

**CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS
FROM VARIOUS PARTS OF *GLADIOLUS CANDIDUS*, *RANUNCULUS MULTIFIDUS*,
ARTEMISIA ABYSSINICA AND *CRINUM ABYSCINICUM***

Dawit Tesfaye¹, Milkyas Endale¹, Emebet Getaneh¹, Eticha Abdisa², Leta Guta² and
Yadessa Melaku^{1*}

¹Department of Applied Chemistry, Adama Science and Technology University, P.O. Box 1888,
Adama, Ethiopia

²Department of Applied Biology, Adama Science and Technology University, P.O. Box 1888,
Adama, Ethiopia

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ABSTRACT. The essential oil compositions of *Gladiolus candidus*, *Ranunculus multifidus*, *Artemisia abyssinica* and *Crinum abyscnicum* were analyzed with gas chromatography-mass spectrometry (GC-MS). The principal components in the leaves, stems and rhizomes of *G. candidus* were eudesmol, 1-naphthalenepropanol and oleic acid, respectively. *α*-Terpineol, alloocimene and *p*-menth-2-en-1-ol from leaves, bulbs and roots were respectively the major constituents of *C. abyscnicum*. The aerial part of *R. multifidus* furnished *p*-mentha-2,8-dien-1-ol. Linalool and terpinenol were identified as the major constituents of *A. abyssinica*. The essential oils were evaluated for their antibacterial activity. Essential oil from leaves of *G. candidus* displayed zone of inhibition (IZ) of 15.1±0.3 and 16.7±0.9 mm against *E. coli* and *S. aureus*, respectively. Leaves essential oil of *C. abyscnicum* exhibited IZ of 17.9±1.1 and 15.6±1.1 mm against *E. coli* and *P. aeruginosa*, respectively, whereas essential oil from aerial part of *R. multifidus* displayed IZ of 18.8±0.8 and 19.4±0.6 mm against *S. aureus* and *S. pyogens*, respectively. At the same concentration, ceftriaxone showed IZ of 15.1±0.1, 16.2±0.8, 14.3±0.9 and 16.1±2.5mm against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. pyogens*, respectively. The findings presented herein support the ethnobotanical uses of these plants against bacteria.

KEY WORDS: *G. candidus*, *R. multifidus*, *A. abyssinica*, *C. abyscnicum*, Antibacterial activity, Essential oil

INTRODUCTION

Infectious diseases are responsible for an immense global burden that affects public health [1]. The diseases are reported to be the cause of deaths for over 9 million people in the world [1] with the major causes reported to be lower respiratory tract infections, diarrheal diseases, malaria and tuberculosis [2]. There are also infectious diseases that have newly appeared or have existed but are found resistant to the existing drugs [2]. Resistance developed by pathogenic microorganisms to the existing drugs is also a serious concern worldwide [3]. This makes infectious diseases harder to treat and consequently causes severe illness and death [3]. Medicinal plants that have been used traditionally for the treatment of infectious diseases remain an important source of pharmaceuticals. Hence, these plants might serve as a source of lead drugs [3]. In Ethiopia, many plant species including *Gladiolus candidus*, *Crinum abyscnicum*, *Ranunculus multifidus* and *Artemisia abyssinica* have been used as traditional medicine for the treatment of various human and animals against various infectious diseases

Gladiolus candidus (Iridaceae), locally named as *Dallo* in Afan Oromo and *Milas Golgul* in Amharic, is a perennial herb that grows in woodland and dry grassland between 1450 and 2250 m in various parts of Ethiopia such as Arsi, Sidamo, Bale and Harerge floristic regions. The species is also occurring in other African countries such as Djibouti, Somalia, Kenya and

*Corresponding author. E-mail: yadessamelaku2010@gmail.com

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Tanzania. The plant has been utilized for the treatment of ailments such as skin infections, upper respiratory tracts, gonorrhoea, dysentery, meningitis and malaria [4]. The use of the plant as a relief of rheumatic pains, headaches and hemorrhoids is documented [5]. The roots of *G. candidus* is traditionally been used for the treatment of several diseases including infectious diseases and cancer in Ethiopia

Crinum abyssinicum (Amaryllidaceae), locally named as *Kulubi Warabessa* in Afan Oromo (Ethiopia), is herbaceous plant with large truncated bulbs which may produce a neck or a pseudo stem made up of sheathing bases of the old leaves. The bulbs of the plant have been considerably used in folk medicine for the treatment of a wide array of diseases including antitumor, anti-coughs, immune stimulating, analgesic, antiviral, anti-malarial, antibacterial and antifungal activities [6]. In Ethiopia, *C. abyssinicum* is used for treatment of hypertension [7], hepatitis B and skin infection [4] in addition to its use to expel internal parasites from animals [8]. Furthermore, the plant is traditionally been used for the treatment of cancer, asthma, colds and malaria [4].

Ranunculus multifidus (Ranunculaceae) is an erect herb known with a vernacular name “*Etsesio*” in Amharic, Ethiopia. The plant is commonly used for the treatment of anti-rheumatics and rubefacient. People use the plant to get relieve from intermittent fever. It is also used as a remedy for anti-hemorrhagic, anti-spasmodic [9], anthelmintic and malaria. The leaves of *R. multifidus* are used to treat chronic skin disorders, hemorrhoids and ascariasis.

Artemisia abyssinica (Asteraceae) is a short-lived perennial plant distributed in open grassland, roadside and juniperus forest and also cultivated in Tigray, Gonder, Gojam, Wello, Shewa, Welega, Ilubababor, Bale and Harerge floristic region between an altitude of 1,800 and 3,500 m. It is used in folk medicine as an anthelmintic, antispasmodic, antirheumatic and antibacterial agent. The leaves of *A. abyssinica* is boiled in water and used as a remedy for heart troubles and as cough cure [10]. The use of the plant for the treatment of rabies, tonsillitis, gonorrhoea, cough and syphilis was also reported [11]. The fresh roots in the form of juice are also employed for the treatment of epilepsy in domestic animal [12].

G. candidus, *C. abyssinicum*, *R. multifidus* and *A. abyssinica* are plants that possess essential oils which play a vital role in the protection of plants against various biotic factors in addition to their use against various life-threatening diseases [13]. These essential oils have been extensively used for bactericidal, fungicidal, anti-parasitic and cosmetic applications [14]. Despite their rich and complex composition, the use of essential oils remains limited to the cosmetics and perfumery domains. Hence, it is worthy to develop a better understanding of the chemistry of essential oils of *G. candidus*, *C. abyssinicum*, *R. multifidus* and *A. abyssinica* and the biological properties of extracts and constituents for their valuable applications in treating bacterial infections. Essential oils could be exploited as effective complements to synthetic compounds of the chemical industry, without inducing the same secondary effects [15]. In this regard, the medicinal flora of Ethiopia still remains virtually unexplored from the point of view of their essential oil constituents and biological activities [16]. Therefore, this paper presents the chemical constituent of the essential oils and antibacterial activities of *G. candidus*, *C. abyssinicum*, *R. multifidus* and *A. abyssinica* selected from flora of Ethiopia.

EXPERIMENTAL

Plant materials

The plant materials such as *G. candidus*, *R. multifidus*, *A. abyssinica* and *C. abyssinicum* were collected from Tiyo Woreda, Arsi zone [7°57'N 39°7'E/7.95°N 39.117°E/7.95] [17] south eastern Ethiopia with an elevation of 2430 m on July 2020. The specimen was authenticated by a botanist and deposited in the National Herbarium, Addis Ababa University with voucher specimen number of G-001, G-002, G-003 and G-004 for *G. candidus*, *R. multifidus*, *A. abyssinica* and *C. abyssinicum*, respectively.

Extraction of essential oil

For the extraction of essential oils from the plant materials by hydro-distillation under optimal operating conditions, a quantity of 50 g of each plant part was added to 400 mL of distilled water in a 1 L flask using Clevenger type apparatus for 3 h. The essential oils were extracted from the distillate using chloroform, dried over anhydrous sodium sulfate, filtered and kept in a glass vial in the fridge at a temperature of 4 °C till analysis.

Yield of essential oils

The yields of essential oil of the *G. candidus*, *R. multifidus*, *A. abyssinica* and *C. abyscincicum* were expressed in g relative to 100 g of dry plant material and it was calculated according to the following equation:

$$\text{Yield (\%)} = \frac{\text{Amount of extracted oil (g)}}{\text{Amount of dry plant material}} \times 100\%$$

Chromatographic analysis of the essential oils

The essential oils obtained from various parts of the plant materials were analyzed by GC-MS at Adama Science and Technology University using an Agilent 6870. GC with Agilent 5977 mass selective detector [MSD operated in the EI mode (70 eV). Scan range 40-400 amu and scan rate 3.99 scan/s and an Agilent Chem. Station data system. The GC column was HP-5 Fused silica capillary with a (5% phenyl-poly methyl siloxane stationary phase film thickness of 0.25 µm a length of 30 m and internal diameter of 0.25 mM). The carrier gas was helium with a column head pressure of 48.7 kPa and a flow rate of 1.0 mL/min. The inlet temperature was 20 °C and interface temperature was 280 °C. The GC oven temperature was used as follows 60 °C initial temperature hold for 10 min. increased at 3 °C/min to 180 °C hold for 6 min. increased 5 °C/min to 220 °C. A 1% w/v solution of the sample in hexane was prepared and 1 µL was injected using a splitter of 1:20.

Identification of compounds

The retention indices (RIs) for all of the essential oils were determined by co-injection of the sample with a mixture of the homologous series of C₈-C₂₅ n-alkanes. Identification of components was based on comparison of their mass spectra (MS) with those of NIST MS search 2.0 and Wiley 275 libraries and with those described by Adams [18].

Antibacterial activity

Clinical bacterial strains with American standards including *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), and *Staphylococcus pyogenes* (ATCC 19615) were used to evaluate the antibacterial activity of the essential oils. The standard bacterial cultures were obtained from Oromia Regional Laboratory and Quality Control, Adama, Ethiopia. McFarland number 0.5 standard was prepared by mixing 9.95 mL 1% H₂SO₄ in distilled water and 0.05 mL 1% BaCl₂ in distilled water in order to estimate bacterial density [19]. The prepared sample was stored in an air tight bottle and used for comparison of bacterial suspension.

The antibacterial susceptibility test was evaluated by the agar disk diffusion method [19]. Essential oil extract of each plant species was prepared in DMSO to give 30, 15, 7.5, and 3.75 µg/mL. Bacteria cell suspensions were adjusted to 0.5 McFarland turbidity standards to prepare

1×10^8 bacterial/mL inoculum. Each bacterial suspension was inoculated on Mueller-Hinton agar plates, and the plates were allowed to dry for 5 min. The sterile filter paper disks were soaked in 30, 15, 7.5, and 3.75 $\mu\text{g/mL}$ of each essential oil. The extract-soaked filter paper disks were then placed on the inoculated Mueller-Hinton agar plates and the analysis were conducted in triplicate. Ceftriaxone 30 $\mu\text{g/mL}$ disk was used as the positive control and DMSO was used as the negative control. The plates were incubated for 18 h at 35 ± 2 °C. After incubation, the zones of inhibition were recorded as the diameter of the growth free zones measured in mm using antibiotic zone reader.

RESULTS AND DISCUSSION

Essential oil yield

Oil yields expressed in relation to dry weight of plant materials. It is apparent through observing the extraction yields, the essential oil yields differ significantly according to their different organs and the species (Table 1).

Table 1. Botanical names and essential oil yields (%w/w) of selected plants.

Species name	Essential oil (% w/w)				
	Leaves	Stems	Roots/ rhizomes	Aerial	Bulb
<i>G. candidus</i>	0.29	0.28	0.44	-	-
<i>C. abyscincicum</i>	0.32	-	0.08	-	0.32
<i>R. multifidus</i>	-	-	-	0.28	-
<i>A. abyssinica</i>	4.63	0.47	3.23	-	-

The essential oil composition from air dried leaves, stems and rhizomes of *G. candidus* were 0.29, 0.28, 0.44% w/w, respectively. The results showed that the yield obtained from the rhizome of *G. candidus* is greater than from the leaves and stems of the same plant. The essential oil yield of the rhizome of *G. atrovioleaceus*, sister species of *G. candidus*, afford an average yield of 0.033 % [20]. This was smaller compared with the essential oil obtained from rhizome of *G. candidus* (0.44%). The essential oil composition from dried leaves, bulbs and roots of *C. abyscincicum* were 0.32, 0.32 and 0.08% w/w, respectively, which were isolated at the maximum growth stage of the plant. The study also exhibit that the essential oil yield obtained from the aerial part of *R. multifidus* was lesser than the yield displayed by various parts of *G. candidus*, *A. abyssinica* and *C. abyscincicum*. It was also found out that the essential oil yields from different parts of *A. abyssinica* such as the leaves, stems and roots were found to be 4.63, 0.47 and 3.23% w/w, respectively. The results clearly showed that the leaves of *A. abyssinica* were rich in essential oil. Previous work has shown different parts of the same plant species had differences in their essential yields [21]. Same parts of plants growing in different geographical regions were also reported to have different essential oil yields. It is noteworthy that the essential oil yield obtained herein from the leaves of *A. abyssinica* was found to be greater in comparison to the yield obtained by Asfaw *et al.* for the same plant [22].

Essential oil composition of *G. candidus*

The constituents of essential oils isolated by hydrodistillation of the leaves, stems and roots of *G. candidus* were examined by GC-MS and the results are presented in Table 2. A total of 6 compounds were identified from the leaves of *G. candidus* essential oil from Ethiopia, accounting for about 81.21% of the compositions. *G. candidus* leaves essential oils were rich in linalool (2.27%) (1), viridiflorol (7.40%) (4), eudesmol (30.06%) (6), hexadecanoic acid (14.30%) (15), 1-naphthalenepropanol (19.52%) (20) and ethyl linoleate (7.67%) (27). A total of 4 compounds

were identified from the stem of *G. candidus* essential oils, accounting for about 94.55% of the essential oil compositions. Essential oils extract from stem of *G. candidus* were rich in linalool (6.73%) (**1**), 1-naphthalenepropanol (**20**) and ethyl linoleate (31.20%) (**27**). GC-MS analysis of the essential oil from rhizome of *G. candidus* revealed a total of 21 compounds with the major constituents of found to be oleic acid (20.3 %) (**26**), abietadiene (2.94 %) and heneicosane (5.19 %) (**7**). It was evident from Table 1 that, linalool, viridiflorol and 1-naphthalenepropanol were the only compounds detected in the leaves, stems and rhizome essential oils of *G. candidus*.

Table 2. Essential oil composition of leaves, stems, and roots of *G. candidus*.

S. No.	Name of Compound	RI	% Area		
			L	S	R
1	Linalool	1087	2.27	6.73	0.17
2	β -Gurjunene	1432	-	-	0.24
3	Epizonarene	1510	-	-	0.73
4	Viridiflorol	1594	7.40	2.09	0.32
5	Humulene epoxide	1604	-	-	0.56
6	Eudesmol	1617	30.06	-	-
7	Cubenol	1617	-	-	0.18
8	Geranyl tiglate	1700	-	-	0.23
9	Tetradecanoic acid	1789	-	-	0.66
10	cis-9-Hexadecenal	1807	-	-	-
11	Nootkatone	1813	-	-	0.21
12	Hexadecanal	1818	-	-	0.21
13	Hexahydrofarnesyl acetone	1845	-	-	1.40
14	(Z) - hexadec-9-enoic acid	1949	-	-	1.39
15	Hexadecanoic acid	1972	14.30	-	-
16	1-Hexadecanol	1893	-	-	0.25
17	Hexadecanoic acid	1968	-	-	0.21
18	Manoyl oxide	1989	-	-	0.68
19	Manool, 13-epi-	2011	-	-	0.39
20	1-Naphthalenepropanol	2055	19.52	54.53	1.26
21	Abietadiene	2089	-	-	2.94
22	1-Octadecanol	2089	-	-	1.57
23	Methyl linoleate	2089	-	-	-
24	Heneicosane	2101	-	-	5.19
25	Methyl octadecanoate	2126	-	-	2.28
26	Oleic acid	2134	-	-	20.3
27	Ethyl linoleate	2180	7.67	31.20	-

L: leaves; S: stems; R: rhizomes; RI: retention index. Results expressed as % area. Those compounds with % area beyond 0.1% were excluded in this report.

The principal component of essential oil from the leaves of *G. candidus* was eudesmol (**6**) (30.06%) (Table 2). This compound is reported to have various pharmacological activities such as anti-tumor, antibacterial and anti-angiogenic [23]. The antitumor property is believed to be by inhibiting angiogenesis by suppressing CREB activation of the growth factor signaling pathway [23]. Therefore, the presence of this compound as a major constituent in the leaves could be the case for the use of this plant as antibacterial and antitumor agent. The other principal component in the essential oil of *G. candidus* is linalool (**1**) (2.27%) which has been reported to exhibit immense biological activities such as antimicrobial, anti-inflammatory, anticancer, anti-oxidant properties [24].

Table 3. Essential oil composition of leaves, barks and roots of *C. abyscincicum*.

Compounds	RI	% Area		
		L	B	R
(Z)-3-Hexenyl acetate	1006	-	3.37	-
Decane	1007	0.82	-	-
D-Limonene	1023	-	-	0.33
Cis-decahydronaphthalene	1099	0.69	1.94	2.60
Cis- <i>p</i> -Menth-2-en-1-ol	1123	-	4.02	3.72
Trans- <i>p</i> -Mentha-2,8-dien-1-ol	1127	-	4.64	-
Alloocimene	1127	0.82	55.49	4.08
2-Furfuryl-5-methylfuran	1157	-	0.39	0.23
Isomenthone	1157	-	-	0.51
Octanoic acid	1182	-	1.20	-
α -Terpineol	1182	3.57	-	-
Thymyl Methyl Ether	1199	0.12	0.55	0.44
1-Decanol	1272	-	-	0.76
Nerol	1272	0.18	-	-
Geranial	1272	-	1.00	-
Citronellylformate	1276	-	-	0.33
Undecan-2-one	1289	-	0.28	-
<i>p</i> -Cymen-7-ol	1289	-	-	0.20
Undecan-2-one	1299	0.39	-	-
Citronellic acid	1301	0.10	-	-
Guaiacol, <i>p</i> -vinyl-	1317	-	-	0.19
Carvyl acetate, trans-	1333	0.28	-	-
Ethyl decanoate	1398	-	-	0.27
14-Methyl Pentane Decanoic Acid Methyl ester	1431	0.77	-	-
Methyl Pentane Decanoic Acid	1431	-	1.35	-
β -Gurjunene	1432	-	-	2.32
α -Humulene	1448	-	-	0.45
Ethyl Cinnamate	1461	0.18	-	-
Tridecan-1-ol	1578	0.18	-	-
Hexadecanoic Acid	1594	0.24	-	-
Hexadecane	1604	1.10	2.12	2.59
Eudesmol	1617	0.40	-	-
Caryophylla-12,13-dien-5-ol	1639	0.12	-	-
Heptadecane	1693	0.21	-	-
cis-9-Hexadecenal	1807	0.35	-	-
Nootkatone	1813	0.21	-	-
Hexahydrofarnesyl Acetone	1845	1.14	1.25	1.92
Pentadecanoic acid	1865	4.71	-	-
Oxacycloheptadec-8-en-2-one	1924	0.43	-	-
Isophytol	1949	1.68	-	-
Hexadecanoic acid	1972	-	1.74	-
1-Hexadecanol	1893	3.86	-	-
Ethyl hexadecanoate	1994	5.75	-	-
Eicosane	2008	0.45	-	-
Octadecanal	2022	0.44	-	-
1-Naphthalenepropanol	2055	2.80	7.32	2.12
Abietadiene	2089	1.35	-	-
Methyl linoleate	2089	-	-	4.72
Octadecanoic acid	2174	0.21	-	-
Ethyl linoleate	2180	1.10	-	-

L: leaves; R: root; B: bulb; RI = retention index and results expressed as % area. Those compounds with % area beyond 0.1% were included in this report.

The essential oil of the stem of *G. candidus* furnished viridiflorol (**4**) (2.09%) as one of the principal constituents. Viridiflorol is a sesquiterpenoid previously reported have antibacterial activity against *Mycobacterium tuberculosis* [25]. Hexadecanoic acid (**15**) possesses some biological activities such as antioxidant, hypocholesterolemia, nematocide and pesticide [21]. Ethyl linoleate (**6**) is used in many cosmetics for its antibacterial and anti-inflammatory properties [25] and is reported to accelerate healing of wounds and clinically proven to be an effective anti-acne agent [26]. Ethyl linoleate was also reported as inhibitor of melanogenesis by inhibiting tyrosinase promoter activity [27]. The presence of eudesmol, linalool, viridiflorol and ethyl linoleate suggest the use of the essential oil of this plant as a potential candidate against disease causing microorganisms and cancer.

Essential oil composition of *C. abyssinicum*

The essential oils of the leaves, bulbs and roots of *C. abyssinicum* were analyzed using GC-MS and the findings are depicted in Table 3. The essential oil obtained from *C. abyssinicum* was colorless with a flowery odor. A total of 31 compounds were identified from essential oil extract of the leaves of *C. abyssinicum* using GC-MS. Essential oil from the leaves of *C. abyssinicum* were rich in ethyl hexadecanoate (5.75%), pentadecanoic acid (4.71%), 1-hexadecanol (3.86%), and α -terpineol (3.57%). On the other hand, a total of 15 compounds were identified from bulb *C. abyssinicum* essential oils, accounting for about 92.65% of the essential oil composition. The major constituents of the essential oil of the bulb were found to *p*-menth-2-en-1-ol, *cis*-(4.02%), *p*-mentha-2,8-dien-1-ol (4.64%), alloocimene (55.49%) and 1-naphthalenepropanol (7.32%).

A total of 18 compounds were identified in the essential oil of the root of *C. abyssinicum*. Essential oils from roots of *C. abyssinicum* were rich in *p*-menth-2-en-1-ol, *cis*-(3.72%), alloocimene (4.08%), and methyl linoleate (4.72%). *cis-p*-menth-2-en-1-ol (3.72%), reported herein as one of principal components from the bulbs of *C. abyssinicum*, have some established biological activities including antifungal, insecticidal, larvicidal, anti-inflammatory and antibacterial [28]. Ocimene, the most common monoterpenes found in nature, had anticonvulsant, antifungal, antitumor, and pest resistance properties [29]. Pentadecanoic acid, 14-methyl ester belonging to fatty acid has antibacterial and antifungal activity [30]. In addition, α -terpineol attracts a great interest as it has a wide array of biological applications including an antioxidant, anticancer, anticonvulsant, antiulcer, antihypertensive, antibacterial and anti-nociceptive compound [31]. Therefore, the presence of these compounds in various parts of *C. abyssinicum* demonstrates the potential use of this plant against many life-threatening diseases.

Essential oil composition of aerial part of *R. multifidus*

The aerial part of *R. multifidus* was hydrodistilled using Clevenger type apparatus to furnish colorless aromatic oil in 0.28% yield. The essential oil was further analyzed with GC-MS to furnish 11 constituents (Table 4), accounting for about 98.70% of the essential oil compositions. Essential oil of the aerial part of *R. multifidus* were rich in *p*-Mentha-2,8-dien-1-ol (38.82%) (**14**), octanal (3.82%) (**17**), nonanal (9.50%) (**18**), protoanemonin (8.59%) (**19**), pentadecanal (14.86%) (**20**), 1-naphthalenepropanol (7.48%) (**5**) and spathulenol (7.48%) (**21**). The chemical constituents detected in *R. marginatus*, sister species of *R. multifidus*, from Turkey were different from those detected herein from the aerial part of *R. multifidus* [32]. *p*-Menth-2-en-1-ol found as a major constituent in *R. multifidus* has some established biological activities including antifungal, insecticidal, larvicidal, anti-inflammatory and anti-bacterial [30]. Trans-calamenene and spathulenol were reported to have antibacterial and anti-carcinogenic activities [33]. Therefore, the presences of these compounds in the aerial part of *R. multifidus* support the traditional uses of the plant against various infectious diseases.

Table 4. Essential oil constituents of aerial parts of *R. multifidus*.

Compounds	RI	% Area
p-Mentha-2,8-dien-1-ol	1127	38.82
β -Pinene	1127	0.92
Thymyl Methyl Ether	1199	0.86
Octanal	1298	3.82
1-Octen-3-one	1398	0.96
Nonanal	1432	9.50
Protoanemonin	1604	8.59
Calamenene	1523	4.13
Pentadecanal	2017	14.86
1-Naphthalenepropanol	2055	7.48
Spathulenol	2197	7.48

RI = retention index and results are expressed as % area. Those compounds with % area beyond 0.1% were included in this report.

Essential oil composition of *A. abyssinica*

The GC-MS analysis of essential oils from the leaves, stems and roots of *A. abyssinica* has led the identification of 70 compounds, accounting for about 65.94 % of the essential oil compositions.

Table 5. Essential oil composition of *A. abyssinica*.

Name of Compound	RI	% Area		
		L	S	R
D-Limonene	1023	3.15	-	-
Linalool	1087	13.96	-	5.16
β -Phellandrene	1133	0.66	-	-
1,8-Cineole	1032	1.06	-	-
Camphor	1157	0.88	-	-
Borneol	1168	0.88	-	-
Artemisyl acetate	1171	0.67	-	-
Limonen-4-ol	1177	0.36	-	-
Terpinen-4-ol	1182	10.36	-	-
Alpha Terpineol	1182	0.20	-	-
Naphthalene	1188	0.37	-	-
Piperitol	1204	0.20	-	-
Octanol acetate	1207	0.46	-	-
β -Cyclocitral	1219	0.82	-	-
Bornylformate	1224	0.14	-	-
Cis-Carveol	1228	0.49	-	-
Cis-7,8-p-Menthadien-2-ol	1231	0.27	-	-
Carvacrol, methyl ether	1244	0.23	-	-
Geraniol	1256	0.28	-	-
(E)-Anethole	1265	0.10	-	-
Trans cinnamaldehyde	1272	3.30	-	-
Nerylformate	1285	0.16	-	-
Menthyl acetate	1295	0.23	-	-
Bornyl acetate	1308	0.48	-	-
(Z)-Methyl cinnamate	1317	0.19	-	-
Linalool propanoate	1336	0.21	-	-
α -Terpinyl acetate	1346	0.14	-	-

2-Phenylpropanoate	1355	0.25	-	-
Cyclosativene	1368	0.33	-	-
Decanoic acid	1375	0.30	-	-
(Z)-Ethyl cinnamate	1378	0.11	-	-
(E)-Methyl cinnamate	1385	0.26	-	-
β -Caryophyllene	1390	1.12	-	-
Ethyl decanoate	1395	0.12	-	-
2-Phenylethylbutanoate	1401	3.22	-	-
α -Gurjunene	1408	0.60	-	-
Geranylacetone	1450	1.35	-	-
Geranylpropanoate	1476	-	0.44	-
β -Chamigrene	1478	0.26	-	-
Phenylethyl-3-methylbutanoate	1490	0.21	-	-
Bicyclgermacrene	1494	0.28	-	-
d-Decalactone	1497	0.20	-	-
α -Muurolene	1498	0.10	-	-
α -Bisabolene	1540	0.17	-	-
(E)-Nerolidol	1560	0.55	-	7.74
(Z)-3-Hexenyl benzoate	1570	1.61	-	-
Dendrolasin	1578	0.62	-	-
Globulol	1582	0.49	-	-
Davanone	1594	0.22	-	17.68
Geranylisovalerate	1599	0.10	-	-
Cubanol	1613	0.14	-	-
Eudesmol	1617	0.46	-	-
Cubanol	1636	5.13	-	-
α -Muurolol	1642	0.74	-	-
Selin-11-en-4-ol	1654	0.35	-	-
Caryophyllenol	1659	0.15	-	-
β -Eudesmol	1674	0.14	-	-
α -Bisabolol	1687	0.94	-	-
Eudesm-7(11)-en-4-ol	1693	0.14	-	-
Edusema-3,5,11-triene	1783	1.32	-	-
(2E,6E)-Farnesyl acetate	1845	0.11	-	-
Pentadecanoic acid	1863	1.61	-	-
1-Hexadecanol	1879	0.18	0.10	-
Methyl hexadecanoate	1923	0.11	-	-
2-Heptadecanone	1907	-	0.24	-
(Z)-Hexadec-9-enoic acid	1949	0.13	-	-
1-Naphthalenepropanol	2055	-	1.10	55.06
Manool	2057	0.15	-	-
Ethyl linoleate	2167	-	-	7.09
Octadecanoic acid	2174	-	97.34	-

L = leaves; S = stems; R = roots; RI = retention index and results expressed as % area. Those compounds with %area beyond 0.1% were included in this report.

The major constituents of the essential oil of the leaves of *A. abyssinica* were D-limonene (3.15%) (**22**), linalool (13.96%) (**1**), 2-phenylethylbutanoate (3.22%) (**23**), and terpinen-4-ol (10.36%) (**24**). On the other hand, a total of 5 compounds were identified in the stems of *A. abyssinica* essential oils extract with the principal component found to be octadecanoic acid (97.34%) (**25**). Likewise, the essential oil of the root comprised of 5 compounds including linalool (5.16%) (**2**), nerolidol, (E)-(7.74%) (**26**), 1-naphthalenepropanol, a-ethenyldecahydro-a,5,5,8a-tetramethyl-2-methylene-, (aR,1S,4aS,8aS)- (55.06%) (**5**), davanone (17.68%) (**27**), cubanol (**28**),

ethyl linoleate (7.09%) (**29**) and octadecanoic acid (**25**). It was found that the essential oil of the leaves and roots had linalool in common as a major constituent with its yield lower in the latter.

The essential oils composition of *A. abyssinica* reported by Nigist *et al.* [22] from same plant collected from different area of Ethiopia were rich in 4-hydroxycyclohexanemethanol (21.3%), α -terpinolene (9.2%), yomogialcohol(38.5%), artemisylacetate (24.9%), and artemisiaalcohol (6.7%) [34] and 4,5-dihydroxyocta-3,5- diene-2,7-dione (55.0%) [35]. As clearly seen, there is a slight difference in the essential oil composition of *A. abyssinica* reported herein compared with the composition of essential oils reported for the same plant by Nigist *et al.* [22]. The variation might be due to geographical location or variation due to environmental conditions [35].

Linalool reported herein as the principal component was reported to have several biological activities such as antibacterial and antiplasmodial effect [22]. Limonene, which is the main ingredient of lemon essential oil, was found to have antimicrobial activities against *L. monocytogenes* in minced beef meat [36]. Nerolidol known to have some established biological activities including antioxidant, antifungal, anticancer, and antimicrobial activities [37]. Davanone also shows good antibacterial and anticytotoxicity against selected microorganisms. Terpinen-4-ol significantly enhances the effect of several chemotherapeutic and biological agents. The possible mechanism of the activity of terpinen-4-ol involves induction of cell death rendering this compound a potential anti-cancer drug alone and in combination in the treatment of numerous malignancies [38].

Antibacterial activity of essential oil extract

The *in vitro* antibacterial activities of the essential oils of the various parts of *G. candidus*, *R. multifidus*, *A. abyssinica* and *C. abyssinicum* were evaluated by the disc diffusion method using Mueller-Hinton agar against four bacterial strains including *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes* with the results given in Table 6.

The essential oils from the leaves, stems and roots of *A. abyssinica* were assessed for their antibacterial activity. Among these, the essential oil displayed by the roots was significant compared with the activity shown by the leaves and stems. The essential oil from root of *A. abyssinica* displayed inhibition zone of about 13.5 ± 0.3 mm against *E. coli* at a concentration of $30 \mu\text{g/mL}$ (Table 6). The result is significant compared with ceftriaxone (15.1 ± 0.1 mm at $30 \mu\text{g/mL}$). The essential oil of the root also displayed antibacterial activity with inhibition zone of 11.1 ± 0.8 and 11.5 ± 0.8 mm against *P. aeruginosa* and *S. pyogenes*, respectively. The activity demonstrated by the essential oil of the roots of *A. abyssinica* could probably be due to the presence nerolidol and davanone as the principal components. In fact, the activity of these compounds against bacteria has been reported previously [37].

The essential oil from leaves and roots of *C. abyssinicum* were shown to have antibacterial activity with inhibition zone of 17.9 ± 1.1 and 11.4 ± 0.7 mm at $30 \mu\text{g/mL}$, respectively against *E. coli*. The activity displayed by the essential oil of the leaves has an inhibition zone of 17.9 ± 1.1 and 15.6 ± 1.1 mm at $30 \mu\text{g/mL}$ against *E. coli* and *P. aeruginosa*, respectively. The results are comparable to the activity shown by ceftriaxone with inhibition zone of about 15.1 ± 0.1 and 16.2 ± 0.8 mm, respectively, at the same concentration. α -Terpineol was reported to have antibacterial activity in addition to its use as anticancer, antiulcer and antihypertensive properties [31]. Indeed, the presence of α -terpineol as one of the principal components of the essential oils might be responsible for the antibacterial activity of the essential oil of the leaves of *C. abyssinicum*.

The essential oils of the leaves, stems, and rhizomes of *G. candidus* were evaluated for their antibacterial activity (Table 6). The essential oil of the leaves displayed activity with inhibition zone of 15.1 ± 0.3 , 13.9 ± 0.9 , 16.7 ± 0.9 and 10.8 ± 1.1 mm at $30 \mu\text{g/mL}$ against *E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes*, respectively. Ceftriaxone displayed zone of inhibition of 15.1 ± 0.1 , 16.2 ± 0.8 , 14.3 ± 0.9 and 16.1 ± 2.5 against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S.*

pyogens, respectively, at 30 µg/mL. The essential oil was shown to have broad spectrum antibacterial activity. Furthermore, the activity displayed by the essential oil of the leaves is superior to the essential oil from the stem and rhizomes but comparable with the positive control. The major constituent of the leaves essential oil was found to be eudesmol (6). This compound was reported to have antibacterial activity in addition to its use against tumor and angiogenic [23]. Hence, the antibacterial activity displayed by the leaves essential oil might to be due to eudesmol (6).

Table 6. Antibacterial activity of essential oils from various plant parts against selected pathogenic microorganisms.

Plant part	Concentration (µg/mL)	Zone of inhibition (mm)			
		<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. pyogenes</i>
<i>A. abyssinica</i> leaves	30	6.9 ± 0.3	8.5 ± 0.8	6.4 ± 0.2	7.0 ± 0.2
	15	6.5 ± 0.3	6.3 ± 0.2	6.4 ± 0.2	6.9 ± 0.3
	7.5	6.5 ± 0.2	6.5 ± 0.4	6.3 ± 0.2	6.8 ± 0.3
	3.25	6.3 ± 0.3	6.3 ± 0.1	6.2 ± 0.1	± 0.2
<i>A. abyssinica</i> stem	30	7.2 ± 0.6	12.4 ± 0.9	6.5 ± 0.2	8.8 ± 0.7
	15	6.6 ± 0.6	6.8 ± 0.9	6.6 ± 0.1	7.0 ± 0.7
	7.5	6.7 ± 0.2	6.7 ± 0.5	6.3 ± 0.1	6.8 ± 0.9
	3.25	6.4 ± 0.4	6.2 ± 0.1	6.2 ± 0.1	± 0.2
<i>A. abyssinica</i> root	30	13.5 ± 0.3	11.1 ± 0.8	7.1 ± 0.5	11.5 ± 0.8
	15	12.6 ± 0.3	8.3 ± 0.5	6.9 ± 0.3	7.4 ± 0.6
	7.5	9.9 ± 0.9	7.3 ± 0.4	6.7 ± 0.1	6.9 ± 0.5
	3.25	6.7 ± 0.3	6.5 ± 0.2	6.1 ± 0.2	6.2 ± 0.3
<i>G. candidus</i> leaves	30	15.1 ± 0.3	13.9 ± 0.9	16.7 ± 0.9	10.8 ± 1.1
	15	14.1 ± 0.1	10.9 ± 0.9	13.6 ± 0.7	8.0 ± 0.8
	7.5	10.2 ± 0.4	10.0 ± 0.9	9.3 ± 0.7	7.4 ± 0.8
	3.25	8.1 ± 1.0	8.3 ± 0.4	8.1 ± 0.6	6.1 ± 0.2
<i>G. candidus</i> stem	30	7.7 ± 0.6	6.5 ± 0.8	12.2 ± 0.8	8.9 ± 0.9
	15	7.6 ± 0.4	7.0 ± 0.6	9.2 ± 0.4	7.2 ± 0.6
	7.5	6.8 ± 0.8	6.4 ± 0.4	6.7 ± 0.4	7.0 ± 0.3
	3.25	6.6 ± 0.3	7.0 ± 0.9	6.6 ± 0.4	6.1 ± 0.1
<i>G. candidus</i> rhizome	30	6.8 ± 0.3	8.7 ± 0.8	6.5 ± 0.4	7.9 ± 1.0
	15	7.6 ± 0.8	7.3 ± 0.4	6.4 ± 0.1	7.5 ± 0.6
	7.5	7.5 ± 0.9	7.2 ± 0.6	6.5 ± 0.4	6.9 ± 0.3
	3.25	6.2 ± 0.3	7.4 ± 0.6	6.9 ± 0.5	6.5 ± 0.1
<i>C. abyssinicum</i> leaves	30	17.9 ± 1.1	15.6 ± 1.1	6.9 ± 0.3	10.4 ± 0.7
	15	13.3 ± 1.1	11.6 ± 0.7	7.1 ± 0.9	8.5 ± 1.0
	7.5	9.9 ± 0.6	9 ± 0.5	6.6 ± 0.4	7.7 ± 1.2
	3.25	7.1 ± 0.3	7.0 ± 0.2	6.5 ± 0.6	6.7 ± 0.5
<i>C. abyssinicum</i> bulbs	30	7.8 ± 1.1	8.2 ± 0.6	6.9 ± 0.3	6.7 ± 0.2
	15	6.5 ± 0.3	6.6 ± 0.1	6.6 ± 0.3	6.4 ± 0.2
	7.5	6.4 ± 0.3	7.5 ± 0.9	6.6 ± 0.3	6.4 ± 0.3
	3.25	6.2 ± 0.2	6.7 ± 0.5	6.2 ± 0.2	6.2 ± 0.1
<i>C. abyssinicum</i> roots	30	11.4 ± 0.7	9.2 ± 0.5	6.6 ± 0.6	7.5 ± 0.5
	15	10.7 ± 1.0	8.4 ± 0.4	6.5 ± 0.1	8.4 ± 1.0
	7.5	7.0 ± 0.3	7.2 ± 0.8	7.1 ± 0.6	6.5 ± 0.3
	3.25	6.8 ± 0.5	6.6 ± 0.4	6.5 ± 0.4	6.3 ± 0.2
<i>R. multifidus</i> aerial	30	9.8 ± 0.5	10.3 ± 0.9	18.8 ± 0.8	19.4 ± 0.6
	15	9.2 ± 0.8	9.3 ± 0.3	16.3 ± 0.9	18.1 ± 0.5
	7.5	8.6 ± 1.0	8.9 ± 0.3	11.7 ± 0.8	16.9 ± 0.9
	3.25	8.7 ± 1.0	7.9 ± 0.5	7.3 ± 0.9	5.9 ± 0.7
Ceftriaxone	30	15.1 ± 0.1	16.2 ± 0.8	14.3 ± 0.9	16.1 ± 2.5

Experiments were done in triplicates and results are expressed as mean ± SD.

The essential oil from the aerial part of *R. multifidus* displayed inhibition zone of 18.8 ± 0.8 and 19.4 ± 0.6 mm at $30 \mu\text{g/mL}$ against *S. aureus* and *S. pyogenes*, respectively. This indicates that the essential oil of *R. multifidus* is active against Gram positive bacteria. Furthermore, the inhibition zone displayed by the essential oil of *R. multifidus* against *S. aureus* and *S. pyogenes* comparable to the activity displayed by ceftriaxone (14.3 ± 0.9 and 16.1 ± 2.5 mm against *S. aureus* and *S. pyogenes*, respectively, at $30 \mu\text{g/mL}$).

One of the main postulated modes of action of essential oil has an ability to disrupt bacterial membranes intracellular materials, such as genetic materials, proteins, and potassium ions, in the event of membrane disruption [39]. The possible mechanism for its activity involves induction of cell death rendering this compound a potential anti-cancer drug and in combination in the treatment of numerous malignancies [38]. The essential oil showed strong antibacterial activity against *S. pyogenes*, while *E. coli*, and appeared to be resistant [40]. The borneol, α -terpineol, and terpinen-4-ol showed strong antibacterial activity against all tested bacteria [32].

CONCLUSION

The antibacterial activity of the essential oils from aerial parts of *R. multifidus* against *S. aureus* and *S. pyogenes* was found to be significant compared with ceftriaxone. This could probably due to the presence of *p*-mentha-2,8-dien-1-ol as a major constituents in the essential oil of *R. multifidus*. The antibacterial activity displayed by the essential oil of the leaves of *C. abyssinicum* was turned out to be higher than ceftriaxone, a standard drug used as positive control. This might be accounted to the presence α -terpineol in the essential oil of the leaves of *C. abyssinicum*, which is cited in many reports as antibacterial agent. It was also found that the IZ displayed by the essential oils of leaves of *G. candidus* is better than ceftriaxone. Remarkable activity displayed by these essential oils could be due to the presence of certain bioactive compounds such as D-limonene, linalool, eudesmol, alloocimene, α -terpineol, viridiflorol, nerolidol and davanone in the plant species essential oil. The aforementioned findings suggest that the essential oils can be used as natural antibacterial remedies. Furthermore, the results presented herein support the traditional uses of these plants against bacteria.

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