Original paper

Chemical composition and anti-microbial analysis of *Mentha arvensis* L. and *Thymus linearis* Benth. essential oils of leaves

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Abstract

This study was performed to evaluate the chemical composition of the essential oils of *Mentha arvensis* and *Thymus linearis* and their antimicrobial activities. The complexity of the essential oils is a basic challenge for determining their reliable and accurate compositional data. Rapid advances in spectroscopic and chromatographic techniques have resolved this challenge to a large extent by examining essential oils. Essential oils were analyzed through Gas Chromatography-Mass Spectrometry (GC-MS) following extraction through steam distillation from their leaves for the first time in Miandam, District Swat, KP, Pakistan. The GC-MS analysis revealed 26 and 25 components in the essential oil of *Mentha arvensis* and *Thymus linearis* respectively. The major components were carvone (23.53%), P-Cymen-2-ol (20.35%), and caryophyllene oxide (18.81%) in *Mentha arvensis* but *Thymus linearis* has Thymol (2-isopropyl-5-methyl phenol) (40%), O-Cymene (2-Isopropyltoluene) (14.95%) and beta-bisabolene (12.54%). The essential oils of both plants showed bactericidal activities against five different bacterial strains (i.e. Escherichia coli, Klebsiella pneumonia, Bacillus subtilis, and Pseudomonas aeruginosa) during disc diffusion method and therefore it is suggested that they may be used as a natural antiseptic and could play important roles in food and pharmaceutical industry.

Keywords

Essential oil, GC-MS, Mentha arvensis, Steam distillation, Thymus linearis


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Introduction

Pakistan is an agricultural country and has a rich flora, having both medicinally and economically important vegetation. There are a huge number of herbs and aromatic plants that have high potential to be employed as a natural mixture in native medicines (Hussain et al., 2008). The essential oils of coriandrum have been in practice by human beings since ancient times (Iscan et al., 2002). Throughout human history, plants are considered as an important source of healing agents and their secondary metabolites (SMs) are used for therapeutic purposes (Wink, 2003).

Mentha arvensis L. and Thymus linearis Benth. are small perennial herbs belong to Lamiaceae (Labiatae) and cultivated in Thailand, Nepal, India, Japan, and Pakistan (Silva et al., 2015). In Pakistan M. arvensis and T. linearis are grown in the wild and agro-economic regions. The coriandrum (Mentha) genus has about 25-30 species, best growing in wet soil (Okut et al., 2017). The leaves are used in spices, carminative, and additives in herbal tea. These plants contain essential oils which were reported to possess biological activity (Karaman et al., 2001). Essential oils are volatile, natural plant compounds and are often classified by strong scent (Bakkali et al., 2008). Members of Lamiaceae deposit their essential oils in specialized plant cells, typically resin ducts, oil cells, or trichomes (Pengelly, 2004). Fragrance and biological activity of these plants are due to volatile essential oils (Friedrich, 1976). Menthol is the major component in the Mentha genus, which is used in cosmetic and pharmaceutical products (Kobak et al., 2010). It also is effective against human liver cancer and has thermogenic, diuretic, antibacterial, antifungal, and expectorant properties (Agboola et al., 2016).

Thymus linearis is an aromatic evergreen herb (Borug et al., 2014), while the leaves have antiseptic, carminative, and anthelmintic properties (Karaman et al., 2001). Cosmetic and pharmaceutical industries use their dried leaves for the extraction of essential oils (Youdim et al., 2002). The fragrant nature of essential oils allows them to be used in the food industry (Swaroop et al., 2010).

The chemical composition of essential oils did not remain constant. Nutrition, environment, and stress are the factors that influence the total yield and chemical composition of essential oils (Hudaib et al., 2002). Analysis of essential oils is an important task to be achieved. Different tools were used for the quantitative analysis of essential oils. Gas Chromatography-Mass Spectrometry (GC-MS) is one of the tools which is exclusively used for this purpose (Baharum et al., 2010). Climatic conditions and chemical composition affect the antimicrobial activity and insecticidal activities of essential oils. Previously from different regions of the world, the Thymus linearis and Mentha arvensis essential oils were reported to be involved in antimicrobial activities (Fazili et al., 2017; Joshi et al., 2019; Naz et al., 2015; Naz et al., 2020). Our research study demonstrates the effectiveness of Thymus linearis and Mentha arvensis plants grown in the Miandam region of Swat, Pakistan which was used medicinal plants. Therefore, the study aims to isolate natural plant products and to investigate the components of the essential oils from leaves of M. arvensis and T. linearis of intact natural flora of Miandam, District Swat and explore their chemical components and potential antimicrobial activities.

Materials and methods

Sample collection

The fresh green leaves (500 gm) of Mentha arvensis and Thymus linearis were collected from Miandam area altitude (10,000ft) of district Swat in Khyber Pakhtunkhwa province of Pakistan during November 22-25, 2017 by the scientific method of (Akinjoh et al., 2006). The plants were identified in the department of the botany University of Peshawar and a voucher specimen of the plant was deposited in the herbarium of the same university.

Isolation of essential oils

Plant material was dried under shade for 10 days and particle size was reduced. 250 grams of dried plant material was loaded in Clevenger extraction apparatus (Clevenger., 1928; Hussain et al., 2008). Distillation was continued for 3 hours until all the oils were distilled. The essential oils were separated from the aqueous phase using a repatory funnel. The extracted oil was dried using Sodium Sulphate (Na2SO4) as a dehydrating agent and kept under refrigeration for further analysis using the GC-MS technique as described by (Patra et al., 2017).

Gas Chromatography-Mass Spectrometry

GC-MS analysis of essential oils was carried out on (GCMS-QP2020 plus series Shimadzu, Italy, gas chromatograph-mass spectrometer equipped with microbore capillary columns (100 %), 2, 3DE56BDMS-β CD in PS086 (50 m×0.25 mm i.d.×0.25 μm thickness (Mega Legnano, Italy) and high-performance quadrupoles by the stranded methods of (Patra et al., 2017). Samples (0.2 μL) were injected by the method of (Rajeswara et al., 2014). The injector (detector) temperatures were 220°C, the transfer line is 230°C, and the ion course is 200°C respectively. The carrier gas (He) flow rate is about 1.44 ml per min, the split ratio was 1:50 and 70 eV ionization energy scan rate is 50-660 amu per second while the mass range is 35-350 m/z. The ion source temperature was 210°C, interface temperature was 210°C, and injection temperature was 240°C for 2 mints at 2°C per mint, while the column temperature was 40°C.

Components identification

The identification of compounds was accomplished based on comparing the GC mass spectra with those obtained by using saturated n-alkanes (C8–C20) in the inbuilt library that is Wiley, USA, which were reported in the preprint (Karaman et al., 2000; El-Sayed., 2012; http://webbook.nist.gov/chemistry library. The mass spectra were further compared with previously reported literature of (Hsleh et al., 1989; Adams, 2007) which were store in NIST http://webbook.nist.gov/chemistry library, peak area percentage was calculated by the method of (Rajeswara et al., 2014).
**Antibacterial activity**

The antimicrobial activity was performed to study the antibacterial potential of the *Mentha arvensis* and *Thymus linearis* by the method of (Ginovyan et al., 2017; Uda et al., 2018). In this assay essential oils were diluted in DMSO and control (Streptomycin) against five bacterial species, *E. coli, P. aeruginosa K. pneumonia, B. subtilis*, and *S. aureus* were applied to the biological activity. The bacterial strains were incubated overnight at 37°C in 20 ml of nutrient broth, on the second day, 2 ml of bacterial culture were transferred to fresh medium for dilution purposes. From the diluted broth, 3 ml of the bacterial culture were transferred to nutrient agar plates and spread it uniformly on the plates to make a bacterial lawn. The essential oils were diluted with DMSO at a concentration of 1:01, 1:02, and 1:03. The 6 mm Whatman filter paper discs were diluted with 6 µL from different concentrations of the diluted essential oils. The dried discs were aseptically placed on the agar media containing bacterial cultures. The Plates were then incubated at 37 for 24 hours. The zone of inhibition was measured in mm the next day. Pure oil was used as a positive control, whereas DMSO was used as a negative control. Studies were conducted in triplicates and the mean values were calculated.

**Results**

Gas Chromatography-Mass Spectrometry (GC-MS) of the *Mentha arvensis* and *Thymus linearis* essential oils showed the presence of different molecular weight fragment components ranging from small to higher molecular weight terpenoids. In *M. arvensis* a total of 26 components were identified (Figure 1), similarly in the *T. linearis* at least 25 components were identified (Figure 2). The GC-MS spectra of the identified component revealed that *M. arvensis* has a high concentration of carvone (23.53%), p-cymen-2-ol (20.35%), and caryophyllene oxide (18.81%) while the other compounds were found in small concentrations (Table 1). In the case of *T. linearis* the major compounds identified were Thymol (40%), α-cymene (14.95%), and beta-Bisabolene (12.54%). Similarly, other compounds were also present in small concentrations like Bergamot (7.94) and carvacrol (5.42%) (Table 1).

However, Carvone, a major component in *M. arvensis* essential oil was quite less (23.53%) in our present report as compared to the previous report (60.25%) from India. These variations can be attributed to biotic and abiotic factors. The retention times and percent composition of *M. arvensis* are presented in (Table 1).

![Figure 1](image1.png)

**Figure 1:** GS-MS chromatogram showing components of *M. arvensis* essential oil.

![Figure 2](image2.png)

**Figure 2:** GS-MS chromatogram showing components of *T. linearis* essential oil.
Table 1: Chemical composition (percentage) of the essential oils of *Mentha arvensis* and *Thymus linearis* based on GC-MS spectrum.

<table>
<thead>
<tr>
<th>No.</th>
<th>Chemical Component</th>
<th>Mentha arvensis</th>
<th>Thymus linearis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Retention indices (Determined)</td>
<td>Retention indices (Literature)</td>
<td>Conc (%)</td>
</tr>
<tr>
<td>1</td>
<td>α -Thujene</td>
<td>920</td>
<td>925</td>
</tr>
<tr>
<td>2</td>
<td>α-Pinene</td>
<td>938</td>
<td>933, 940</td>
</tr>
<tr>
<td>3</td>
<td>Thujene</td>
<td>952</td>
<td>NR</td>
</tr>
<tr>
<td>4</td>
<td>β -Terpineol.cis</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>5</td>
<td>o-Methylthyl</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>6</td>
<td>Thymol methyl ether</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>7</td>
<td>β -Pinene</td>
<td>972</td>
<td>973</td>
</tr>
<tr>
<td>8</td>
<td>Bergamio</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>9</td>
<td>p-Cymen-3-ol</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>10</td>
<td>d-Limonene</td>
<td>989</td>
<td>NR</td>
</tr>
<tr>
<td>11</td>
<td>p-Menta-6,8-dien-2-one</td>
<td>1083</td>
<td>1080</td>
</tr>
<tr>
<td>12</td>
<td>γ -Terpinene</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>13</td>
<td>β -Terpineol.cis</td>
<td>1103</td>
<td>NR</td>
</tr>
<tr>
<td>14</td>
<td>β -Linalool</td>
<td>1126</td>
<td>NR</td>
</tr>
<tr>
<td>15</td>
<td>Borneol</td>
<td>1145</td>
<td>NR</td>
</tr>
<tr>
<td>16</td>
<td>p-Menta-1-en-4-ol</td>
<td>1169</td>
<td>NR</td>
</tr>
<tr>
<td>17</td>
<td>p-Cymen-8-ol</td>
<td>1205</td>
<td>NR</td>
</tr>
<tr>
<td>18</td>
<td>p-Methyl-1-en-8-ol</td>
<td>1242</td>
<td>NR</td>
</tr>
<tr>
<td>19</td>
<td>Caryophyllene oxide</td>
<td>1275</td>
<td>1581</td>
</tr>
<tr>
<td>20</td>
<td>p-Cymen-2-ol</td>
<td>1363</td>
<td>NR</td>
</tr>
<tr>
<td>21</td>
<td>Cyclohexanone, 2-Isopropylidene</td>
<td>1395</td>
<td>NR</td>
</tr>
<tr>
<td>22</td>
<td>Thymol</td>
<td>1434</td>
<td>1302</td>
</tr>
<tr>
<td>23</td>
<td>Carvacrol</td>
<td>-</td>
<td>NR</td>
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<tr>
<td>24</td>
<td>α-Caryophyllene</td>
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<td>NR</td>
</tr>
<tr>
<td>25</td>
<td>Germacrene d-4-ol</td>
<td>-</td>
<td>1574</td>
</tr>
<tr>
<td>26</td>
<td>Globulol</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>27</td>
<td>β -Bisabolene</td>
<td>1590</td>
<td>NR</td>
</tr>
<tr>
<td>28</td>
<td>Caryophyllene</td>
<td>1601</td>
<td>NR</td>
</tr>
<tr>
<td>29</td>
<td>Humulen-(v)</td>
<td>1629</td>
<td>NR</td>
</tr>
<tr>
<td>30</td>
<td>Germacrene D</td>
<td>1652</td>
<td>NR</td>
</tr>
<tr>
<td>31</td>
<td>β -Myrcene</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>32</td>
<td>(+)-4-Carene</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>33</td>
<td>O-Cymene</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>34</td>
<td>Gemecerene B</td>
<td>1765</td>
<td>NR</td>
</tr>
<tr>
<td>35</td>
<td>Spathulenol</td>
<td>1790</td>
<td>NR</td>
</tr>
<tr>
<td>36</td>
<td>Carvone</td>
<td>1855</td>
<td>NR</td>
</tr>
<tr>
<td>37</td>
<td>γ -Eudesmol</td>
<td>1895</td>
<td>NR</td>
</tr>
<tr>
<td>38</td>
<td>(+)-selin 7(11)-en-4,α-ol</td>
<td>1949</td>
<td>NR</td>
</tr>
<tr>
<td>39</td>
<td>α-Bisabolol</td>
<td>1993</td>
<td>NR</td>
</tr>
</tbody>
</table>

Spectra, NIST and WILEY libraries spectrophotometry with previous literature on QP2020 plus series Shimadzu, microbore capillary column. Retention indices (Determined) was relative to homologous series of n-alkanes (C8–C20) on QP2020 D capillary column. Retention indices (Determined) from literature (Rajeswara Rao et al 2000; Joshi et al., 2019; Naz et al., 2020), NR; not reported in previus finding, Dish (-); mean not detected.
Antimicrobial activity

The plant secondary metabolites have been broadly examined for their biological activities in search for new antimicrobial drugs to control new and remerging diseases in recent years. In the present study, we have evaluated the antimicrobial activity of *Thymus linearis* and *Mentha arvensis* essential oils. The GC-MS analysis of the essential oils showed that they contain active components like carvone, p-cymen-2-ol, caryophyllene oxide, Thymol, o-cymene, beta-Bisabolene with the application against the microorganisms. In the present research study, the antimicrobial activities of *T. linearis* and *M. arvensis* essential oil were evaluated against 5 bacterial strains including *Escherichia coli*, *Staphylococcus aureus*, *Klesiella pneumonia*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. The results showed that the *M. arvensis* and *T. linearis* essential oil possessed broad-spectrum antibacterial activity. *Thymus linearis* essential oil showed largest zones of inhibition against *P. aeruginosa*, the zones of inhibition were 37mm for pure oil, and for diluted oil the mean zones of inhibition were 20mm, 18mm, and 15mm respectively. The *T. linearis* oil also showed the best activity against *K. pneumoniae*, *E. coli*, and *B. subtilis* while it showed moderate activity against *S. aureus* (Figure 3).

The *M. arvensis* essential oil showed moderate activity against all the bacterial strains (Figure 3). It showed maximum activity against *B. subtilis* while showed the lowest activity against *S. aureus*. The zones of inhibition for both the oils were less than the control ciprofloxacin 31mm. Now a day the essential oils can be used for the treatment of hospital-acquired diseases and to combat the multi-resistant bacteria like the methicillin-resistant *Staphylococcus aureus* (MRSA). To characterize the clinical efficacy of these essential oils the pharmacokinetic and pharmacodynamics studies should also be conducted.

![Figure 3: Mean zones of Inhibition in millimeter (mm) obtained with *T. linearis* essential oils against 5 bacterial strains. The largest zones of inhibition were recorded against *B. subtilis* and the moderate zones were obtained against *S. aureus*. Pure oil shows highest zones of inhibition against all bacterial strains.](image)

![Figure 4: Mean zones of Inhibition in millimeter (mm) obtained with *M. arvensis* essential oils against 5 bacterial strains. The maximum zones of inhibition were obtained against the *B. subtilis*](image)
Discussions

The thymol and carvacrol molecules present in the extracted essential oils could be used against bacterial mediated infections without provoking bacterial resistance (Garc et al., 2018). Major components identified in our present research findings were in contrast with the previous research studies conducted by (Pino and Fuentes, 1996), they identified menthol (51.68%) and menthone (26.08%) as major components in Mentha arvensis. Another study reported by Hussain et al., (2013) identified thymol (36.5%) and carvacrol (9.50%) in Thymus linearis as major components. Vivek et al., (2009) reported carvone as a major component in Mentha arvensis essential oil from Patiala region India Hussain et al., (2013) and Vivek et al., (2009) reports were in good covenant with our present findings. Our results were in agreement with earlier reports about antimicrobial activity of Origanum essential oils and their main constituents, carvacrol and thymol (Esen et al., 2007; Lee et al., 2007; Bendahou et al., 2008).

Indiscriminate use of antimicrobial drugs leads to antimicrobial resistance (Silva et al., 2015). Pathogens can modify its genome against the antibiotics through various mechanisms. Historically herbal plants as such and their derived products are in use for the treatment of diseases (Sokovi et al., 2007). Essential oils containing ketones and terpenes were less efficient, whereas essential oils containing aromatic phenols possessed higher antimicrobial activity (Mayaud et al., 2008).

The higher antimicrobial activity of the Thymus linearis oil can be attributed to the presence of Thymol which disrupts the membrane of bacterial cells (Chandan et al., 2014). Other components of the essential oils interfere with the electron transport chain and alter ATP production (Biswa et al., 2014). Several essential oils were reported to be highly effective against the multi-resistant bacteria like the eucalyptus, white thyme, clove bud, and cinnamon essential oils (Golparvar et al., 2013). The activity of these oils may be due to their main chemical constituents or due to the synergistic effects among the major and minor components (Warnke et al., 2009).

Conclusions

Although the Mentha arvensis and Thymus linearis leave essential oils have differences in their components composition, however, both the oils shared very effective antibacterial activity against all tested bacterial strains. In conclusion, this finding shows that both selected plants essential oils have the shreds of evidence of antibacterial activity against Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Bacillus subtilis, and Pseudomonas aeruginosa. Therefore, it suggested that these essential oils of both plants (Mentha arvensis and Thymus linearis) leaves could represent a key tool for the attainment of hopeful candidates for further pharmacological, and clinical studies in the developing formulations of new natural antibacterial agents as well as a potential tool drugs for the treatment of infectious diseases. Our results further suggested that these essential oils should be further investigated for their therapeutic efficacy.

Acknowledgments

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Statement of interest

None.

References

9. Chandan, k., S. Vishwakarma, R. Caroline. J and S. Khushbu, (2014). Antimicrobial activity of Origanum essential oils and their derived products are in use for the treatment of diseases (Sokovi et al., 2007). Essential oils containing ketones and terpenes were less efficient, whereas essential oils containing aromatic phenols possessed higher antimicrobial activity (Mayaud et al., 2008).


