	Sabinol isovalerate	$C_{15}H_{24}O_2$	5.11	1515	0.60
2	Borneol	$C_{10}H_{18}O$	23.99	1167	0.69
3	α-Terpeneol	$C_{10}H_{18}O$	25.25	1189	2.50

4	Carveol	$C_{10}H_{16}O$	26.61	1229	0.43
5	*cis*-Geraniol	$C_{10}H_{18}O$	27.10	1228	0.94
6	Lavandulol	$C_{10}H_{18}O$	28.35	1170	3.45
7	Perillal	$C_{10}H_{14}O$	29.12	1272	2.72
8	Perillyl alcohol	$C_{10}H_{16}O$	30.30	1296	13.08
9	Piperitenone	$C_{10}H_{14}O$	32.14	1340	7.06
10	Geranyl isovalerate	$C_{15}H_{26}O_2$	54.23	1606	10.29
Sesquiterpenes and derivatives					
11	(-)-Aromadendrene	$C_{15}H_{24}$	35.48	1440	0.48
12	*trans*-β-Ionone	$C_{13}H_{20}O$	38.28	1486	0.86
13	β-Bisabolene	$C_{15}H_{24}$	39.20	1509	0.31
14	Epicubebol	$C_{15}H_{26}O$	39.37	1493	0.52
15	*δ*-Cadinene	$C_{15}H_{24}$	39.76	1524	0.63
16	2-Methyl-9-(prop-1-en-3-ol-2-yl)-bicyclo [4.4.0]dec-2-ene-4-ol	$C_{15}H_{24}O_2$	40.52	1555	0.51
17	Nerolidol	$C_{15}H_{26}O$	41.37	1544	0.91
18	(+)-Spathulenol	$C_{15}H_{24}O$	41.85	1576	7.45
19	Caryophyllene oxide	$C_{15}H_{24}O$	42.04	1581	4.83
20	(+)-Ledene	$C_{15}H_{24}$	42.35	1493	0.53
21	Mintketone	$C_{15}H_{24}O$	42.44	1595	0.91
22	Cedrol	$C_{15}H_{26}O$	42.67	1598	1.03
23	Costol	$C_{15}H_{24}O$	42.97	1778	1.09
24	Aromadendrene oxide	$C_{15}H_{24}O$	44.26	1678	1.86
25	*τ*-Cadinol	$C_{15}H_{26}O$	44.40	1640	5.76
26	β-Acorenol	$C_{15}H_{26}O$	44.50	1649	0.67
27	Mustakone	$C_{15}H_{22}O$	45.09	1687	1.92
28	Germacra-4(15),5,10(14)-trien-1*α*-ol	$C_{15}H_{24}O$	45.30	1695	9.65
29	Dehydrosaussurea lactone	$C_{15}H_{20}O_2$	47.30	1838	0.69
30	Nootkatone	$C_{15}H_{22}O$	48.11	1808	0.87
31	Diepicedrene-1-oxide	$C_{15}H_{24}O$	48.86	1551	0.67
32	Phytone	$C_{18}H_{36}O$	48.98	1844	1.00
33	*trans*-Longipinocarveol	$C_{15}H_{24}O$	49.27	1618	0.30
Diterpenes					
34	Phytol	$C_{20}H_{40}O$	53.76	2114	1.53
Aromatics					
35	8,9-Dehydrothymol	$C_{10}H_{12}O$	26.45	1221	0.34
36	Cuminol	$C_{10}H_{14}O$	29.93	1289	0.26
37	Eugenol	$C_{10}H_{12}O_2$	32.88	1357	0.72
38	Dihydroactinolide	$C_{11}H_{16}O_2$	39.90	1532	0.86
Fatty acids					
39	Palmitic acid	$C_{16}H_{32}O_2$	51.24	1968	3.91
Hydrocarbons					
40	Heptacosane	$C_{27}H_{56}$	56.55	2700	0.29
Others					
41	4-(2-Methyl-3-oxocyclohexyl)-butanal	$C_{11}H_{18}O_2$	39.60	1515	0.42
42	8*α*-11-Elemadiol	$C_{12}H_{24}O_2$	40.71	1745	0.28
43	3-Deoxyestradiol	$C_{18}H_{24}O$	51.80	2259	0.48
Total					94.30

RI; retention index**,** RT; retention time.

The GC-MS analysis (Figure 2B) of the roots EO of *C. adenocaule* revealed a total of 75 components of which 48 compounds which account for 97.08% of the oil composition were reported based on their areal records (compounds with area of 0.20% and above were reported) (Table 2). Phytone (12.64%), geranyl isovalerate (12.15%), phytol (10.50%), palmitic acid

(8.60%), germacra-4(15),5,10(14)-trien-1α-ol (4.73%), τ-Cadinol (4.21%), neophytadiene (4.02%) and shyobunol (3.81%) were among the major components of the roots EO of the plant. Among the identified compounds, sesquiterpenes and derivatives (39.87%) afford the highest composition followed by fatty acids and derivatives (19.34%), diterpenes and derivatives (16.60%), monoterpenes and derivatives (13.03%), others (4.64%), hydrocarbons (1.88%) and aromatics (1.72%). The chemical composition of the roots EO of *C. adenocaule* showed slight differences from those identified in the leaves of the plant. This is due to the fact that the composition of plant EOs collected from different parts varies with the age of the plant, type of soil, experimental conditions and genetic differences [35].

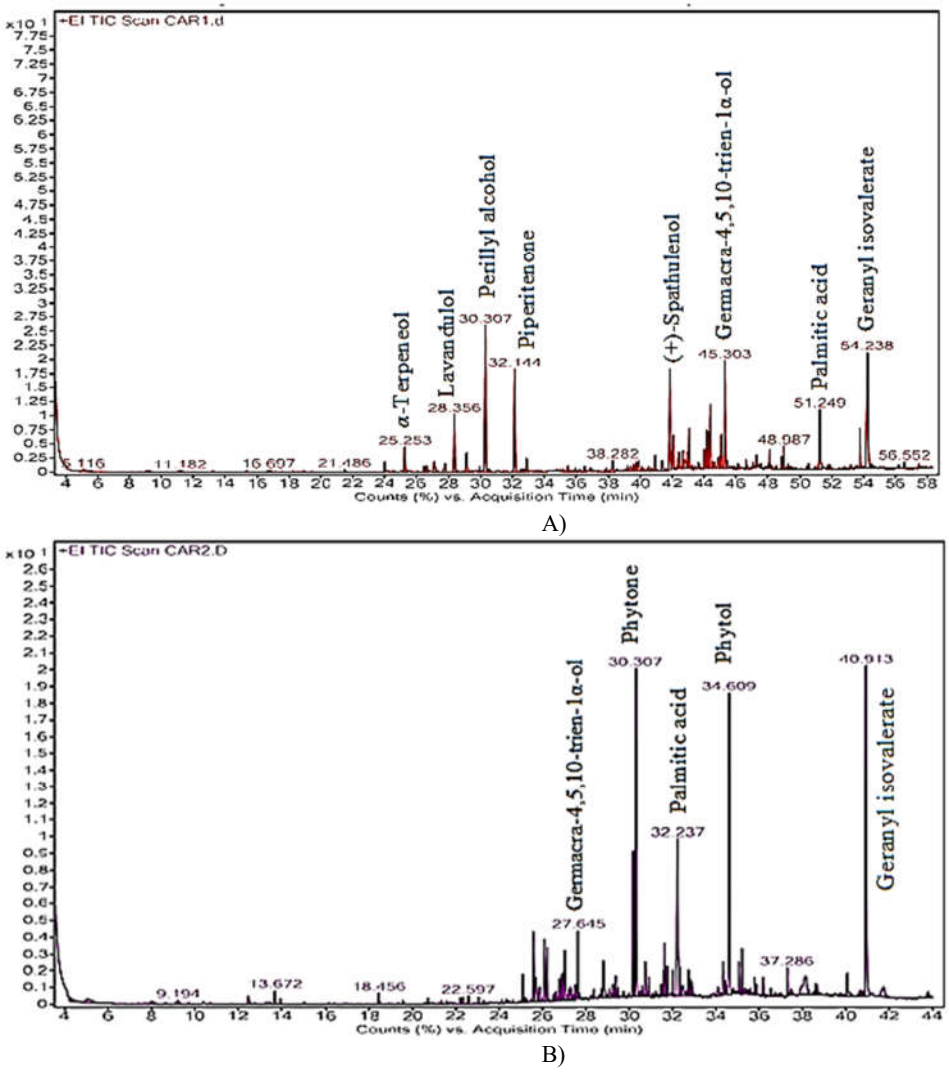


Figure 2. Gas chromatogram of the leaves (A) and roots (B) EOs of *C. adenocaule*.

To the best of our knowledge, the chemical composition and biological activities of EOs from *C. adenocaule* are reported herein for the first time. Nevertheless, prior works on related species found a total of 39 compounds comprising 80% of the total oil from the EOs of *C. juttae* among which phytol (29.6%), neophytadiene (6.6%), hexadecanoic acid (5.5%) and isophytol (4.6%) were identified as the most abundant constituents [22]. The findings of the present study showed some similarities with the EO profile of the related species reported previously.

Table 2. Essential oil composition of the roots of *C. adenocaule*.

No.	Compound name	Molecular formula	RT	RI	Relative abundance (%)
Monoterpenes and derivatives					
1	Sabinol isovalerate	$C_{15}H_{24}O_2$	5.02	1515	0.88
2	Geranyl isovalerate	$C_{15}H_{26}O$	40.91	1606	12.15
Sesquiterpenes and derivatives					
3	Cedrene	$C_{15}H_{24}$	22.20	1422	0.23
4	Caryophyllene	$C_{15}H_{24}$	22.34	1419	0.36
5	*cis*-Thujopsene	$C_{15}H_{24}$	22.59	1429	0.24
6	*α*-Bisabolene	$C_{15}H_{24}$	23.06	1504	0.20
7	(+)-Spathulenol	$C_{15}H_{24}O$	25.58	1576	2.11
8	Isoaromadendrene epoxide	$C_{15}H_{24}O$	25.71	1589	1.25
9	Caryophyllene oxide	$C_{15}H_{24}O$	25.83	1581	0.41
10	(-)-Globulol	$C_{15}H_{26}O$	25.87	1580	0.69
11	Cedrol	$C_{15}H_{26}O$	26.09	1598	3.07
12	β-Costol	$C_{15}H_{24}O$	26.87	1778	0.81
13	Ledene oxide	$C_{15}H_{24}O$	26.95	1631	1.16
14	*τ*-Cadinol	$C_{15}H_{26}O$	27.03	1640	4.21
15	Humulenol-II	$C_{15}H_{24}O$	27.26	1650	1.32
16	*trans*-Bisabolene epoxide	$C_{15}H_{24}O$	27.42	1586	0.18
17	Thujopsenal	$C_{15}H_{22}O$	27.51	1724	0.56
18	Germacra-4(15),5,10(14)-trien-1*α*-ol	$C_{15}H_{24}O$	27.64	1695	4.73
19	Hexahydro-farnesol	$C_{15}H_{32}O$	29.37	1571	0.67
20	Phytone	$C_{18}H_{36}O$	30.30	1844	12.64
21	Shyobunol	$C_{15}H_{26}O$	38.12	1701	3.81
22	Longiborneol	$C_{15}H_{26}O$	41.72	1592	1.22
Diterpenes and derivatives					
23	Neophytadiene	$C_{20}H_{38}$	30.19	1837	4.02
24	Isophytol acetate	$C_{22}H_{42}O_2$	32.00	2064	0.73
25	Phytol	$C_{20}H_{40}O$	34.60	2114	10.50
26	Phytyl-acetate	$C_{22}H_{42}O_2$	35.21	2064	1.35
Aromatics					
27	Resorcinol	$C_6H_6O_2$	12.44	1372	0.47
28	1-Isocyanato-2-methyl-Benzene	C_8H_7NO	13.67	1131	0.58
29	*o*-Toluidine	C_7H_9N	13.91	1070	0.27
30	Chavicol	$C_9H_{10}O$	18.45	1255	0.40
Fatty acids and derivatives					
31	Lauric acid	$C_{12}H_{24}O_2$	25.09	1568	1.00
32	Ethyl-linolenate	$C_{20}H_{34}O_2$	28.72	2169	0.28
33	Myristic acid	$C_{14}H_{28}O_2$	28.81	1768	2.56
34	di-Linoleylmethylketone	$C_{19}H_{34}O$	30.92	2075	0.64
35	Methyl-palmitate	$C_{17}H_{34}O_2$	31.64	1926	1.53
36	Palmitic acid	$C_{16}H_{32}O_2$	32.23	1968	8.60
37	Oleic acid	$C_{18}H_{34}O_2$	32.35	2141	1.80
38	*cis*-13-Eicosenoic acid	$C_{20}H_{38}O_2$	32.72	2366	0.95

39	Methyl-linoleate	$C_{19}H_{34}O_2$	34.33	2097	1.09
40	Linoleic acid	$C_{18}H_{32}O_2$	36.17	2133	0.53
41	Octyl-palmitoleate	$C_{24}H_{46}O_2$	40.64	2559	0.36
Hydrocarbons					
42	Heptacosane	$C_{27}H_{56}$	40.05	2700	1.88
Others					
43	Xanthinin	$C_{17}H_{22}O_5$	29.27	2383	0.46
44	2-Methylene cholestan-3-ol	$C_{28}H_{48}O$	30.74	2317	1.22
45	7-Hexadecenal	$C_{16}H_{30}O$	31.48	1798	0.34
46	Aspidocarpine	$C_{22}H_{30}N_2O_3$	31.58	-	0.68
47	1,13-Tetradecadien-3-one	$C_{14}H_{24}O$	31.76	1571	0.82
48	2-Hexadecenal	$C_{16}H_{30}O$	32.90	1878	1.12
Total					97.08

RI; retention index, RT; retention time.

The GC-MS analysis (Figure 3) of the leaves EO of *Z. spinachristi* showed a total of 65 components, of which 41 compounds were identified comprising 95.26% of the total oil composition. The identification technique was based on their areal records (compounds with area of 0.20% and above were reported). Nootkatone (30.12%), palmitic acid (23.53%), nuciferyl acetate (8.47%) and germacra-4(15),5,10(14)-trien-1α-ol (7.51%) were among the predominant compounds identified from the leaves of the plant (Table 3). The relative compositions of the classes of compounds exhibited that sesquiterpenes and derivatives (52.74%) afford the highest composition followed by fatty acids and derivatives (28.84%), others (10.15%), aromatics (2.34%), and monoterpenes and derivatives (1.19%).

Table 3. Essential oil composition of the leaves of *Z. spinachristi*.

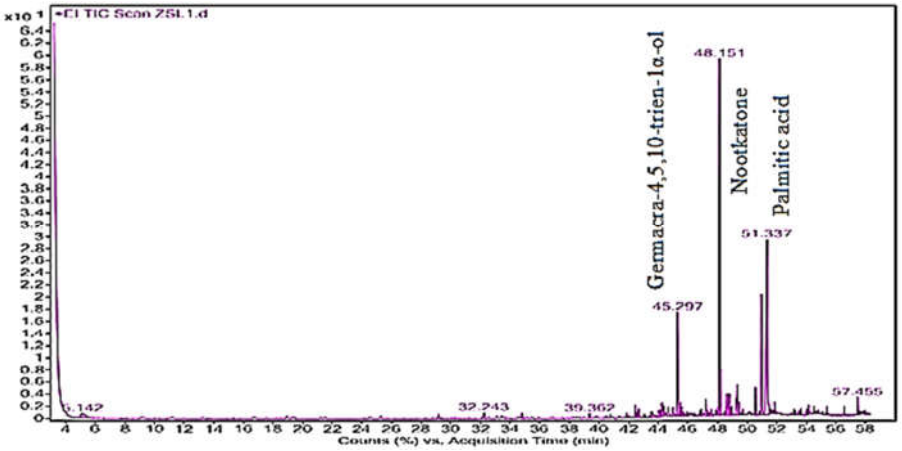
No.	Compound name	Molecular formula	RT	RI	Relative abundance (%)
Monoterpenes and derivatives					
1	*cis*-Piperitol	$C_{10}H_{18}O$	32.24	1195	0.58
2	Geranyl isovalerate	$C_{15}H_{26}O_2$	51.86	1606	0.61
Sesquiterpenes and derivatives					
3	8,14-Cedranoxide	$C_{15}H_{24}O$	41.86	1545	0.39
4	*cis*-Thujopsene	$C_{15}H_{24}$	42.45	1429	0.78
5	Cedrol	$C_{15}H_{26}O$	42.66	1598	0.58
6	β-Longipinene	$C_{15}H_{24}$	43.83	1403	0.23
7	β-Guaiene	$C_{15}H_{24}$	44.04	1490	0.87
8	τ-Cadinol	$C_{15}H_{26}O$	44.40	1640	0.78
9	(+)-Ledene	$C_{15}H_{24}$	44.48	1493	0.16
10	β-Costol	$C_{15}H_{24}O$	44.66	1778	1.57
11	*trans*-Longipinocaveol	$C_{15}H_{24}O$	45.00	1618	0.89
12	Germacra-4(15),5,10(14)-trien-1α-ol	$C_{15}H_{24}O$	45.30	1695	7.51
13	Epizizanone	$C_{15}H_{22}O$	45.59	1669	0.52
14	Thujopsenal	$C_{15}H_{22}O$	46.80	1724	0.35
15	Aromadendrene oxide	$C_{15}H_{24}O$	47.21	1678	1.34
16	β-Santalol	$C_{15}H_{24}O$	47.56	1715	1.13
17	Caryophyllene oxide	$C_{15}H_{24}O$	47.87	1581	0.35
18	α-Atlantone	$C_{15}H_{22}O$	48.03	1717	0.46
19	Nootkatone	$C_{15}H_{22}O$	48.15	1808	30.12
20	Ylangenal	$C_{15}H_{22}O$	48.63	1675	1.53
21	Isolongifolene	$C_{15}H_{20}$	48.80	1544	2.54
22	Diepicedrene-1-oxide	$C_{15}H_{24}O$	50.84	1551	0.26

23	Hexahydro farnesol	$C_{15}H_{32}O$	56.55	1571	0.38
Aromatics					
24	Tolune	C_7H_8	5.14	763	1.76
25	Resorcinol	$C_6H_6O_2$	16.69	1372	0.23
26	2,4-Di-*tert*-Butylphenol	$C_{14}H_{22}O$	39.36	1519	0.35
Fatty acids and derivatives					
27	Capric acid	$C_{10}H_{20}O_2$	29.17	1373	0.42
28	10,12-Tricosadiynoic acid, methyl ester	$C_{24}H_{40}O_2$	47.29	2832	0.28
29	Methyl-palmitate	$C_{17}H_{34}O_2$	50.56	1926	1.39
30	Palmitic acid	$C_{16}H_{32}O_2$	51.33	1968	23.53
31	Isopropyl palmitate	$C_{19}H_{38}O_2$	51.59	2023	0.29
32	Methyl arachidonate	$C_{21}H_{34}O_2$	53.15	2274	0.39
33	Erucic acid	$C_{22}H_{42}O_2$	53.27	2547	0.30
34	Linoleic acid	$C_{18}H_{32}O_2$	54.15	2133	0.74
35	Stearic acid	$C_{18}H_{36}O_2$	54.51	2172	0.45
36	Oleamide	$C_{18}H_{35}NO$	57.45	2386	1.05
Others					
37	Methyl *o*-tolylcarbamate	$C_9H_{11}NO_2$	34.81	1379	0.40
38	α-Calacorene	$C_{15}H_{20}$	40.50	1542	0.25
39	1-Methyl-3-ethyladamantane	$C_{13}H_{22}$	42.53	1263	0.25
40	Retinal	$C_{20}H_{28}O$	49.48	2466	0.78
41	Nuciferyl acetate	$C_{17}H_{24}O_2$	50.97	1837	8.47
Total					95.26

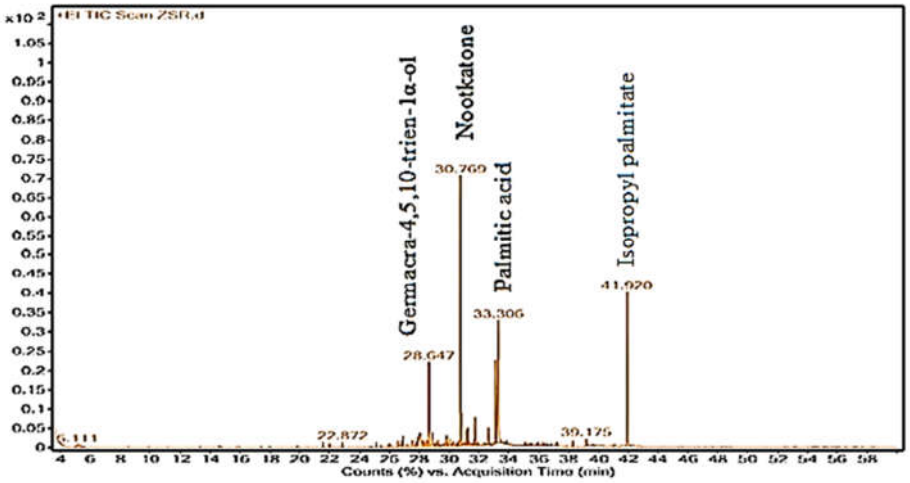
RI; retention index, RT; retention time.

The GC-MS analysis (Figure 3) of the roots EO of *Z. spinachristi* showed a total of 60 components, of which 32 compounds representing 96.15% of the total oil composition were reported based on their areal records (compounds with area of 0.20% and above were reported) (Table 4). Unidentified components were present in such low amounts that either no mass spectrum was recorded or the spectrum was too poor for interpretation. The relative abundance of the identified compounds showed that nootkatone (26.52%), palmitic acid (16.46%), isopropyl palmitate (13.26%), germacra-4(15),5,10(14)-trien-1α-ol (8.73%) and nuciferyl acetate (6.28%) were found abundantly in the roots of the plant. Of the obtained compounds, sesquiterpenes and derivatives (60.18%) form the highest composition followed by fatty acids and derivatives (31.76%), aromatics (1.79%), others (1.31%), monoterpenes and derivatives (0.73%) and hydrocarbons (0.38%). The majority of the compounds identified from the roots EO of the plant were in good agreement with the composition of the leaves.

The chemical composition of the EOs of *Z. spinachristi* along with its biological activities were the subject of previous reports [16, 24, 28]. Previous study on the EOs of *Z. spinachristi* collected from Egypt identified a total of 21 compounds constituting 99.3% of the oil, of which dodecanoic acid (22.4%), oleic acid methyl ester (17.1%) and octanoic acid (10.3%) [16] were among the major ones. Another report by Fard *et al.* [24], revealed the presence of 11 compounds (92.14%) in the EOs (leaves) of the plant, of which carotol (42.20%), hexadecanoic acid (13.75%), linoleic acid (11.76%) and vetivenic acid (9.56%) were found as the most abundant components. The reports showed some correlations with the EO composition of the present study. The presence of 13.75% hexadecanoic acid (palmitic acid) in the report was also in good agreement with the the composition of palmitic acid in the roots EO of *Z. spinachristi* in our work (16.46%). Linoleic acid was identified in the EOs (leaves; 0.74% and roots; 0.43%) of *Z. spinachristi* but with significant difference compared to the findings of Fard *et al.* [24]. The differences in percentage compositon of the EOs are also related to various environmental factors, such as geographical location, climatic conditions, genetic factors, phisiological variations and method of extraction [36].



A)



B)

Figure 3. Gas chromatogram of the leaves (A) and roots (B) EOs of *Z. spinachristi*.

Table 4. Essential oil composition of the roots of *Z. spinachristi*.

No.	Compound name	Molecular formula	RT	RI	Relative abundance (%)
Monoterpenes and derivatives					
1	*cis*-Piperitol	$C_{10}H_{18}O$	21.56	1195	0.45
2	Geranyl isovalerate	$C_{15}H_{26}O_2$	33.87	1606	0.28
Sesquiterpenes and derivatives					
3	*α*-Panasinsen	$C_{15}H_{24}$	24.81	1527	0.22
4	*cis*-Thujopsene	$C_{15}H_{24}$	26.91	1429	0.70
5	β-Guaiene	$C_{15}H_{24}$	27.80	1490	0.58
6	γ-Himachalene	$C_{15}H_{24}$	27.87	1477	0.74
7	(+)-Ledene	$C_{15}H_{24}$	28.04	1493	3.23

8	*trans*-Longipinocaveol	$C_{15}H_{24}O$	28.44	1618	0.82
9	Germacra-4(15),5,10(14)-trien-1α-ol	$C_{15}H_{24}O$	28.65	1695	8.73
10	β-Costol	$C_{15}H_{24}O$	28.87	1778	4.10
11	α-Atlantone	$C_{15}H_{22}O$	29.66	1717	0.47
12	Isolongifolene	$C_{15}H_{20}$	29.82	1544	2.13
13	β-Santalol	$C_{15}H_{24}O$	30.30	1715	1.54
14	Nootkatone	$C_{15}H_{22}O$	30.77	1808	26.52
15	Nuciferyl acetate	$C_{17}H_{24}O_2$	31.12	1837	6.28
16	Thujopsenal	$C_{15}H_{24}O$	31.15	1724	2.19
17	4,5,9,10-Dehydro-isolongifolene	$C_{15}H_{20}$	31.26	1544	1.27
18	β-Curcumene	$C_{15}H_{24}$	35.10	1514	0.41
19	Hexahydro farnesol	$C_{15}H_{32}O$	39.66	1571	0.25
Aromatics					
20	Toluene	C_7H_8	5.11	763	1.49
21	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	25.13	1519	0.30
Fatty acids and derivatives					
22	Methyl-palmitate	$C_{17}H_{34}O_2$	32.64	1926	0.94
23	Palmitic acid	$C_{16}H_{32}O_2$	33.31	1968	16.46
24	Linoleic acid	$C_{18}H_{32}O_2$	35.96	2133	0.43
25	Oleamide	$C_{18}H_{35}NO$	39.18	2386	0.67
26	Isopropyl palmitate	$C_{19}H_{38}O_2$	41.92	2023	13.26
Hydrocarbons					
27	Tricosane	$C_{23}H_{48}$	38.28	2300	0.38
Others					
28	2-Indolinone	C_8H_7NO	14.58	1487	0.18
29	3-Quinuclidinol	$C_7H_{13}NO$	14.74	1157	0.19
30	Methyl-*o*-tolylcarbamate	$C_9H_{11}NO_2$	22.87	1379	0.34
31	1-Methyl-3-ethyladamantane	$C_{13}H_{22}$	26.83	1263	0.34
32	2-Hexadecanol	$C_{16}H_{34}O$	36.59	1571	0.26
Total					96.15

RI; retention index, RT; retention time.

Antibacterial activities

In the present study, the EOs of *C. adenocaule* (roots) and *Z. spinachristi* (leaves) afforded promising antibacterial activities against the four bacterial pathogens (*E. coli*, *S. aureus*, *P. aeruginosa* and *S. pyogenes*). Besides, the EOs of *C. adenocaule* (leaves) and *Z. spinachristi* (roots) exhibited weak to moderate activities against the bacterial strains and activities were dose dependent (Table 5 and Figure 4). The highest inhibitory activities were observed by the EOs of *C. adenocaule* (roots) at 10 mg/mL against *S. pyogenes, E. coli* and *S. aureus* (13.67 ± 0.34, 13.34 ± 0.22 and 13.34 ± 0.27, respectively). Whereas, EOs from the leaves of *Z. spinachristi* displayed highest inhibition zones against *S. pyogenes* (12.67 ± 0.10) and *S. aureus* (12.33 ± 0.32) at 10 mg/mL, compared to ciprofloxacin (29.67 ± 0.48 and 28.66 ± 0.38, respectively). The leaves and roots EOs of *C. adenocaule* and *Z. spinachristi* showed significantly larger inhibition zones against the evaluated bacterial strains at 10 mg/mL concentrations than at lower concentrations ($p < 0.5$). There were no significant statistical difference in growth inhibition of the bacterial strains with 2.5 mg/mL and 5 mg/mL of the leaves EO of *Z. spinachristi* ($p > 0.5$). In contrary, there were significant statistical differences in inhibitory diameters of the tested bacterial pathogens with 2.5 and 5 mg/mL, and 5 and 10 mg/mL of the roots EO of *C. adenocaule* ($p < 0.5$) (Table 5).

The antibacterial properties can be related to the presence of abundant components in the EOs of the two plants, such that, the high composition of perillyl alcohol [37], phytone [38] and spathulenol [39] in *C. adenocaule* and nootkatone [40], palmitic acid [41] and β-costol [42] in *Z. spinachristi* are responsible for the attractive antibacterial activities. Perillyl alcohol (the most

abundant compound in the leaves EO of *C. adenocaule*) is a hydroxylated monoterpene which is mainly found in the EOs of sage, perilla, spearmint, lavandin, peppermint, cherries, lemongrass, cranberries, gingergrass and celery seeds. It has shown to be implicated against different stages of tumor such as, gastric, lung, colon, breast, skin and liver cancers in rodent models [43]. It also plays significant roles in pathophysiologic processes like oxidative stress, thymidine incorporation in to DNA and inflammation [37]. Spathulenol (another abundant compound in the leaves EO of *C. adenocaule*), is a tricyclic sesquiterpene with 5,10-cycloaromadendrane skeleton [44]. Literature surveys revealed that spathulenol possess significant biological applications such as antihyperalgesic, antioxidant, anticholinesterase, antibacterial, antiproliferative, anti-inflammatory, antioedematogenic, chemotherapy of MDR cancer and cytotoxicity [39]. Thus, the high composition of spathulenol in the leaves EO also support the aforementioned activities of *C. adenocaule*. Phytone (Hexahydrofarnesyl acetone), one of the most abundant compound in the roots EO of *C. adenocaule* identified in this study, is a sesquiterpene, exhibited various biological activities such as anti-inflammatory, antibacterial and anti-nociceptive activities [38]. Phytol is an acyclic hydrogenated diterpenoid alcohol which can be used as a precursor for the manufacture of vitamin E and vitamin K1. It is frequently available in the EOs of certain aromatic plants. Some insects, such as sumac flea beetle uses the compound as chemical deterrents against predation. Phytol is also used for commercial applications such as fragrance industry (shampoos, cosmetics, household cleaners, toilet soaps and detergents [45, 46].

Nootkatone (the major compound identified from the EOs of *Z. spinachristi*) is a natural product which is widely found in grape fruit and other plants and function as an insecticide and repellant against mosquito vectors of parvoviruses. It is a sesquiterpene and is safely used in products such as cosmetics and juices to enhance the fragrance and flavor. Nootkatone possess the advantage of being neither genotoxic nor carcinogenic and is approved for use in and on people. It could offer consumers a favorable alternative than synthesized compounds like N,N-diethyl-*m*-toluamide (DET), since nootkatone at >98% purity causes no skin and other health problems [40]. Palmitic acid (the second abundant compound in the EOs of *Z. spinachristi*), is a saturated fatty acid which is mainly found in palm oil, olive oil and animal products and possess antibacterial properties. It is also known as n-hexadecanoic acid and is the most common saturated fatty acid which can be provided in the diet or synthesized from other fatty acids, amino acids and carbohydrates [41]. β-costol, was also another compound reported in the leaves EO of *Z. spinachristi* with significant composition. It belongs to the class of organic compounds called eudesmane, isoeudesmane sesquiterpenoids. The compound was reported in the EOs of various plants and exhibit antibacterial and antifungal activities [42]. Significant composition of (+)-ledene was also observed in the GC-MS analysis of the roots EO of *Z. spinachristi*. The compound is also known as leden, which belongs to the class of compounds called 5, 10-cycloaromadendrane sesquiterpenoids and was reported to possess antibacterial, antifungal, antioxidant and anticancer activities [47].

The antibacterial activities of the EOs of *Z. spinachristi* were in good agreement with the previous reports towards different bacterial pathogens [24]. According to the report of Fard *et al.* [24], the EO of the plant evaluated against six bacterial and fungal strains exhibited good activity against one bacterial strain (*K. pneumonia*) and two fungal strains (*A. niger, P. digitatum*). The report also revealed that, the EO of *Z. spinachristi* displayed good antioxidant potential with IC_{50} value of 53.91 ± 2.43, and its DPPH scavenging potential was related to the presence of potentially bactericidal compounds in the EOs of the plant, such as linoleic acid. Reports on the antibacterial capacity of the EOs of *C. adenocaule* are very limited, and thus in the present work, none of the results was compared with previous findings. This is due to the fact that the habitat of the plant is limited to certain countries of the world. Thus, we recommend further works on the antibacterial and other biological activities of the plant to support its ethno medicinal values.

Table 5. Inhibition zones (mean ± SD) of the essential oils of *C. adenocaule* and *Z. spinachristi*.

Plant name and parts used	Concentration (mg/mL)	Zone of inhibition (mm)			
		E. coli	*S. aureus*	*P. aeruginosa*	*S. pyogenes*
C. adenocaule (leaves)	10	7.75 ± 0.36[a]	7.50 ± 0.41[a]	7.50 ± 0.22[a]	10.00 ± 0.31[a]
	5	7.12 ± 0.22[b]	7.00 ± 0.19[b]	7.45 ± 0.20[b]	8.00 ± 0.28[b]
	2.5	NA	NA	NA	7.00 ± 0.33[c]
C. adenocaule (roots)	10	13.34 ± 0.22[a]	13.34 ± 0.27[a]	12.67 ± 0.30[a]	13.67 ± 0.34[a]
	5	10.34 ± 0.48[b]	11.67 ± 0.18[b]	11.34 ± 0.20[b]	11.33 ± 0.27[b]
	2.5	8.34 ± 0.12[c]	9.67 ± 0.43[b]	9.67 ± 0.15[c]	10.00 ± 0.00[b]
Z. spinachristi (leaves)	10	12.00 ± 0.00[a]	12.33 ± 0.32[a]	12.00 ± 0.18[a]	12.67 ± 0.10[a]
	5	9.33 ± 0.11[b]	10.66 ± 0.29[b]	10.66 ± 0.16[a]	11.00 ± 0.21[b]
	2.5	8.00 ± 0.21[c]	9.00 ± 0.00[c]	9.00 ± 0.33[b]	9.67 ± 0.22[c]
Z. spinachristi (roots)	10	9.00 ± 0.13[a]	8.50 ± 0.25[a]	8.00 ± 0.20[a]	8.00 ± 0.25[b]
	5	8.00 ± 0.36[b]	8.10 ± 0.26[b]	7.50 ± 0.31[b]	7.00 ± 0.15[c]
	2.5	NA	NA	NA	NA
Ciprofloxacin (30 µg/disc)	0.5	29.66 ± 0.44[a]	28.66 ± 0.38[a]	30.34 ± 0.41[a]	29.67 ± 0.48[a]

NA: no activity. Values are displayed as mean ± SEM. Columns with the same letter did not differ significantly ($p < 0.05$) according to ANOVA analysis.

Antioxidant activities

The EOs of the leaves and roots of *C. adenocaule* and *Z. spinachristi* were also subjected to *in-vitro* antioxidant activities against DPPH free radical and results were promising. The antioxidant activity tests of the EOs of both plants along with the standard (ascorbic acid) were performed in four different concentrations (500, 250, 125 and 62.5 µg/mL) which were prepared from their stock solutions (1000 µg/mL). The results displayed that the absorbance values measured and the radical scavenging activity (%) calculated were attractive, and activities were dose dependent which showed smooth relationships with concentrations (Figure 5). Both *C. adenocaule* (roots) and *Z. spinachristi* (leaves) exhibited better scavenging activity (90.52 ± 0.10 and 90.61 ± 0.25 respectively) against DPPH radical relative to ascorbic acid (98.30 ± 0.00) at 1000 µg/mL and their IC_{50} values were calculated as 7.41 and 4.15 µg/mL, respectively. Moreover, the EOs of *C. adenocaule* (leaves) and *Z. spinachristi* (roots) displayed good DPPH scavenging activities (88.52 ± 0.33 and 84.69 ± 0.10 respectively) at 1000 µg/mL compared to ascorbic acid and their IC_{50} values were calculated as 11.22 and 19.86 µg/mL, respectively. According to Molyneux, 2004 [48], EOs displaying $IC_{50} < 50$ µg/mL are strong antioxidants and is in good agreement with the present work. The EOs of both plants showed encouraging antioxidant activities and thereby could be the subject of further investigations. The antioxidant activities of the EOs of *Z. spinachristi* were also the subject of previous studies. Our findings concur with Fard *et al.* [24], who studied the scavenging capacity of the EOs (leaves) of the plant. According to the report, the EOs of *Z. spinachristi* collected from Iran revealed antioxidant activity with an IC_{50} of 53.91 µg/mL and the activity was related to presence of linoleic acid [24]. In comparison, our results showed better IC_{50} values and thereby better scavenging potential than the previous report. Unfortunately, no study was reported on the antioxidant capacity of the EOs extracted from the leaves and roots of *C. adenocaule*.

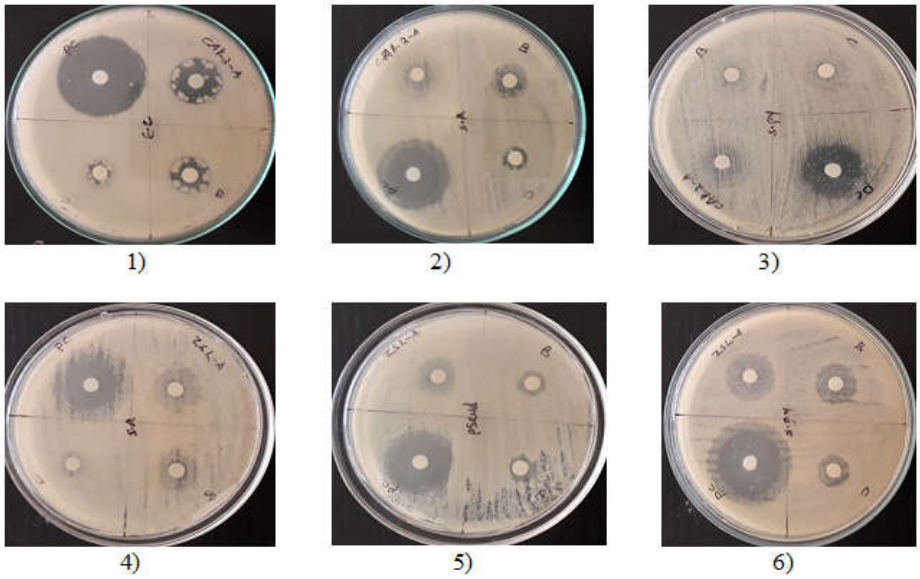


Figure 4. Inhibition zones of the essential oils of *C. adenocaule* and *Z. spinachristi*. Where, 1; *C. adenocaule* (roots) against *E. coli*, 2; *C. adenocaule* (roots) against *S. aureus*, 3; *C. adenocaule* (roots) against *S. pyogenes*, 4; *Z. spinachristi* (leaves) against *S. aureus*, 5; *Z. spinachristi* (leaves) against *P. aeruginosa*, 6; *Z. spinachristi* (leaves) against *S. pyogenes*, A; 10 mg/mL, B; 5 mg/mL, C; 2.5 mg/mL, *E. c*; *E. coli, S. a; S. aureus, P. a; P. aeruginosa, S. p; S. pyogenes* and PC; Positive control (ciprofloxacin).

In general, the antibacterial mechanism of action of EOs might be associated with the presence of secondary metabolites that produce synergism. EOs are able to bind with the bacterial cell wall and disrupt it, causes the damage of cell components in the microorganism and enhance permeability. Possible mechanisms include, inhibition of protective enzymes and weakening of biochemical pathway. Most of the reported compounds from the EOs of the two plants are terpenes and derivatives, and it is most probable that the hydrophobicity and/or lipophilicity of hydroxyl containing terpenes have much effects on the antibacterial mechanism of action and leads to possible synergetic effects [49]. Antioxidants are compounds that trap and neutralize free radicals, thereby minimize and/or control health effects of the body caused by free radicals. It is well documented that, the antioxidant potential of EOs is correlated to their oxygenated monoterpenes and to the availability of various phytocompounds which work synergistically to scavenge free radicals. In the present study, several classes of compounds including fatty acids, fatty acid esters, terpenoids, phenolic compounds and alcohols were detected. Reports showed that the aforementioned classes of compounds are responsible to exert antioxidant capability through multi step radical reactions, such as initiation, propagation and finally termination [50].

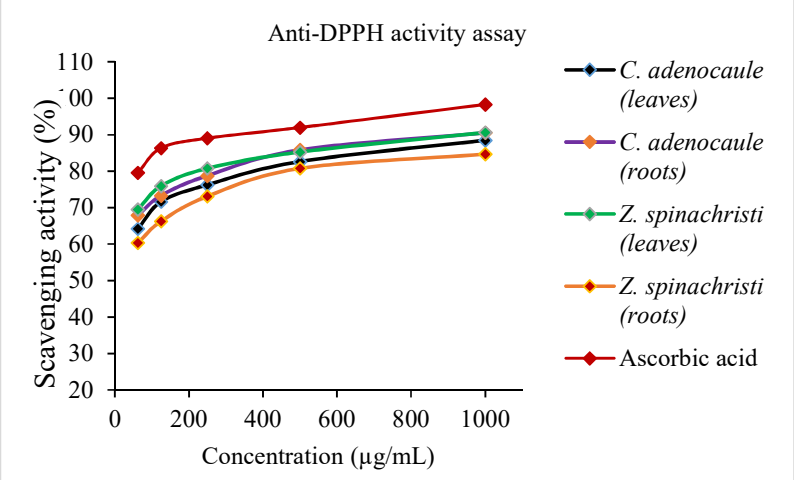


Figure 5. DPPH radical scavenging activity (%) of the essential oils of *C. adenocaule* and *Z. spinachristi* against different concentrations.

CONCLUSION

In the present work, EOs extracted from the leaves and roots of *C. adenocaule* and *Z. spinachristi* were evaluated for their chemical composition, antibacterial and antioxidant properties. Results showed that the EOs of the two plants possessed significant activities which are correlated to the presence of potentially antibacterial and antioxidant compounds in the EOs of the plants, such as, perillyl alcohol, germacra-4(15),5,10(14)-trien-1α-ol, phytol, spathulenol and τ-cadinol in *C. adenocaule* and nootkatone, palmitic acid and β-costol in *Z. spinachristi*. The EOs from the leaves and roots of the two plants exhibited a significant antibacterial and antioxidant compounds, and may thus have a potential application for pharmaceutical formulations, such as mouth and tooth washes. Therefore, the antibacterial and antioxidant investigations and other bioassay tests of the major compounds of these plant oils are recommended for future studies. Additionally, further efforts are needed to broaden the biological activities of the plants against other bacterial pathogens which were not studied in this work. The toxicological properties of the EOs of the two plants should also be the concern of future works for safety purposes.

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