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Phytochemical investigation of saponifiable matter & volatile oils and antibacterial activity of *Moluccella laevis* L., family Lamiaceae (Labiatae)

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Abstract

The current study was aimed to evaluate some parts of *Moluccella laevis viz*., phytochemical and antibacterial. Regarding to GC/MS of the saponifiable matters of petroleum ether fraction of total ethanolic extract of the aerial parts (TEE), the main recognized unsaturated fatty acids were methyl linolenate (25.58%) and methyl linoleate (15.87%). Whereas, the major saturated fatty acids were, palmitic (25.4%) followed by stearic acid (10.48). A comparative analysis of the volatile constituents of *M. laevis* flowers and leaves was performed by Head Space GC/MS. The volatile mixtures of both plant parts displayed comparable amounts of hydrocarbons and oxygenated compounds, with a noticeable greater contribution of the latter in the leaves.Besides, *α*-pinene (40.84%), chrysanthenyl acetate (17.89%) and isobornyl acetate (10.64%), were identified as the major volatile components in the flowers. While, isobornyl acetate (35.09%) was characterized as the major constituent followed by, 2-methyl-4-butanolide (22.12%), 1-heptene oxide (7.47%) and benzoic acid, methyl ester (4.05%) of the volatile oil composition of the leaves. Moreover, this study included the antibacterial activity of TEE and its different fractions against Gram positive and negative bacteria. TEE exhibited MIC values of 326 476, and 541 µg/mL against *E. coli, K. pneumonia* and *P. aeruginosa,* respectively, while the aqueous fraction showed MICs of 410, 633, 748 and 10713 µg/mL against *P. aeruginosa*, *K. pneumonia*, *E. coli* and *S. aureus*, respectively. Finally, the EtOAc fraction displayed MICs 449, 541 and 1085 µg/mL against *K. pneumonia, E. coli* and *P. aeruginosa*, respectively.

Key words

Moluccella laevis, GC/MS, saponifiable matter, volatile oil, antimicrobial activity

1. Introduction

One of the most important families containing volatile oils is Lamiaceae. It was previously called Labiatae, or the mint family. It included 236 genera and more than 7,000 species, therefore, itis considered as one of the largest plant families. Due to ease of cultivation, many plants of Lamiaceae are cultivated for their fragrant characters. They are used in perfume manufacture, food and medicine. They are used in folkloric medicine as antiemetic, anti-inflammatory, antispasmodic, carminative, choleretic, diaphoretic, emmenagogue and antimicrobial agents [1]. One of the most important genera of this family is Moluccella (syn. Lamium). It is native to Europe, Asia and North Africa. It comprises approximately 40 species [2]. *Moluccella laevis* L. is one of the interesting research plants for chemical and biological investigation, due to the presence of very few studies on it. The volatile oil of *M. laevis* was analyzed by capillary gas chromatography coupled to GC/MS. Forty-one components were identified. The major components of the oil were *α*-pinene (20.9%), pinocarvone (27%), methyl chavicol (20%) and *E-β*-caryophyllene (10%) [3]. On the other hand, the previously reported cytotoxic activity showed that *M. laevis* aerial parts and roots had no cytotoxic activity against HT-29 and DLD-1 [4]. This encouraged us to carry out an extensive study of this plant including characterization of the saponifiable matter of the aerial parts, in addition to, performing a comparative study of the volatile oil constituents of the leaves and flowers. Finally, evaluating the antibacterial activities of the aerial parts.

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2. Materials and Methods

2.1. Plant material

The aerial parts and flowers of *M. laevis* L. were taken from the Nursery of Faculty of Agriculture, Minia University in March 2016. It was recognized by Prof. Dr. Nasser Barkat, Department of Botany, Faculty of Science, Minia University. A voucher specimen under registration number (Mn-ph-Cog-35) was kept in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Minia University, Minia, Egypt.

2.2. Preparations of saponifiable and antibacterial samples

The fresh aerial parts of *M. laevis* were air-dried in the shade then reduced to fine powder. The powered plant material (1 kg) was then extracted by maceration in 95% ethanol (3 L, 3x, within one-week interval) and the total ethanolic extract (TEE, 80 g) was concentrated under vacuum to a syrupy consistency. TEE was transferred to a separating funnel and suspended in the least amount of distilled water (50 mL). It was successively fractionated with petroleum ether (pet. ether, 200 mL, 3x) and ethyl acetate (EtOAc, 200 mL, 3x) to afford two fractions: (pet. ether (18 g) and EtOAc (5 g). While, the remaining mother liquor was the aqueous fraction (50 g).

2.2.1. Preparation of the saponifiable matter

2.2.1.1. Preparation of the unsaponifiable matter

A part of the dried pet. ether farcation (2.0 g) was subjected to alkali hydrolysis by refluxing with (50 mL of N/2 ethanolic NaOH) for 8 h on a boiling water bath. The major part of the ethanol was distilled off and the liquid left was diluted with twice its volume distilled water, extracted with several portions of dichloromethane (DCM) until exhaustion. The combined fractions of DCM were washed with NaOH solution (5%), then with distilled water until the washings were free from any alkalinity [5-7]. The DCM residue was dehydrated over anhydrous Na_2SO_4 and then the solvent was distilled off. The obtained brownish residue was (0.6 g).

2.2.1.2. Preparation of the fatty acids

The aqueous alkaline solution (soap), remained after the removal of the unsaponifiable matter was acidified with (10%) H_2SO_4. The liberated fatty acids were extracted with successive portions of DCM. The combined DCM portions were washed with distilled water till the washing was neutral to litmus paper. The solvent was distilled off and saponifiable matter (the residue of the total fatty acids) was dried. It was semi-solid and brown in color [5-7].

2.2.1.3. Preparation of the fatty acid methyl esters

The fatty acids were converted to their methyl esters by refluxing with 50 mL of CH_3OH in presence of 1.5 mL of H_2SO_4 acid for 2 h on a boiling water bath. The major part of the CH_3OH was distilled off and the liquid remained was diluted with twice its volume of distilled water, extracted with several portions of DCM until exhaustion. The collective DCM portions were washed with distilled water till the washing became neutral to litmus paper. The DCM was distilled off and the remaining residue representing the fatty acids methyl esters, was dried over anhydrous Na_2SO_4. It formed a semi-solid brownish yellow residue and was reserved for further investigation [5-7].

2.3. Analysis of the volatile constituents of leaves and flowers

A small piece (about one square centimeter) from the fresh flowers or leaves of *M. laevis* (each separately in a special vial) was left for a certain period of time and then the overhead gas was used directly for GC/MS analysis of their volatile constituents using the Head Space technique [8].

2.4. GC/MS analysis of fatty acid methyl esters and the volatile oils

The column was a capillary column (30 m length x 0.25 mm ID), packed with Rtx-5MS (diphenyl dimethyl polysiloxane). The injected volume was 1 µL. The analysis was carried out at a programmed temperature. The initial temperature was 70 °C, then increased at a rate of 3 °C/min and final temperature 220 °C (kept for 5 min) using a flame ionization detector. The injector and detector temperature were 240 °C. The total run time was 45 min for saponifiable matter and 60 min for volatile oils. Helium was used as the carrier gas with a flow rate of 0.8 mL/min. EI (Electron impact) mode of ionization with a mass range of *m/z* 40-500 was applied.

2.5. Antibacterial activity

2.5.1. Bacterial strains

All the bacterial strains used in the study were clinical isolates, obtained from Microbiology Department, Faculty of Pharmacy, Minia University. Bacterial strains were cultured on Müller Hinton agar. *Staphylococcus aureus* (*S. aureus*) [Gram positive, facultative anaerobic bacteria], *Escherichia coli* (*E. coli*) and *Klebsiella pneumonia* (*K. pneumonia*) [Gram negative, facultative anaerobic bacteria] and *Pseudomonas aeruginosa* [Gram negative, facultative aerobic bacteria] were used in the current study.

2.5.2. Preparation of samples

TEE and its fractions of *M. laevis* aerial parts were weighed and dissolved in DMSO to obtain the desired concentrations.

2.5.3. Screening of the antibacterial activity using agar-well diffusion technique

The cultures were adjusted to 0.5 mL of 1×10^6 CFU/mL (0.5 McFarland turbidity). Sterile, molten and cooled 20 mL of the Nutrient agar media were added to the petri dishes, then rotated slowly and allowed to solidify on a flat surface. The media were then incubated with the microorganisms using a sterile swab to evenly distribute bacteria over the appropriate medium. The plates were allowed to dry for 15 min before use then four equidistant and circular wells of 10 mm diameter were carefully punched into the agar medium using a sterile cork borer [9,10]. The wells were then filled with 100 µL of tested samples in addition to the standard drug, after that the plates were allowed to stand for one h to allow the prediffussion, then they were incubated overnight at 37 °C. The antibacterial activity was evaluated by measuring the zone of inhibition against the tested organisms [11].

2.5.4. Determination of the MIC

Two-fold serial dilutions were performed on TEE and its fractions. The initial concentrations of the extract and fractions were 10 mg/mL. Equal volumes of the TEE and fractions were applied separately to each well using a micropipette [12]. All plates were incubated overnight at 37 °C, then the plates were collected and the developed inhibition zones were measured. The MIC for well diffusion method was calculated by plotting the natural logarithm of the concentration of each dilution of the tested sample against the square of inhibition zones.

3. Results and discussion

3.1. GC/MS analysis of fatty acid methyl esters

Identification of the compounds was confirmed by comparing their fragmentation pattern (mass spectra) with those of the reference compounds [13] in addition to matching them with the database of reference compounds [13] in addition to matching them with the database of the National Institute Standard and Technology [14,15]. The quantitation was based on peak area integration. The results are illustrated in Figure 1 and listed in Table 1.

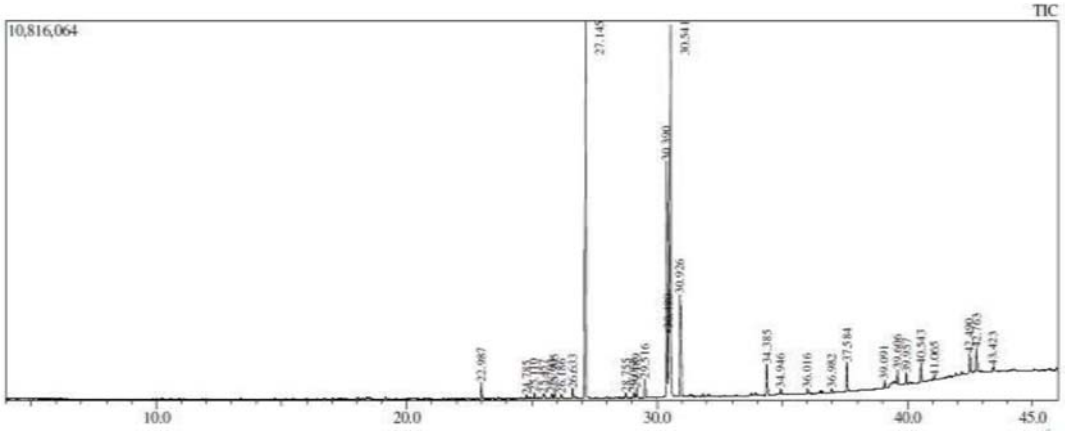

Figure 1: GLC chromatogram of fatty acid methyl esters of pet. ether fraction of TEE of *M. laevis* aerial parts.

Table 1: Identification of fatty acids as methyl esters.

No.	Compound name	M. formula	M. weight	Rt (min)	RRt	Area (%)	Base peak	Characteristic peaks
1	Myristic acid, methyl ester	$C_{15}H_{30}O_2$	242	22.99	0.75	0.99	74.05	199(11.76%),143(17%),87(64.7%), 74(100%),41(26.47%)
2	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256	25.11	0.82	0.32	74.05	213(11.76%),143(14.7%),87(67.64%), 74(100%),41(26.47%)
3	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	270	27.14	0.88	25.04	74.05	87(82.35%),74.05(100%),57(23.53%), 43(29.4%),41(20.59%)
4	Margaric acid, methyl ester	$C_{18}H_{36}O_2$	284	29.0	0.95	0.34	74.05	87(82.35%),74(100%),57(23.53%), 43(23.53%),41(14.7%)
5	Linoleic acid, methyl ester	$C_{19}H_{34}O_2$	294	30.39	0.99	15.87	67.05	95(61.76%),(85.29%),67(100%), 55(47.05%),41(44.12%)
6	Oleic acid, methyl ester	$C_{19}H_{36}O_2$	296	30.48	0.99	3.85	55.01	97(55.88%),69(76.47%),55(100%), 41(58.42%)
7	Linolenic acid, methyl ester	$C_{19}H_{32}O_2$	292	30.54	1.00	25.58	79.01	95(48.53%),79(100%),67(58.82%), 55(41.18%),41(44.12%)
8	Stearic acid, methyl ester	$C_{19}H_{38}O_2$	298	30.92	1.01	10.48	74.01	87(67.65%),74(100%),57(20.59%), 43(44.12%),41(35.29%)
9	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326	34.38	1.13	2.12	74.05	326(29.41%),143(20.59%), 87(64.71%),74(100%),43(38.24%)
10	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	340	36.01	1.18	0.27	74.05	143(20.59%),87(67.65%), 74(100%),57(20.59%),43(35.29%)
11	Behenic acid, methyl ester	$C_{23}H_{46}O_2$	354	37.58	1.23	1.95	74.05	354(32.35%),143(20.58%), 87(64.70%),74(100%),43(41.17%)
12	Tricosanoic acid, methyl ester	$C_{24}H_{48}O_2$	368	39.09	1.28	0.52	74.05	143(23.53%),87(70.58%), 74(100%),57(26.47%),43(55.58%)
13	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382	40.54	1.32	1.26	74.05	382(38.24%),143(23.53%), 87(61.76%),74(100%),55(29.41%)
14	Cerotic acid, methyl ester	$C_{27}H_{54}O_2$	410	43.42	1.42	0.38	74.05	143(20.59%),87(70.59%), 74(100%),43(70.59%),41(35.29%)
	Total identified fatty acids (88.97%)	***Unsaturated (45.3%)***		Mono (3.85%)				
				Poly (41.45%)				
		Saturated (43.67%)						
	Total unidentified fatty acids (11.03%)							

R_t: Retention time. RRt: Relative Retention time.

GC/MS analysis of the saponifiable matter of *M. laevis* aerial parts, shown in Table 1 and Figure 1, revealed the presence of twenty-nine fatty acids, of which fourteen fatty acids constituting 88.97% were identified, whereas the fifteen fatty acids representing 11.03% could not be identified. Besides, a high percentage of unsaturated fatty acids (45.3%) were observed, On the other hand, linolenic acid, methyl ester (25.58%) and linoleic acid, methyl ester (15.87%) represented the major proportion of the unsaturated fatty acids. While, the saturated acids formed only 43.67% of the saponifiable matter. Palmitic acid (25.04%) was identified as the major saturated fatty acid, followed by stearic acid (10.48%), whereas the remaining saturated fatty acids were detected in minor amounts. Linolenic acid, methyl ester was previously reported in the family Lamiaceae [16], but not previously identified from this genus. It is an essential fatty acid (Omega-3) and had various activities as, anti-inflammatory, neuroprotective and reduction of stroke risk [17]. Moreover, it induces protection against ischemia in spinal injury, preventing necrosis and apoptosis of motor neurons and has antiarrhythmic properties [18]. Likewise, palmitic acid was previously reported in family Lamiaceae [16], but detected for the first time in this genus. It had various biological activities as anti-inflammatory and analgesic [19]. Furthermore, it showed selective cytotoxicity to human leukemic cells, but no cytotoxicity to normal HDF cells. Also, it induced apoptosis in the human leukemic cell line MOLT-4 and showed *in vivo* antitumor activity in mice [20].

3.2. GC/MS of volatile oils

3.2.1. Volatile oil of flowers

Identification of the volatile oils constituents was carried out by direct comparison fragmentation pattern of each of the separated compounds with those of the reference [13]. The quantitation was based on peak area integration. The results are demonstrated in Figure 2 and presented in Table 2.

Head Space GC/MS analysis of the volatiles of *M. laevis* flowers revealed the presence of twenty-six compounds, where twenty-two of them were identified. The oxygenated compounds represented 47.15%, whereas the hydrocarbons were 44.00% (Figure 2 and Table 2).

3.2.2. Volatile oil of leaves

On the other hand, Head Space GC/MS analysis of the volatile constituents of the leaves revealed the presence of twenty-seven compounds, where twenty-five compounds were identified (Figure 3 and Table 3). The oxygenated compounds constituted 91.97%, while the hydrocarbons formed only 5.25%.

Amongst the identified oxygenated volatile compounds in the flowers, different chemical classes were detected, which included esters (33.44%), ketones (2%), aldehydes (9.8%), carboxylic acids (1.22%) and alcohols (0.14%). Besides, α-pinene (40.84%), chrysanthenyl acetate (17.89%) and isobornyl acetate (10.64%) were identified as the major volatile components in the flowers (Table 4).

While, the major detected groups in leaves volatile oil were esters (68.03%), followed by phenolic ethers (8.35%), ketones (7.21%), aldehydes (4.79%), alcohols (1.14%). Where, isobornyl acetate (35.09%) was characterized as the major constituent followed by 2-methyl-4-butanolide (22.12%) (Table 4).

Comparative analysis of the volatile constituents of *M. laevis* flowers and leaves (Tables 4-5). The dominant proportion of the volatile principles identified from flowers and leaves was chiefly occupied by ester (33.44% and 68.03%, respectively).

Moreover, both plant parts showed varying levels of alcohols, aldehydes and ketones. Minor concentrations of carboxylic acids were found only in the volatile content of the flower, while phenolic ethers were found only in the volatile constituents of leaves (Table 4).

Additionally, some compounds were characterized as common constituents in the volatile constituents of both organs, including 2,2,4,6,6-pentamethyl heptane, benzene acetaldehyde, benzoic acid methyl ester, chrysanthenyl acetate, *E*-β-ionone, 1,2-benzene dicarboxylic acid diethyl ester and methyl linoleate (Table 5). It is also worth mentioning that the volatile principles of flowers demonstrated a higher percentage of monoterpenoid compounds (42.24%) in comparison with those of the leaves (1.14%). On the other hand, no sesquiterpenoids could be detected in the volatile principles of either organ. These results are displayed in (Tables 2 and 3).

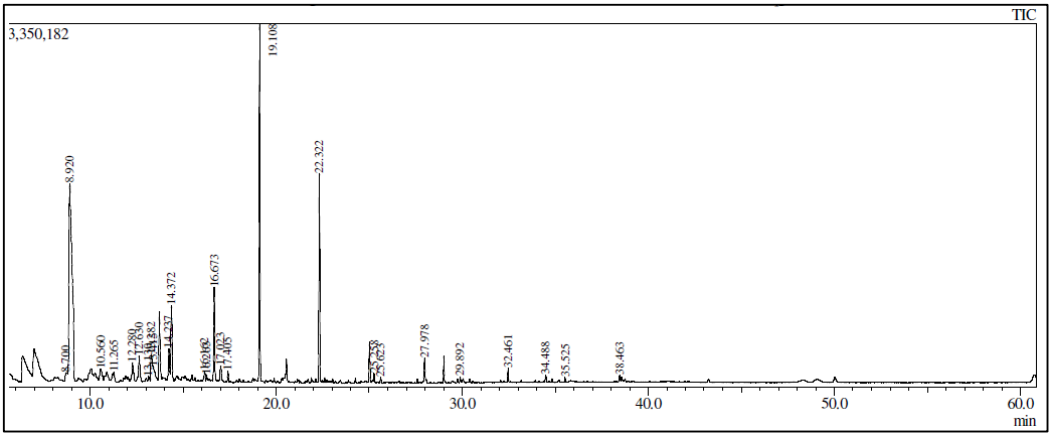

Figure 2: GLC chromatogram of the volatile constituents of *M. laevis* flowers.

Table 2: Volatile constituents of *M. laevis* flowers.

No.	Compound name	M. formula	M. weight	R_t (min)	RR_t^*	Area (%)	Base peak
1	α-Thujene	$C_{10}H_{16}$	136	8.700	0.975	1.16	93.10
2	**α-Pinene**	**$C_{10}H_{16}$**	**136**	**8.920**	**1.000**	**40.84**	**93.10**
3	2,2,4,6,6-Pentamethyl heptane	$C_{12}H_{26}$	170	10.560	1.180	1.48	57.10
4	Octanoic acid	$C_8H_{16}O_2$	144	11.260	1.260	1.22	60.05
5	3-Methoxy phenyl butyrate	$C_{11}H_{14}O_3$	194	12.280	1.380	1.34	124.00
6	Benzene acetaldehydee	C_8H_8o	120	12.630	1.410	1.97	91.05
7	β-Ocimene-x (3,7-Dimethyl-1,3,6-octatriene)	$C_{10}H_{16}$	136	13.130	1.470	0.24	121.10
8	Unidentified	----------	194	13.282	1.490	2.22	71.05
9	*O*-Tolylaldehyde	C_8H_8o	120	13.420	1.500	0.56	91.05
10	Benzoic acid, methyl ester	$C_8H_8O_2$	136	14.240	1.590	1.83	105.10
11	Unidentified	----------	----	14.370	1.600	5.72	86.10
12	6,6-Dimethylbicyclo[3.1.1]-2-heptene-2-ethyl-ol (Nopol)	$C_{11}H_{18}O$	166	16.160	1.810	0.41	95.10
13	2-Acetyl-4-methylpyridine	C_8H_9NO	135	16.260	1.820	0.29	92.05
14	Ethyl benzaldehyde	$C_9H_{10}O$	134	16.670	1.870	6.35	134.10
15	3-Methyl acetophenone	$C_9H_{10}O$	134	17.020	1.900	1.21	119.10
16	*n*-Decanal	$C_{10}H_{20}O$	156	17.400	1.950	0.64	57.10
17	Chrysanthenyl acetate	$C_{12}H_{16}O_2$	192	19.110	2.140	17.89	119.10
18	Isobornyl acetate	$C_{12}H_{20}O_2$	196	22.320	2.500	10.64	95.10
19	*E*-β-Ionone	$C_{13}H_{20}O$	192	25.260	2.800	0.43	177.15
20	Tetradecanal	$C_{14}H_{28}O$	212	25.620	2.870	0.27	57.10
21	1,2-Benzenedicarboxylic acid, diethyl ester	$C_{12}H_{14}O_4$	222	27.980	3.130	1.42	149.10
22	2-Methyl-heptadecane	$C_{18}H_{38}$	254	29.890	3.350	0.26	57.10
23	Unidentified	----------	----	32.460	3.640	0.68	73.05
24	2,2-Dimethoxy-2-phenylacetophenone	$C_{16}H_{16}O_3$	256	34.490	3.866	0.36	151.10
25	Unidentified	----------	----	35.520	3.980	0.23	73.05
26	Methyl linoleate	$C_{19}H_{34}O_2$	294	38.460	4.300	0.32	67.05
	Total identified compounds (91.15%)	***Oxygenated*** (47.15 %)					
		Hydrocarbons (44.00 %)					
	Total unidentified compounds (8.85%)						

R_t: Retention time. RRt: Relative Retention time.

Table 3: Volatile constituents of *M. laevis* leaves.

No.	Compound name	M. formula	M. weight	R_t (min)	RR_t^*	Area (%)	Base peak
1	4-Hydroxy-3-methyl butanal	$C_5H_{10}O_2$	102	7.030	0.314	0.56	56.10
2	2-Methyl-4-butanolide	$C_5H_8O_2$	100	9.791	0.438	22.12	41.05
3	Tetrahydro-2-pyranone	$C_5H_8O_2$	100	10.137	0.453	2.07	42.05
4	2,2,4,6,6-Pentamethyl heptane	$C_{12}H_{26}$	170	10.557	0.472	2.20	57.10
5	4-Methyl-5*H*-furan-2-one	$C_5H_6O_2$	98	10.872	0.487	2.49	41.05
6	Heptene 1,2-oxide	$C_7H_{14}O$	114	12.223	0.547	7.47	71.05
7	Benzene acetaldehyde	C_8H_8O	120	12.641	0.566	0.86	91.05
8	Tertiary butylphenyl ether	$C_{10}H_{14}O$	150	13.305	0.595	0.88	94.05
9	2-Acetylpyrrole	C_6H_7NO	109	13.690	0.613	1.78	94.05
10	Benzoic acid, methyl ester	$C_8H_8O_2$	136	14.238	0.637	4.05	105.05
11	Nonanal	$C_9H_{18}O$	142	14.348	0.642	1.63	57.10
12	Endo-borneol	$C_{10}H_{18}O$	154	16.161	0.723	1.14	95.10
13	2,5-Dimethyl benzaldehyde	$C_9H_{10}O$	134	16.689	0.747	1.74	134.10
14	Unidentified	----------	----	16.999	0.761	1.07	97.05
15	Hexahydro-2,5-methano-1*H*-inden-7(4*H*)-one	$C_{10}H_{14}O$	150	17.414	0.779	0.86	57.10
16	3-Ethyl-4-methyl-1*H*-pyrrole-2,5-dione	$C_7H_9NO_2$	139	18.698	0.837	0.67	139.10
17	Chrysanthenyl acetate	$C_{12}H_{16}O_2$	192	19.117	0.856	1.58	119.10
18	Unidentified	----------	----	20.395	0.913	1.18	86.05
19	α-Terpinyl propionate	$C_{13}H_{22}O_2$	210	21.152	0.947	0.60	121.10
20	**Isobornyl acetate**	**$C_{13}H_{20}O_2$**	**208**	**22.329**	**1.000**	**35.09**	**95.10**
21	*E*-β-Ionone	$C_{13}H_{20}O$	192	25.011	1.120	1.20	177.15
22	Dihydroactinidiolide	$C_{11}H_{16}O_2$	180	25.263	1.131	1.64	111.05
23	1,2-Benzenedicarboxylic acid, diethyl ester	$C_{12}H_{14}O_4$	222	26.222	1.192	0.60	149.05
24	Eicosane	$C_{20}H_{42}$	282	27.984	1.253	3.05	57.10
25	2,2-Dimethoxy-2-phenyl acetophenone	$C_{16}H_{16}O_3$	256	29.896	1.338	0.59	151.10
26	Methyl oleate	$C_{19}H_{36}O_2$	296	38.471	1.722	0.80	55.10
27	Methyl linoleate	$C_{19}H_{34}O_2$	294	38.496	1.544	1.55	67.10
	Total identified compounds (97.22%)	***Oxygenated*** (91.97%)					
		Hydrocarbons (5.25%)					
	Total unidentified compounds (2.25%)						

R_t: Retention time. RRt: Relative Retention time.

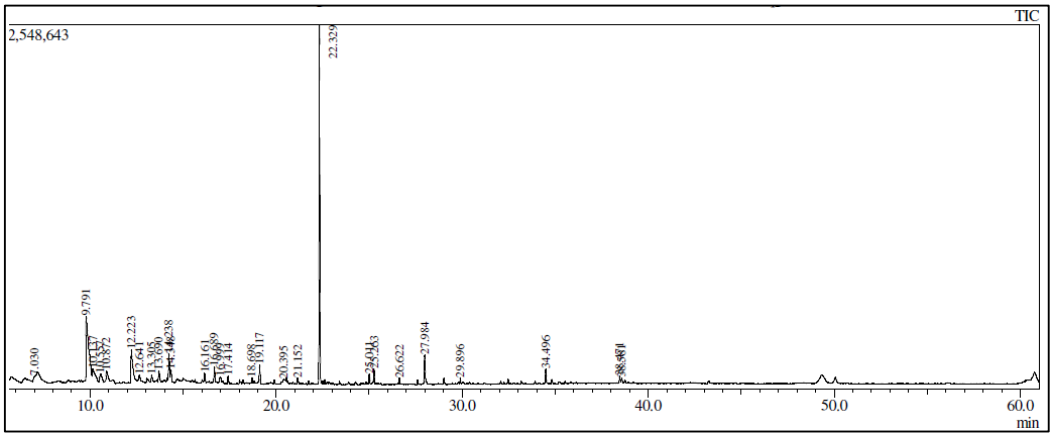

Figure 3: GLC chromatogram of the volatile constituents of *M. laevis* leaves.

Table 4: Different chemical classes identified in the volatile constituents of *M. laevis* flowers and leaves.

No.	Chemical class	Flowers		Leaves	
		No. of compounds	%	No. of compounds	%
1	Hydrocarbons	5	43.98	2	2.79
2	Oxygenated:	17	47.15	23	91.97
	a-Alcohols	1	0.14	1	1.14
	b-Aldehydes	5	9.80	4	4.79
	c-Ketones	3	2.00	5	7.21
	d-Phenolic ethers	----	----	2	8.35
	e-Esters	6	33.44	9	68.03
	f-Carboxylic acids	1	1.22	----	----
	g-Others	1	0.29	2	2.45

Table 5: Relative abundance of the major volatile constituents of *M. laevis* flowers and leaves.

No.	Compound name	Flower (%)	Leaf (%)
1	2,2,4,6,6-Pentamethyl heptane	1.48	2.2
2	Benzene acetaldehyde	1.97	0.86
3	Benzoic acid, methyl ester	1.83	4.05
4	Chrysanthenyl acetate	17.89	1.58
5	*E-β*-Ionone	0.43	1.20
6	1,2-Benzene dicarboxylic acid, diethyl ester	1.42	0.60
7	Methyl linoleate	0.32	1.55
	Total	**25.34**	**12.04**

Comparison of the volatile oil constituents of *M. laevis* flowers with the reported results of the same plant oil [3] using a different GC/MS method of analysis, revealed some differences such as the presence of pinocarvone (27%), methyl chavicol (20%), *α*-pinene (20.9%) and *β*-caryophyllene (10%), while, applying Head Space GC/MS, the percentage of *α*-pinene increased (40.84%) and the presence of different constituents as chrysanthenyl acetate (17.89%), isobornyl acetate (10.64%) and ethyl benzaldehyde (6.35%) appeared [21].

α-Pinene was detected in both *M. laevis* using the two different methods and *M. spinosa* [21], which had various biological activities as antioxidant, antifungal (*C. albicans*), antimalarial [22], antibacterial, antispasmodic and anti-inflammatory [23]. Furthermore, isobornyl acetate was found in the highest concentration in the volatile oil of leaves of *M. laevis*. It was previously reported in family Lamiaceae [24]. It demonstrated antibacterial and antifungal activities [25].

3.3. Evaluation of the antibacterial activity

The continuous use of antibiotics for a long period of time leads to antibiotic resistance, which is considered a serious global problem. Therefore, it is important to search for new sources of antibacterial agents [26]. The medicinal plants represent a rich source of antimicrobial agents as they produce certain active principals that react with microorganisms present in the environment, inhibiting their growth [27,28].

The antibacterial activity of the TEE and its different fractions of *M. laevis* aerial parts were investigated using the agar well diffusion method to determine their inhibition zones and minimum inhibitory concentrations (MICs) compared to standard antibiotics.

The inhibition results recorded in Table 6 showed that the TEE and its different fractions of *M. laevis* aerial parts exhibited moderate inhibitory activity against the tested Gram negative bacterial strains and little or no effect on Gram positive bacteria

growth. The highest inhibition zone was exhibited by TEE against *K. pnuemoniae* (19 mm) followed by aqueous fraction against *P. aeruginosa* (18 mm). Also, the aqueous fraction showed maximum inhibition zone (17 mm) against *E coli* and lastly the pet. ether fraction exhibited the least inhibitory activity against *E. coli*, with inhibition zone of 12 mm.

The antibacterial activity of the TEE and different fractions of *M. laevis* aerial parts may be attributed to the presence of sterols [29] and flavonoids [30,31].

The MICs results revealed that the TEE exhibited the lowest MICs (326 µg/mL) against *E. coli* followed by *K. pneumonia* (476 µg/mL) and finally, against *P. aeruginosa* (541 µg/mL), while the aqueous fraction showed MICs (410, 633, 748 and 10713 µg/mL) against *P. aeruginosa*, *K. pneumonia*, *E. coli* and *S. aureus*, respectively. Finally, the EtOAc fraction showed MICs of 449, 541 and 1085 µg/mL against *K. pneumonia, E. coli* and *P. aeruginosa*, respectively. The results were interpreted according to CLSI as illustrated in Tables 7 and 8.

Table 6: Inhibition zones of the TEE and its different fractions.

Sample	Inhibition zones (mm)/tested microorganism			
	S. aureus	***E. coli***	***K. pneumonia***	***P. aeruginosa***
TEE	NA	16	19	15
Pet. ether fraction	NA	12	NA	NA
EtOAc fraction	NA	16	18	16
Aqueous fraction	15	17	17	18

NA= No activity.

Table 7: MICs of the TEE and its different fractions of *M. laevis* aerial parts.

Sample	MICs (µg/mL)/tested microorganism			
	S. aureus	***E. coli***	***K. pneumonia***	***P. aeruginosa***
TEE	NA	326	476	541
Pet. ether fraction	NA	NA	NA	NA
EtOAc fraction	NA	541	449	1085
Aqueous fraction	10713	748	633	410

NA= No activity.

Table 8: MICs interpretive standards for the tested microorganisms according to CLSI.

Antibiotic	Microorganism								
	S. aureus			***P. aeruginosa***			***E. coli and K. pneumonia***		
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Ampicillin	≤ 0.25	----	≥ 0.5	----	----	----	≤ 8.0	16	≥ 32
Gentamicin	≤ 4.0	8.0	≥ 16	≤ 4.0	8.0	≥ 16	≤ 4.0	0.8	≥ 16
Amikacin	≤ 16	32	≥ 64	≤ 16	32	≥ 64	≤ 16	32	≥ 64
Augmentin	≤ 4.0/2.0	----	≥ 32/16	≤ 64/4.0	----	≥ 128/4.0	≤ 8.0/4.0	16/0.8	≥32/16

S: Susceptible. **I**: Intermdiate. **R**: Resistant.

4. Conclusion

This study demonstrated that the high percentage of unsaturated fatty acids of *M. laevis* saponifiable matters. Also, α-pinene and isobornyl acetate were identified as the major volatile components in the flowers and leaves, respectively. Therefore, further research on this plant is recommended to develop new anti-inflammatory, neuroprotective and stroke risk reducing agents. Furthermore, this plant may be a good source for discovering new antibacterial agents.

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Conflict of interests

The authors declare that there is no conflict of interests regarding these studies.

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