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, it is 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, choleric, 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.

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 Na2SO4 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%) H2SO4. 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 CH3OH in presence of 1.5 mL of H2SO4 acid for 2 h on a boiling water bath. The major part of the CH3OH 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 until 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 Na2SO4. It formed a semi-solid brownish yellow residue and was reserved for further investigation [5-7].

2.2.1.4. Preparation of the volatile oils

The cultures were adjusted to 0.5 mL of 1 × 10⁶ 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 prediffusion, 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. 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 × 10⁶ 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 prediffusion, 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.

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_{12}H_{24}O_{2} | 242       | 22.99    | 0.75| 0.99     | 74.05            | 199(11.76%), 143(17%), 87(64.7%), 74(100%), 43(26.47%)   |
| 2   | Pentadecanoic acid, methyl ester      | C_{15}H_{30}O_{2} | 256       | 25.11    | 0.82| 0.32     | 74.05            | 213(11.76%), 143(14.7%), 87(67.64%), 74(100%), 43(26.47%) |
| 3   | Palmitic acid, methyl ester           | C_{16}H_{32}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_{17}H_{34}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_{18}H_{36}O_{2} | 294       | 30.39    | 0.99| 15.87    | 67.05            | 95(61.76%), 85(29.29%), 67(100%), 55(47.05%), 41(44.12%) |
| 6   | Oleic acid, methyl ester              | C_{19}H_{38}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_{20}H_{40}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_{20}H_{42}O_{2} | 298       | 30.92    | 1.01| 10.48    | 74.01            | 87(67.65%), 74(100%), 57(20.59%), 43(44.12%), 35(29.52%) |
| 9   | Eicosanoic acid, methyl ester         | C_{21}H_{44}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_{46}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_{48}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_{50}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  | Tetrasanoic acid, methyl ester        | C_{25}H_{52}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  | Ceric 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(57.09%), 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%)

Rt: Retention time. RRT: Relative Retention time.

Figure 1: GLC chromatogram of fatty acid methyl esters of pet. ether fraction of TEE of *M. laevis* aerial parts.
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.](image-url)
Table 2: Volatile constituents of *M. laevis* flowers.

| No. | Compound name | M. formula | M. weight | R<sub>t</sub> (min) | RRt | Area (%) | Base peak |
|-----|---------------|------------|-----------|-------------------|-----|----------|-----------|
| 1 | α-Thujene | C<sub>10</sub>H<sub>16</sub> | 136 | 8.700 | 0.975 | 1.16 | 93.10 |
| 2 | α-Pinene | C<sub>10</sub>H<sub>16</sub> | 136 | **8.920** | **1.000** | **40.84** | **93.10** |
| 3 | 2,2,4,6,6-Pentamethyl heptane | C<sub>10</sub>H<sub>22</sub> | 170 | 10.560 | 1.180 | 1.48 | 57.10 |
| 4 | Octanoic acid | C<sub>8</sub>H<sub>16</sub>O | 144 | 11.260 | 1.260 | 1.22 | 60.05 |
| 5 | 3-Methoxy phenyl butyrate | C<sub>10</sub>H<sub>16</sub> | 194 | 12.280 | 1.380 | 1.34 | 124.00 |
| 6 | Benzene acetaldeyde | C<sub>8</sub>H<sub>8</sub>O | 120 | 12.630 | 1.410 | 1.97 | 91.05 |
| 7 | β-Ocimene-x (3,7-Dimethyl-1,3,6-octatriene) | C<sub>10</sub>H<sub>16</sub> | 136 | 13.130 | 1.470 | 0.24 | 121.10 |
| 8 | Unidentified | | 194 | 13.282 | 1.490 | 2.22 | 71.05 |
| 9 | O-Tolylaldehyde | C<sub>8</sub>H<sub>10</sub>O | 120 | 13.420 | 1.500 | 0.56 | 91.05 |
| 10 | Benzoic acid, methyl ester | C<sub>8</sub>H<sub>8</sub> | 136 | 14.240 | 1.590 | 1.83 | 105.10 |
| 11 | Unidentified | | | | | | |
| 12 | 6,6-Dimethylbicyclo[3.1.1]-2-heptene-2-ethyl-ol (Nopol) | C<sub>11</sub>H<sub>18</sub>O | 166 | 16.160 | 1.810 | 0.41 | 95.10 |
| 13 | 2-Acetyl-4-methylpyridine | C<sub>8</sub>H<sub>9</sub>N0 | 135 | 19.260 | 1.920 | 0.29 | 92.05 |

R<sub>t</sub>: Retention time. RRt: Relative Retention time.

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

| No. | Compound name | M. formula | M. weight | R<sub>t</sub> (min) | RRt | Area (%) | Base peak |
|-----|---------------|------------|-----------|-------------------|-----|----------|-----------|
| 1 | 4-Hydroxy-3-methyl butanal | C<sub>5</sub>H<sub>10</sub>O<sub>2</sub> | 102 | 7.030 | 0.314 | 0.56 | 56.10 |
| 2 | 2-Methyl-4-butanolide | C<sub>5</sub>H<sub>8</sub>O<sub>2</sub> | 100 | 9.791 | 0.438 | 22.12 | 41.05 |
| 3 | Tetrahydro-2-pyranone | C<sub>5</sub>H<sub>8</sub>O<sub>2</sub> | 100 | 10.137 | 0.453 | 2.07 | 42.05 |
| 4 | 2,2,4,6,6-Pentamethyl heptane | C<sub>12</sub>H<sub>26</sub> | 170 | 10.557 | 0.472 | 2.20 | 57.10 |
| 5 | 4-Methyl-5-H-furan-2-one | C<sub>5</sub>H<sub>6</sub>O<sub>2</sub> | 98 | 10.872 | 0.487 | 2.49 | 41.05 |
| 6 | Heptene 1,2-oxide | C<sub>7</sub>H<sub>14</sub>O | 114 | 12.223 | 0.547 | 7.47 | 71.05 |
| 7 | Benzene acetaldehyde | C<sub>8</sub>H<sub>8</sub>O | 120 | 12.641 | 0.566 | 0.86 | 91.05 |
| 8 | Tertiary butylphenyl ether | C<sub>10</sub>H<sub>14</sub>O | 150 | 13.305 | 0.595 | 1.63 | 57.10 |
| 9 | 2-Acetylpyrrole | C<sub>6</sub>H<sub>7</sub>N0 | 109 | 13.690 | 0.613 | 1.78 | 94.05 |
| 10 | Benzoic acid, methyl ester | C<sub>8</sub>H<sub>8</sub> | 136 | 14.238 | 0.637 | 4.05 | 105.05 |
| 11 | Nonanal | C<sub>9</sub>H<sub>18</sub> | 142 | 14.348 | 0.642 | 1.63 | 57.10 |
| 12 | Endo-borneol | C<sub>10</sub>H<sub>20</sub> | 154 | 16.161 | 0.723 | 1.14 | 95.10 |
| 13 | 2,5-Dimethyl benzaldehyde | C<sub>9</sub>H<sub>18</sub> | 134 | 16.689 | 0.747 | 1.74 | 134.10 |
| 14 | Unidentified | | | | | | |
| 15 | Hexahydro-2,5-methano-1H-inden-7(4H)-one | C<sub>5</sub>H<sub>10</sub>O<sub>2</sub> | 150 | 17.414 | 0.799 | 0.86 | 57.10 |
| 16 | 3-Ethyl-4-methyl-1H-pyrrrole-2,5-dione | C<sub>5</sub>H<sub>8</sub>NO | 139 | 18.698 | 0.837 | 6.07 | 139.10 |
| 17 | Chrysanthemyl acetate | C<sub>12</sub>H<sub>18</sub>O<sub>2</sub> | 122 | 19.117 | 0.856 | 1.58 | 119.10 |
| 18 | Unidentified | | | | | | |
| 19 | α-Terpinyl propionate | C<sub>13</sub>H<sub>22</sub>O<sub>2</sub> | 210 | 21.152 | 0.947 | 0.60 | 121.10 |
| 20 | Isobornyl acetate | C<sub>8</sub>H<sub>18</sub>O<sub>2</sub> | 208 | **22.329** | **1.000** | **35.09** | **95.10** |
| 21 | E-β-Ionom | C<sub>10</sub>H<sub>18</sub> | 192 | 25.011 | 1.120 | 1.20 | 177.15 |
| 22 | Dihydroactinidolide | C<sub>9</sub>H<sub>16</sub> | 180 | 25.263 | 1.131 | 1.64 | 111.05 |
| 23 | 1,2-Benzenedicarboxylic acid, diethyl ester | C<sub>11</sub>H<sub>18</sub>O<sub>3</sub> | 222 | 26.222 | 1.192 | 0.60 | 149.05 |
| 24 | Eicosane | C<sub>20</sub>H<sub>42</sub> | 282 | 27.984 | 1.253 | 3.05 | 57.10 |
| 25 | 2,2-Dimethoxy-2-phenyl acetophenone | C<sub>12</sub>H<sub>18</sub>O<sub>2</sub> | 256 | 34.490 | 3.866 | 0.36 | 151.10 |
| 26 | Methyl oleate | C<sub>19</sub>H<sub>36</sub>O<sub>2</sub> | 296 | 38.471 | 1.722 | 0.80 | 55.10 |
| 27 | Methyl linoleate | C<sub>19</sub>H<sub>34</sub>O<sub>2</sub> | 294 | 38.496 | 1.544 | 1.55 | 67.10 |

R<sub>t</sub>: Retention time. RRt: Relative Retention time.
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. pneumoniae* (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 | *S. aureus* | *P. aeruginosa* | *E. coli* and *K. pneumonia* |
|------------|-------------|-----------------|-------------------------------|
|            | S | I | R | S | I | R | S | I | R | S | I | R |
| Amoxicillin| ≤ 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: Intermediate. 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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