Radical Scavenging, Antibacterial Activities and Chemical Composition of Volatile Oil of Edible *Mentha longifolia* (L.) Hudson, Subs. *Schimperi* (Briq.) Briq, from Yemen

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Received: July 28, 2017 ; Accepted: December, 15, 2017

**Abstract:** The study was planned to investigate the content and the composition of the volatile oil of *Mentha longifolia*, subspecies *schimperi*, growing in Yemen. Antibacterial activity and radical scavenging activity (RSA) of the oil were evaluated as well. Chemical analysis of oil was achieved by IR spectroscopy and GC/MS. DPPH radical was used to estimate RSA of oil extract. Antibacterial activity was tested using agar diffusion method against four standard bacterial strains. Seventeen components, representing 99.97\% of the total oil composition were identified. Piperitone oxide isomers were found to be the main components, representing 99.48\% of the total oil composition. Oil content determination refers to the presence of 4.1\% v/w. The chemical composition of the analysed oil in the current study is noticeably different from all the oils previously analyzed, especially in the percentage of trans-piperitone oxide and oil content. The bioassays showed that the present study oil, possess significant antibacterial activity and promising antioxidant. These results indicate that oil could serve as safe natural additives in the food and pharmaceutical industries and as a potential source for production of piperitone oxide.

**Keywords:** *Mentha longifolia* (L.) subspecies *schimperi*, volatile oil composition, trans-piperitone oxide, radical scavenging activity, antibacterial activity.

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Introduction:

*Mentha longifolia* (L) Huds., is one of mint species grown in Yemen and there was only one subspecies recorded for *M. longifolia* species in Yemen, which is subsp. *schimperi* (Wood, 1997; Al-Dubaie and El-Khulaidi, 2005). Recently, a new subspecies was recorded, which is subsp. *typhoides*. This subspecies was grown wild in one area in Yemen (Jabal An-Nabi Shu’ayb, Sana’a Governorate) [PhD Thesis, Faculty of Science, Sana’a University, Sana’a, Yemen; 2013].

Several published works on the volatile oil chemical composition of *M. longifolia* from different countries around the World, revealed that the existence of the main components are qualitatively and quantitatively variable (Viljoen et al., 2006; Gulluce et al., 2007; Sharopov et al., 2012; Aksit et al., 2013; Barzin et al., 2014; Al-Okbi et al., 2015; Verma et al., 2015; Abedi et al., 2015; Diop et al., 2016; Ghasemi et al., 2016). Numerous reports suggest strong antibacterial and antifungal activities of a wide range of volatile oils aromatic plants, especially those belonging to the Lamiaceae family (Gobert et al., 2002; Mahmoudi et al., 2016; Dehghanpour-Farashah and Taheri, 2016). As one of this plant family, volatile oils of *M. longifolia* from different geographical locations have been found to possess antioxidant activity (Nikšić et al., 2012; Santos et al., 2010; Nickavar et al., 2008), antioxidant along with antifungal activities (Džamić et al., 2010; Marino et al., 2001) and antioxidant along with antimicrobial activities (Nikšić et al., 2012; Stanisavljević et al., 2014; Gulluce et al., 2007). The major use of the extracted volatile oils of *Mentha* species, is in cosmetics and food industry for the production of different sweets and beverages (Mimica–Dukić and Božin, 2008).

However, because no information records on the content, chemical composition, antibacterial activity and radical scavenging activity (RSA) of volatile oil of *Mentha longifolia* subsp. *schimperi*., grown in Wadi Bana area of Abb province, Yemen and to explain why in Yemen, the fresh or dried leaves of *M. longifolia* subsp. *schimperi* and *Pulicaria jaubertii* were used with milk and certain kind of bread in the preparation of delicious traditional daily meal called Shafoot, the objectives of this study were to investigate the content, the composition and to evaluate the antibacterial activity, as well as RSA of the volatile oil of the dry herbal material of this plant.
Materials and Methods

Plant material

Sample collection

Upper parts of *Mentha longifolia* (flowers, leaves and stems) were collected in January and February 2015, from Wadi Bana-Abb, Yemen. Its identification was clarified by Dr/ H. Ibrahim, the staff member of Plant Taxonomy Unit, Department of Biology, Faculty of Science, Sana'a University, Yemen, and according to Wood, 1997, Al-Dubaie and El-Khulaidi, 2005. A voucher specimen was deposited under the number Bot. 722Kh., in the herbarium of the Department of Biology, Faculty of Science, Sana'a University, Sana'a, Yemen.

Plant preparation for extraction

Fresh herb of aerial parts of the plant were cut into small pieces and left to dry naturally on laboratory benches at room temperature (23-27°C) in shade and dry environment for fifteen days until they were crisp. The dried plant materials were ground into fine powder using a mortar and pestle, just before submitting to hydro distillation.

Volatile oil content

According to the European pharmacopoeia method, the fine powder of the dried aerial part of *M. longifolia subsp. schimperi*. (536 g), was hydro distilled for 3.5 hours using modified Clevenger-type apparatus to give about 22 mL volatile oil. The oil was dried over anhydrous sodium sulfate and stored in sealed dark colored vial at 4 °C before analysis.

Volatile oil analysis

IR analysis

IR spectrum of the investigated volatile oil was recorded on a Shimadzu-FTIR-410 Spectrometer (Japan) in the range 400 to 4000 cm\(^{-1}\). Sodium chloride plate (optically polished plates) technique was used to achieve this analysis. The spectrum was plotted as intensity versus wave number (cm\(^{-1}\)).

GC/MS analysis

Chemical analysis of the studied volatile oil composition was carried out by Shimadzu gas chromatography. Gas chromatography was equipped with DB-5 wax cross–linked fused silica capillary column (30 m long × 0.25 mm internal diameter) covered with film thickness (0.5μm) of polydimethylsiloxane. Oven temperature was automatic, from 40 0°C for three minutes with an
increase of 4 °C/min to 250 °C and isothermally for 10 minute at 250 °C. Injections were performed with injector temperature of 200 °C and ion source temperature rest at 250 °C. The injection volume was 1 μL. Flow rate of a carrier gas (Helium), was fixed at 1 mL/minute. The type of mass spectrometer was an electron impact (EI) (70 eV), computerized from m/e 40 to m/e 500 (National research center Dokki, Cairo, Egypt). Retention indices were calculated using standards n-alkanes (C₅–C₃₀) and then compared with the data available in literature (Adams, 1995).

Bioassays

Assay of radical scavenging activity (RSA)

A preliminary assessment of RSA of volatile oil of *M. longifolia*, subspecies *schimperi*, was conducted by the spectrophotometric DPPH assay method (Đorđević et al., 2007). The stable radical 2.2’-diphenyl-1-picrylhydrazyl (DPPH) was used to estimate the electron donation ability of the investigated volatile oil. The control solution (DPPH solution) and six samples of increasing concentrations of the volatile oil were prepared by diluting 0, 10, 20, 40, 60, 80 and 100 μg of volatile oil with methanol to a total volume of 1 mL. To each sample, 2 mL of 90 μM methanol solution of DPPH was added. Samples mixtures were incubated for one hour period at room temperature and after that, the absorbance of each sample was recorded against the absorbance of the control solution at 517 nm. A parallel RSA assay on ascorbic acid with the same set of concentrations was also performed. Inhibition percent of DPPH radical (I %) was calculated as follow:

\[ I\% = 100 \left( \frac{A° - A}{A°} \right) \]

Where A° is the absorbance of the control solution (DPPH solution) and A is the absorbance of individual investigated samples. The test was carried out in triplicate.

Antibacterial assay

Antibacterial activity of volatile oil of *M. longifolia*, subspecies *schimperi* from Yemen, was evaluated in vitro by traditional antibiotic susceptibility test, using agar disc diffusion method (Bauer et al., 1966). One drop of pure volatile oil (10 μL) was poured on the agar prepared as required. After incubation (18 hours at 37°C), diameters of inhibition zones (in mm.) were measured. The evaluation of the antibacterial activity was carried out against four standard bacterial strains: *Staphylococcus aureus* ATCC 6538, *Streptococcus pyogens* ATCC 10541, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 25619. Gentamycin as a therapeutically important antibiotic in treating infections caused by microorganisms was used as a comparative substance (as positive control). The tested bacterial strains were supplied from Microbiology Unit, Department of Biology, Department of Biology,
Faculty of science, Sana'a University. The tests were carried out in triplicate. Data of the investigated oil samples were expressed as means ± S.D.

Results

Volatile oil content

Based on plant sample dry weight, the volatile oil content of this plant is 4.1% v/w. The resulting volatile oil is colorless oil. It has, changeable color (colorless to pale yellow), specially after long period of time.

Volatile oil analysis

IR Analysis

Infrared spectroscopy is certainly one of the most important analytical techniques available to today’s scientists. One of the great advantages of infrared spectroscopy is that virtually any sample in virtually any state may be studied. Liquids, solutions, essential oils, pastes, powders, films, fibres, gases and surfaces can all be examined with a judicious choice of sampling technique. FT-IR was previously used in the purity analysis of adulterated essential oils and in the analysis of essential oils components (Yan-qun Li et al., 2013; Bizuneh, 2014). Therefore, FT-IR technique has been used as a reliable and supportive analysis of GC/MS analysis results.

The FTIR spectrum of volatile oil was recorded in the region (500-4000 cm\(^{-1}\)). A useful step in the analysis of IR spectrum entails looking at the absorption bands in the vicinity of \(~3000\) cm\(^{-1}\). The volatile oil in its IR spectrum exhibits a bands (medium & sharp) in the vicinity of \(~3000\) cm\(^{-1}\) slightly to the left of that value (at \(3050\) cm\(^{-1}\)) and some bands (medium & sharp) slightly to the right (at \(2950-2865\) cm\(^{-1}\)). Absorption bands in this region tells us that the compound has hydrogen bonded to sp\(^2\) carbons, and hydrogen bonded to sp\(^3\), respectively, but none bonded to sp carbons. In double bond region of the IR spectrum, single absorption band at \(~1710\) cm\(^{-1}\) and a band (weak & sharp) at \(~1600\) cm\(^{-1}\) are detected. The single absorption band at \(~1710\) cm\(^{-1}\) tells us that the compound has ketonic carbonyl group whereas an alkene C=C bond is indicated by a band at \(~1600\) cm\(^{-1}\). The two equivalent absorption bands (weak & sharp) at \(1382\) cm\(^{-1}\) and \(1360\) cm\(^{-1}\) indicates the presence of an isopropyl group. Epoxies ring usually give three absorption bands, so it was found that the volatile oil in its IR spectrum exhibits absorption bands at \(1215\) cm\(^{-1}\) and two absorption bands (weak & sharp) at \(920\) and \(840\) cm\(^{-1}\).
The compatibility between the FT-IR analysis results and the chemical structure of the common components of the investigated oil showed that the technique was intended to confirm the results of the GC/MS analysis and to confirm that this technique is effective not only as a tool of analysis but also as a tool of confirming the results of an analysis obtained from another technique.

**GC/MS Analysis**

A total of nineteen chemical components with retention time between 10.70 and 33.10 minutes, are recognized on the gas chromatogram of this volatile oil. Seventeen of these components, representing 99.97% of the total oil content were identified by GC/MS analysis. These components are listed in Table 1 along with their peak numbers, retention time (RT), composition percentage and their retention indices (RI) values (calculated and literature). GC/MS analysis showed that the dominant components are the isomers (trans and cis) of the oxygenated monoterpene piperitone oxide (99.48%). GC/MS analysis also showed that the composition percentage of the rest of the identified volatile components (15 components), comprising seven oxygenated monoterpenes (0.38%); one monoterpene hydrocarbon (0.01%); two aromatic compounds (0.02%); three sesquiterpene hydrocarbons (0.04%) and two oxygenated sesquiterpene (0.03% ), not exceed 0.04% for each one component of them.

The individual components of *M. longifolia* subsp. schimperi volatile oil were quantified by GC and identified by matching their mass spectra with data already available in the GC-MS computer libraries (NIST and Wiley libraries) and comparing their calculated retention indices (RI) with the literature values measured on columns with identical polarities (Adams, 1995). Our analysis results are not in accordance with the previously published data, especially, with regard to the presence of trans-piperitone oxide, which have concentration higher than usual (Sharopov et al., 2012; Nikšić et al., 2012; Aksit et al., 2013; Baser et al., 1999; Singh et al., 2008; Verma et al., 2015; Koliopoulos et al., 2010).

However, in agreement with our findings, trans-piperitone oxide and cis-pipertion oxide as main components has been found in the volatile oil of *M. longifolia* collected from several countries around the world. The presence pipertion oxide in its cis form (as one of the main components) has been found in the volatile oil of *M. longifolia* collected from Morocco (Goulami et al., 2001), South Africa (Viljoen et al., 2006), Turkey (Gulluce et al., 2007) and Iran (Barzin et al., 2014), whereas, its existence as the main component was detected in the volatile oil of *M. longifolia* collected from Tajikistan (Sharopov et al., 2012). Similarly, the presence of piperitone oxide in its trans form as one
of the main components and as the main component was detected in the volatile oil of *M. longifolia* collected from India (Singh et al., 2008; Verma et al., 2015).

On the other hand, *trans*-piperitone oxide and *cis*-piperitone oxide as isomers, was also previously reported as the main components of the volatile oil of *M. longifolia* (Sharopov et al., 2012; Koliopoulos et al., 2010).

Although, several published works reported the presence of *trans*-piperitone oxide and/or *cis*-piperitone oxide as a major components in the volatile oil of *M. longifolia* collected from different geographical regions around the World, the essential oil of the presently studied *M. longifolia* differed noticeably with earlier reports, especially in the percentage of *trans*-piperitone epoxide.

As expected, quantitative and qualitative variations in the chemical components of essential oils of *Menthe longifolia* throughout the world are attributed to several factors specifically, geographical location, age of the plant, harvesting season, collection time, collected part, method of drying, volatile oil extraction method, in addition to the type of plant, (wild or cultivated) (Arumugam et al., 2016; Hussain et al., 2010; Viljoen et al., 2006).

**Radical scavenging activity**

Many plant species were found to have antioxidant constituents in their tissues. Medicinal aromatic plants used in traditional medicine and healing are among these natural antioxidant sources. For this reason, volatile oil of *M. longifolia* subsp. *schimperi*, was screened for its possible radical scavenging activity (RSA) using the stable radical 2,2′-diphenyl-1-picrylhydrazyl (DPPH, violet). Recorded results in Table 2 show that the investigated oil has high RSA as revealed by its ability to reduce the stable radical 2,2′-diphenyl-1-picrylhydrazyl (DPPH, violet) to the yellow colored. RSA can be considered as a measure of the ability of investigated oil samples or one of its components to act as “radicals scavengers“.

Earlier published work reported that a strong RSA and antimicrobial activity of volatile oil extract, required chemical component(s) with structural feature containing characterize functional group(s) (Nikšić et al., 2012; Ezoubeiri et al., 2005). It is well known that the antioxidant activity of essential oil containing phenolic and/alcoholic components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals. In addition, RSC was found to be correlated to the presence of mainly oxygenated monoterpenes, such as ketones, epoxides and aldehydes (Nikšić et al., 2012; Barbieri et al., 2016). Moreover, there are some reports that show that oxygenated monoterpenes, such as a-terpineol, linalool, carvacrol, piperitenone, piperitone oxide and
piperitenone oxide, were mainly responsible for the antioxidant potential of plant oils (Barbieri et al., 2016; Nikšić et al., 2012; Bicas et al., 2011; Miguel, 2010). On the other hand, Barbieri et al., (2016), reported that the difference in the oxygenated monoterpenes (piperitenone oxide) amounts between two Clinopodium gilliesii (Benth.) Kuntze (Lamiaceae) oils could explain their different antioxidant activity.

Considering the results reported in the literature (Nikšić et al., 2012; Barbieri et al., 2016), as well as our FT-IR, GC/MS analysis and RSA results, we can suggest that the scavenging activity of the investigated volatile oil on DPPH free radicals could be due to the presence of remarkable percentage of the oxygenated monoterpane trans-piperitone oxide (97.71%) within chemical components of the studied oil. Therefore, one could conclude that RSA is due to the ability of trans-piperitone oxide to form reactive oxygen species during this bioassay. Hence, it's sensible to hypothesize that this oxygenated monoterpane has a potent antioxidant “radicals scavengers“.

Based on RSA results of our study and because it's generally understood that ingestion of foods which include or prepared with natural volatile oils or aromatic plant extracts is expected to prevent the risk of free radical reliant diseases (Alejandro et al., 2011), the investigated volatile oil could serve not only as flavor agent but also as safe alternative antioxidant of foodstuff and edible liquid products and sterile additive in pharmaceuticals. RSA was done for both M. longifolia subsp. schimperi volatile oil and L-ascorbic acid (a widely used natural antioxidant) for comparison purpose.

**Antibacterial activity**

In addition to the antioxidant activity of the studied Mentha longifolia subsp. schimperi volatile oil, and because some human diseases caused by pathogenic bacteria and the resistance that microorganisms have built against antibiotics, in the current work the antibacterial activity of M. longifolia subsp. schimperi volatile oil has been examined to evaluate the potential uses of this volatile oil in the industrial and medicinal fields as a natural preservative, as well as for finding potential new source for therapeutic use.

However, the results of the antibacterial activity of the studied volatile oil, is shown in **Table 3**. The investigated oil showed significant antibacterial activity in comparison with gentamycin. The highest sensitivity to volatile oil was shown by Streptococcus pyogenes ATCC 10541. Significant antimicrobial activity of volatile oil was observed against Pseudomonas aeruginosa ATCC 25619.
Based on the published studies, antibacterial activity of the volatile oil was found to be correlated to the presence of mainly oxygenated monoterpenes such as ketones, epoxides and aldehydes (Nikšić et al., 2012).

Similarly, previous reported studies, indicate that essential oils rich in oxygenated terpenes generally show higher antimicrobial activity compared with oils rich in hydrocarbons (Stanisavljević et al., 2014; Ali et al., 2012; Nikšić et al., 2012).

On the other hand, the effectiveness of *M. longifolia* L. essential oil against Gram–positive and Gram–negative bacteria has been well described (Ghoulami et al., 2000; Rasooli and Rezaei, 2002; Mimica-Dukic et al., 2003). In addition, it was found that Gram–positive bacterial are selectively sensitive to the volatile oils containing high percentage of thymol (Hussein et al., 2017a).

However, antibacterial activity results of the investigated volatile oil are in agreement with the previously published data except compound *trans*-piperitone oxide whose concentration is relative higher than usual. Therefore, it is possible to suggest here that the antibacterial activity of the studied volatile oil is due to some kind of the synergistic effect between the traces amount of components, such as *cis*-piperitone oxide, *cis*-sabinene hydrate, carvenone, thymol, linalool, 4-terpineol, spathulenol and *α-epi*-cedanol and the main component (*trans*-piperitone oxide).

Antioxidant alongside antimicrobial activities of volatile oils are among the most examined features and are important for both food preservation and control of human and animal diseases, specifically those caused by free radicals and those of microbial origin (Nikšić et al., 2012; Džamić et al., 2010; Marino et al., 2001; Stanisavljević et al., 2014; Gulluce et al., 2007). Finally, all results of the present work, may explain why in Yemen? A mixture of fresh or dried leaves of two aromatic plants (*M. longifolia* subsp. *schimperi* and *Pulicaria jaubertii*) are used with milk and certain kind of bread to make delicious traditional daily meal called Shafoot (Hussein et al., 2017b).

**Conclusions:**

This work is the first report on the content, chemical composition, chemotype and RSA of volatile oil of wild *M. longifolia* subsp. *schimperi*, grown in Wadi Bana area of Abb province, Yemen. The work showed that the investigated volatile oil has high oil content (4.1% v/w) than usual and its composition characterized by the presence of the isomers of pipertone oxide as main constituents. The unexpected percent yield of the main component (97.71%) and the unpredicted highest oil content, indicate that the volatile oil has commercial potential for production of piperitone oxide as industrial product, and reveals that this oil belong to piperitone oxide type. The present work showed
that the volatile oil has significant antibacterial activity and a potent antioxidant as ascorbic acid (vitamin C). These results indicate also that RSA of the investigated oil samples is related to the presence of mainly monoterpene ketone oxide (piperitone oxide). Therefore, the investigated oil could serve as safe natural alternative for synthetic antioxidants in food industry and pharmaceuticals.

Table 1. Composition of volatile oil from Mentha longifolia (L.) Huds., subspecies schimperi (Briq.) Briq. from Yemen.

<table>
<thead>
<tr>
<th>Componentsa</th>
<th>PNb</th>
<th>RTc (min.)</th>
<th>CPd (%)</th>
<th>RFe</th>
<th>CRIf</th>
<th>LRIg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabinene</td>
<td>1</td>
<td>10.70</td>
<td>0.01</td>
<td>973</td>
<td>976</td>
<td></td>
</tr>
<tr>
<td>p-Cymene</td>
<td>2</td>
<td>12.74</td>
<td>0.01</td>
<td>1028</td>
<td>1026</td>
<td></td>
</tr>
<tr>
<td>cis-Sabinene Hydrate</td>
<td>3</td>
<td>14.63</td>
<td>0.28</td>
<td>1070</td>
<td>1068</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>4</td>
<td>15.50</td>
<td>0.01</td>
<td>1086</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>Linalool</td>
<td>5</td>
<td>15.62</td>
<td>0.01</td>
<td>1096</td>
<td>1098</td>
<td></td>
</tr>
<tr>
<td>Isomenthone</td>
<td>6</td>
<td>17.36</td>
<td>0.01</td>
<td>1165</td>
<td>1164</td>
<td></td>
</tr>
<tr>
<td>4-Terpineol</td>
<td>7</td>
<td>18.38</td>
<td>0.01</td>
<td>1178</td>
<td>1177</td>
<td></td>
</tr>
<tr>
<td>Carvenone (p-Menth-3-en-2-one)</td>
<td>8</td>
<td>20.17</td>
<td>0.02</td>
<td>1248</td>
<td>1252</td>
<td></td>
</tr>
<tr>
<td>cis-Piperitone oxide</td>
<td>9</td>
<td>21.83</td>
<td>1.77</td>
<td>1258</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>trans-Piperitone oxide</td>
<td>10</td>
<td>22.23</td>
<td>97.71</td>
<td>1274</td>
<td>1277</td>
<td></td>
</tr>
<tr>
<td>Thymol</td>
<td>11</td>
<td>23.18</td>
<td>0.01</td>
<td>1294</td>
<td>1290</td>
<td></td>
</tr>
<tr>
<td>6-Hydroxy-carvotanacetone</td>
<td>12</td>
<td>23.38</td>
<td>0.04</td>
<td>1309</td>
<td>1304</td>
<td></td>
</tr>
<tr>
<td>Piperitenone oxide</td>
<td>13</td>
<td>25.03</td>
<td>0.01</td>
<td>1368</td>
<td>1363</td>
<td></td>
</tr>
<tr>
<td>β-Bourbonene</td>
<td>14</td>
<td>25.36</td>
<td>0.01</td>
<td>1386</td>
<td>1384</td>
<td></td>
</tr>
<tr>
<td>trans-Caryophyllene</td>
<td>15</td>
<td>26.44</td>
<td>0.01</td>
<td>1421</td>
<td>1418</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>16</td>
<td>27.84</td>
<td>0.02</td>
<td>1462</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>Germacrene D</td>
<td>17</td>
<td>28.35</td>
<td>0.02</td>
<td>1478</td>
<td>1480</td>
<td></td>
</tr>
<tr>
<td>Spathulenol</td>
<td>18</td>
<td>31.25</td>
<td>0.01</td>
<td>1572</td>
<td>1576</td>
<td></td>
</tr>
<tr>
<td>α-Epi-Cadinol</td>
<td>19</td>
<td>33.10</td>
<td>0.02</td>
<td>1644</td>
<td>1640</td>
<td></td>
</tr>
</tbody>
</table>

Notes: a Components are listed in order of their elution from a DB-5 column; b Peak numbers; c Retention time in minute; d Composition percentage; e Retention indices; f Calculated retention indices relative to C5-C30 n-alkanes; g Literature retention indices (Adams, 1995).
Table 2. Radical scavenging activity of volatile oil from *Mentha longifolia* (L.) Huds., subspecies *schimperi* (Briq.) Briq. from Yemen.

<table>
<thead>
<tr>
<th>Samples concentration μg/ml</th>
<th>Absorbance and Radical scavenging activity</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The investigated oil †</td>
<td>Ascorbic acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Absorbance at 517 nm</td>
<td>RSA Activity %</td>
<td>Absorbance at 517 nm</td>
</tr>
<tr>
<td>0</td>
<td>0.4308</td>
<td>0</td>
<td>0.4308</td>
</tr>
<tr>
<td>10</td>
<td>0.2741 ± 0.0003</td>
<td>36.37 ± 0.07</td>
<td>0.0386</td>
</tr>
<tr>
<td>20</td>
<td>0.1831 ± 0.0017</td>
<td>57.50 ± 0.39</td>
<td>0.0380</td>
</tr>
<tr>
<td>40</td>
<td>0.1268 ± 0.0010</td>
<td>70.57 ± 0.24</td>
<td>0.0377</td>
</tr>
<tr>
<td>60</td>
<td>0.0812 ± 0.0011</td>
<td>81.15 ± 0.25</td>
<td>0.0350</td>
</tr>
<tr>
<td>80</td>
<td>0.0486 ± 0.0016</td>
<td>88.71 ± 0.38</td>
<td>0.0341</td>
</tr>
<tr>
<td>100</td>
<td>0.0275 ± 0.0013</td>
<td>93.62 ± 0.31</td>
<td>0.0336</td>
</tr>
</tbody>
</table>

**Note:** † Data of the investigated oil samples were expressed as means ± S.D. (S.D. = Standard deviation).

Table 3. Antibacterial activity of volatile oil from *Mentha longifolia* (L.) Huds., subspecies *schimperi* (Briq.) Briq. from Yemen.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Inhibition zone (mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Investigated oil</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 6538</td>
<td>17.25 ± 0.34</td>
</tr>
<tr>
<td><em>Escherichia coli</em> ATCC 8739</td>
<td>12.00 ± 0.56</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 25619</td>
<td>15.50 ± 0.47</td>
</tr>
<tr>
<td><em>Streptococcus pyogens</em> ATCC 10541</td>
<td>41.25 ± 0.61</td>
</tr>
</tbody>
</table>
Figure 1. GC chromatogram (with peak numbers of the main components) of volatile oil of Mentha longifolia (L.) Huds., subspecies schimperi (Briq.) Briq. from Yemen.
Figure 2. FTIR spectrum of volatile oil of *Mentha longifolia* (L.) Huds., subspecies *schimperi* (Briq.) Briq. from Yemen.

Competing interests:
The authors declare that they have no competing interests.

Acknowledgments:
This work was supported by Sana'a University, Faculty of Science. One of the authors; Khaled Hussein is indebted to Alsaeed Foundation for Science and Culture for financial support. Authors are grateful to Dr/Nabil Sultan for editing the manuscript.

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