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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-bisbolene (12.54%). The essential oils of both plants showed bactericidal activities against five different bacterial strains (i.e. Escherichia coli, Staphylococcus aureus, Klesiella 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 cornmint 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 cornmint (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 specified 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 (Kokab et al., 2010). It is also 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 lineris* 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 lineris* 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 (Akinyemi *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 reparatory funnel. The extracted oil was dried using Sodium Sulphate (Na_2SO_4) 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, 3DE56TBDMS-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 mint, 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 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%), o-cymene (14.95%), and beta-Bisbolene (12.54%). similarly, other compounds were also present in small concentrations like Bergamiol (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).

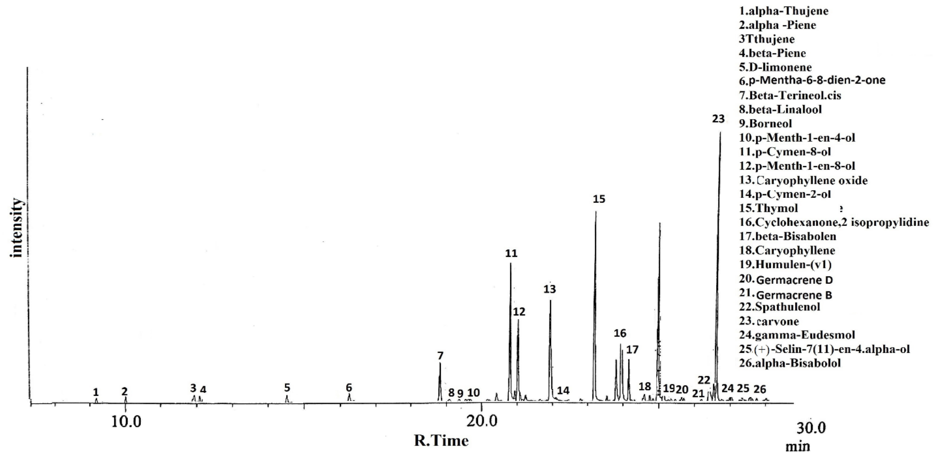

Figure 1: GS-MS chromatogram showing components of *M. arvensis* essential oil.

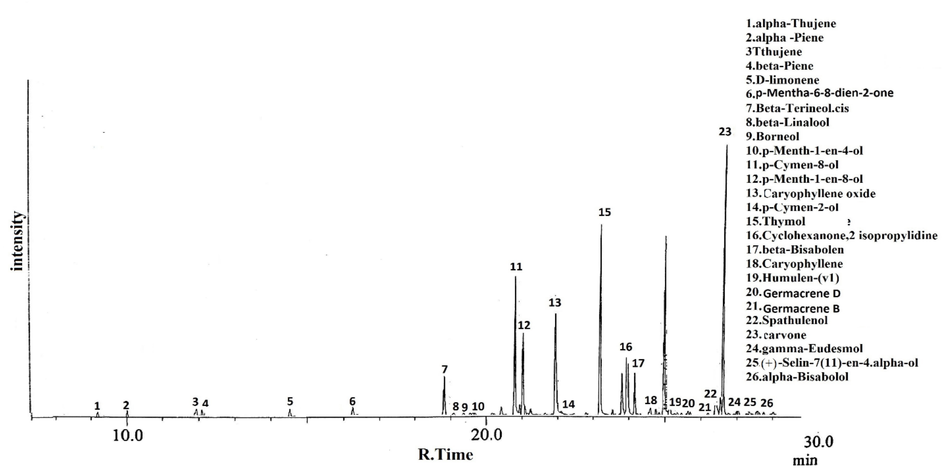

Figure 2: GS-MS chromatogram showing components of *T. linearis* essential oil.

Table 1: Chemical composition (percentage) of the essential oils of ***Mentha arvensis*** and ***Thymus linearis*** based on GC-MS spectrum.

No.	Chemical Component	Mentha arvensis			Thymus linearis		
		Retention indices (Determined)[a]	Retention indices (Literature)[b]	Conc (%)	Retention indices (Determined)	Retention indices (Literature)	Conc (%)
1	α -Thujene	920	925	0.17	938	935	0.07
2	α-Pinene	938	933, 940	0.17	940	NR	0.06
3	Thujene	952	NR	0.05	-	925	-
4	β -Terpineol.cis	-	NR	-	949	NR	0.20
5	o-Methythyol	-	NR	-	959	NR	2.95
6	Thymol methyl ether	-	NR	-	964	NR	0.1
7	β- Pinene	972	973	0.05	-	978	-
8	Bergamiol	-	NR	-	990	NR	7.94
9	p-Cymen-3-ol	-	NR	-	998	NR	9.01
10	d-Limonene	989	NR	0.06	-	NR	-
11	p-Mentha-6,8-dien-2-one	1083	1080	0.05	-	NR	-
12	γ -Terpinene	-	NR	-	1030	NR	2.84
13	β -Terpineol.cis	1103	NR	0.09	-	NR	-
14	β -Linalool	1126	NR	0.1	1065	NR	0.25
15	Borneol	1145	NR	9.56	1159	1167	0.47
16	p-Menth-1-en-4-ol	1169	NR	0.41	1176	NR	0.14
17	p-Cymen-8-ol	1205	NR	1.08	1186	1182	0.12
18	p-Methyl-1-en-8-ol	1242	NR	0.32	-	NR	-
19	Caryophyllene oxide	1275	1581	18.81	-	1584	-
20	p-Cymen-2-ol	1363	NR	20.35	-	NR	-
21	Cyclohexanone, 2-Isopropylidene	1395	NR	13.98	-	NR	-
22	Thymol	1434	1302	0.12	1294	1292	40.9
23	Carvacrol	-	NR		1306	1302	5.42
24	α-Caryophyllene	-	NR		1359	NR	0.31
25	Germacrene d-4-ol	-	1574		1402	NR	0.29
26	Globulol	-	NR		1426	NR	0.06
27	β -Bisabolene	1590	NR	0.18	1450	NR	12.54
28	Caryophyllene	1601	NR	1.61	-	NR	-
29	Humulen-(v)	1629	NR	0.07	1474	NR	0.18
30	Germacrene D	1652	NR	1.54	1494	NR	0.05
31	β -Myrcene	-	NR	-	1502	NR	0.14
32	(+)-4-Carene	-	NR	-	1540	NR	0.17
33	O-Cymene	-	NR	-	1576	NR	14.95
34	Gemecerene B	1765	NR	0.15	-	NR	-
35	Spathulenol	1790	NR	0.69	-	1590	-
36	Carvone	1855	NR	23.53	-	NR	-
37	γ -Eudesmol	1895	NR	0.05	1620	NR	0.49
38	(+)-selin_7(11)-en-4,α-ol	1949	NR	0.26	1697	NR	0.13
39	α-Bisabolol	1993	NR	0.04	-	NR	-

Spectra, NIST and WILEY libraries spectrophotometry with previous literature on QP2020 plus series Shimadzu, microbore capillary column. Retention indices (Determined)[a] was relative to homologous series of n-alkanes (C8–C20) on QP2020 D capillary column. Retention indices (Determined)[b] from literature (Rajeswara Rao et al 2000; Joshi et al., 2019; Naz et al., 2020), NR; not reported in previs 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-Bisbolene 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.

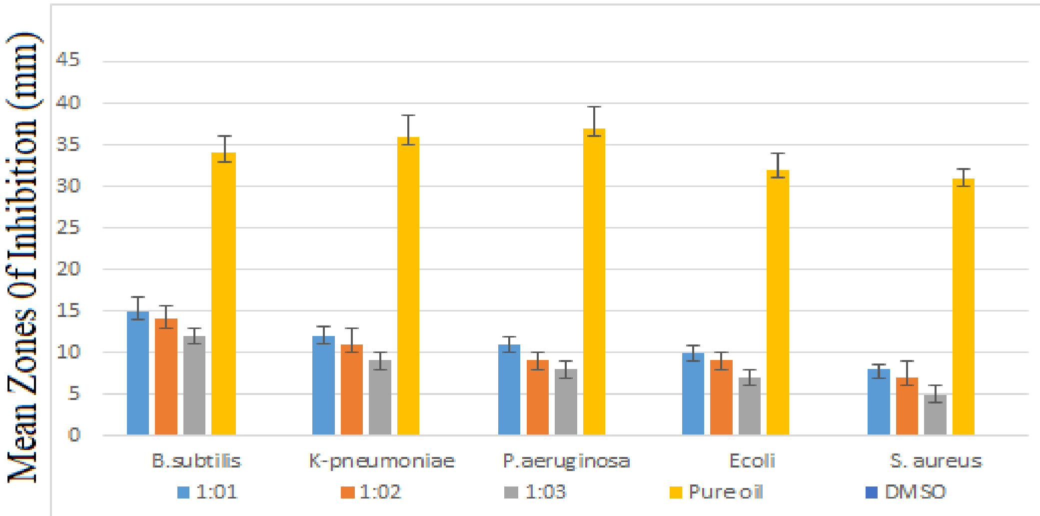

Figure 3: Mean zones of Inhibition in millimeter (mm) obtained with *T. linearis* essential oils against 5 bacterial strains. The largest zones of inhibition were recorded against *B. subtilis* and the moderate zones were obtained against S. aureus. Pure oil shows highest zones of inhibition against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

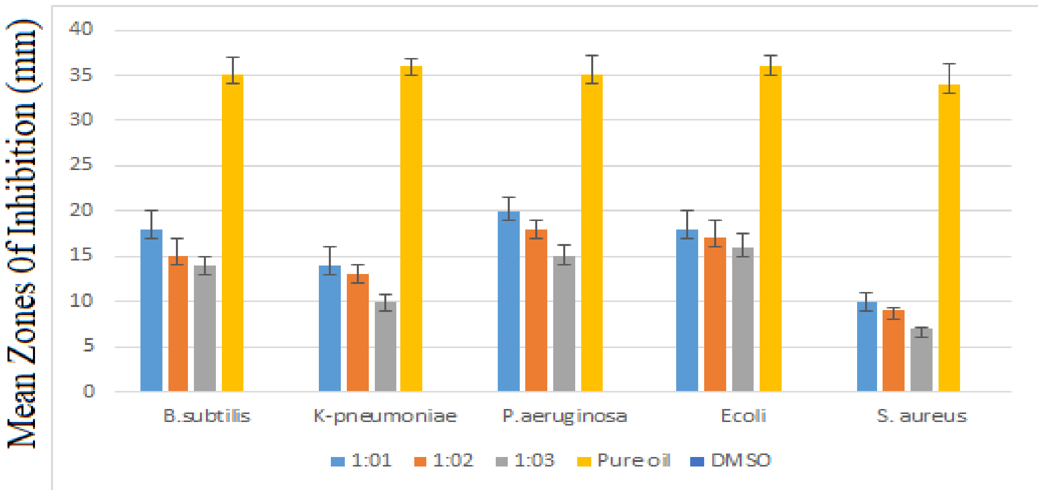

Figure 4: Mean zones of Inhibition in millimeter (mm) obtained with *M. arvensis* essential oils against 5 bacterial strains. The maximum zones of inhibition were obtained against the *B. subtilis*

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 (Biswas *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*, *Klesiella 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 obtainment 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.

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Statement of interest

None.

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