**Major Compounds and Antimicrobial Activity of Essential Oils from Five Iranian Endemic Medicinal Plants**

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**ABSTRACT**

Background: Essential oils are one of the most active components which can be found in medicinal plants. These compounds which are made up of many different volatile compounds possess antimicrobial activity against various microorganisms and have been used since the earliest reported history. Objective: To investigate essential oils of five plant materials (Artemisia sieberi, Cymbopogon olivieri, Haplophyllum tuberculatum, Salvia macrosiphon, Teucrium polium) for antibacterial activities. Materials and Methods: The GC/MS analyses were carried out on herbal extract using a Hewlett-Packard 6890. For investigating the anti-microbial effect, Streptococcus pneumoniae, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Staphylococcus epidermidis were used. Results: Main components of each plant were: Cymbopogon olivieri: piperitone (67.79%); Haplophyllum tuberculatum: borneol (25.73%); Salvia macrosiphon: piperitone (33.16%); Teucrium polium: limonene (37.70%) and Artemisia sieberi: piperitone (34.05%). The best antimicrobial activity by agar diffusion method was respectively belonged to Salvia macrosiphon; Artemisia sieberi and the mixture that had more activity than positive standard. Agar dilution method was used to identify MIC and MBC for each essential oil and the mixture. Best results were: Haplophyllum tuberculatum, Cymbopogon olivieri and Teucrium polium that showed MIC and MBC in 5 µl concentration on Streptococcus pneumoniae and MIC in similar concentration on Staphylococcus aureus. Salvia macrosiphon and Artemisia sieberi showed MIC in 5 µl concentration on Streptococcus pneumoniae. The mixture had good activities on Streptococcus pneumoniae and Staphylococcus aureus. Conclusion: our results and previous works indicate that these essential oils have good antimicrobial activity and have potentials for future works in this field.

Keywords: Artemisia sieberi, Cymbopogon olivieri, Haplophyllum tuberculatum, Salvia macrosiphon, Teucrium polium

**INTRODUCTION**

Essential oils are one of the most active components which can be found in medicinal plants. These compounds which are made up of many different volatile compounds possess antimicrobial activity against various microorganisms and have been used since the earliest reported history. Moreover antifungal, antiviral, insecticidal, food preservation and antioxidant properties are reported from essential oils. Haplophyllum tuberculatum Forssk. (Rutaceae) (Sodabi Jonubi in Persian) is used as a remedy for headaches and arthritis.

The juice is also applied in some countries as a wart removal, skin discoloration, infections and parasitic diseases.
antipyretic effects have been also reported from India.\[^{13}\] Moreover it is one of the main components of cold preparations in Iranian folk medicine.\[^{9}\] Antimalarial\[^{16}\] and antibacterial effects has been reported for several genus of \textit{Artemisia}.\[^{17,18}\] \textit{Artemisia sieberi} Besser (Poaceae) (Dermaneh) is used in cold preparations and also has been reported as an anthelmintic medicine.\[^{9}\] On the other hand its antimicrobial, anti diabetic, poison antidote, antihypertensive and emmenagogue effects have been noted in the literature.\[^{19}\] The combination of all these plants is used as a remedy for after delivery infections. Considering the long time use of above medicinal plants in Traditional Iranian Medicine, in this study essential oils of these plant materials were investigated for antibacterial activities.

**MATERIALS AND METHODS**

**Plant materials:** Plant materials (\textit{Haplophyllum tuberculatum}, \textit{Teucrium polium}, \textit{Salvia macrosiphon}, \textit{Cymbopogon olivieri} aerial parts and \textit{Artemisia sieberi} flowers) have been collected from late May until the end of June 2008 in Evaz of Larestan (south of Fars province, Iran). Plant materials were identified and voucher specimens deposited in Shiraz Faculty of Pharmacy herbarium.

**Essential oil isolation:** Plant materials were air dried at room temperature, powdered (25 g) and subjected to hydrodistillation (250 ml water) for 4 h using a Cleve-enger-type apparatus according to the method recommended in British Pharmacopoeia.\[^{20}\] Mixture was made by mixing similar amount of each essential oil.

**GC-MS analyses:** The GC/MS analyses were carried out using a Hewlett–Packard 6890. The gas chromatograph was equipped with a HP-5M capillary column (phenyl methyl siloxan, 25 m × 0.25 mm i.d., Hewlett–Packard Part No. 190915.433, USA). The oven temperature was programmed from 50 C (3 min) to 250 C at the rate of 3 C/min and finally held for 10 min at 250 C. The carrier gas was helium with the flow rate of 1.2 ml/min. The mass spectrometer (Hewlett–Packard 5973, USA) was working in EI mode at 70 eV. The interface temperature was 250 C; mass range was 30-600 \(m/z\). Identification of components was based on a comparison of their RI and mass spectra with Willey (275) and Adams libraries spectra.\[^{21}\]

**Antimicrobial assay:** For investigating the anti-microbial effect, \textit{Streptococcus pneumoniae}, \textit{Klebsiella pneumoniae}, \textit{Staphylococcus aureus}, \textit{Escherichia coli}, \textit{Staphylococcus epidermidis} and standard agar diffusion method and agar dilution method were used.\[^{22}\]

Blank discs in 6 mm diameter were used in disc plate method. A suspension containing 10\(^8\) of bacteria per ml equivalent to 0.5 standard of McFarland’s tube was prepared from each bacterial strain and was read by spectrophotometer. Then dilutions 1/10 and 1/100 and 1/1000 were prepared from the suspension of initial bacterial population. Desired bacteria were placed on the surface of the plates containing Muller Hinton agar medium (Merck, Darmstadt, De) disk blank. It was done by sterile forceps in a suitable distance of bacteria from each other and from the edge of the contaminate plate. Ten, 5 and 2.5 \(\mu\)l of essential oils and their mixture were poured on the plate environment. Gentamicin was applied as positive control. Plates were incubated at 37°C. Subsequently after 18-24 hours non-growth zone diameter was measured using a caliper.

Pour plate method was used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of essential oils. At first, a suspension of bacteria were made equivalent to McFarland’s pipe number 0.5. Then dilutions 1/10 and 1/100 and 1/1000 were prepared enough with the dilution of about 200 colonies of bacteria on Muller agar plate. The desired dilution of bacteria was 1/1000. Twenty ml of sterile Mueller agar were poured in plates, and then 100, 50, 20, 30 and 10 \(\mu\)l of essential oil of each plant and mixture were added to the plates. After preparing the medium, 0.1 ml of suspension with dilution of 1/1000 bacteria was shed to each plate. The plates were placed in the incubator of 37°C for 24 hours. Each plate that indicates 10 percent of the initial colony amount, this concentration, will be provided as the MIC.

MBC was determined by removing the agar from MIC plate and placing it on a Mannitol Salt Agar (MSA, Merck) and incubated at 37°C for 18 to 24 hours. Unchanged of media color was defined as the lowest concentration of crude extract with no bacterial growth.\[^{22}\]

**RESULTS AND DISCUSSION**

Essential oil of \textit{Cymbopogon olivieri} was obtained with yield of 1.1% (v/w). The identified components account for 96.91% of total. The major components of essential oil were piperitone (67.79%), elemol (12.27%) and \(\beta\)-eudesmol (3.67%) (Table 1). Monoterpene hydrocarbons were 2.67%, Oxygen containing monoterpenes were 65.36%, Sesquiterpene hydrocarbons were 3.99% and Oxygen containing sesquiterpenes were 19.92%. Other reports show that main components of plant’s essential oil are piperitone, \(\alpha\)-terpinene, limonene, elemol,\[^{13}\] \(\alpha\)-3-carene and \(\beta\)-eudesmol\[^{23}\] or torreyol and \(\alpha\)-cadinol.\[^{24}\] Disk diffusion method showed that \textit{C. olivieri} have antimicrobial activity against all microorganisms and the best result was for \textit{Streptococcus pneumoniae} where the effects of essential oil and gentamicin were similar. MIC and MBC tests showed that the best
Major components of essential oil were piperitone (33.16%), β-caryophyllene (26.04%) and caryophyllene oxide (14.39%); (Table 1). Monoterpane hydrocarbons were 7.25%, Oxygen containing monoterpenes were 28.1%, Sesquiterpene hydrocarbons were 31.34% and Oxygen containing sesquiterpenes were 14.03%. Major components of essential oil in other reports are β-cubebene, cyperene, germacrene B, β-selinene, α-farnesene, γ-gurjrene and (+)-aromandendrene[25] or linalool, hexyl hexanoate, hexyl isovalerate, hexyle-2-methyl-
butanoate, hexyl octanoate and sclareol.[26] Disk diffusion method showed that *S. macrosiphon* has antimicrobial activity against all tested microorganisms and the best result was for *Streptococcus pneumoniae* where the effect of essential oil was better than gentamicin. MIC and MBC test showed that the best result was for *Streptococcus pneumoniae* and results for *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* were similar. To the best of our knowledge no particular antimicrobial activity study for *Salvia macrosiphon* has been done.

*Artemisia sieberi* essential oil was obtained by 0.43% (v/w) yield and 90.11% of components were identified. Major compounds components were piperitone (34.05%), camphor (17.68 %) and 1, 8-cineole (9.55%) and their categories were monoterpenes hydrocarbons 9.13%, Oxygen containing monoterpenes 61.93% and Oxygen-containing sesquiterpenes 6.06% (Table 1). Major components which have been derived from *A. sieberi* in other studies are 1,8-cineol, myrcene, eudesm-7(11)-en-4-ol, 4-terpinyl acetate, davanone and γ-cymene[27] or bornyl acetate[28] or α-pinene and comphene[29] or α-thujone, β-thujone, camphor, verbenol and γ-mentha-1,5-dien-8-ol[30] or terpinen-4-ol.[31] Table 2 shows that *Artemisia sieberi* has potent activity against *Streptococcus pneumoniae* when compared to control and *Staphylococcus aureus*. Minimum concentration in MIC and MBC tests were for *Streptococcus pneumoniae* (Table 3). Some popular microorganisms which are sensitive to *A. sieberi* are *Pseudomonas aeruginosa*,[32] *Listeria monocytogenes*, *Bacillus cereus* and *Streptococcus mutans*.[33]

Oil yield was 0.33% for *Haplophyllum tuberculatum* and 91.26% of oil components were identified (Table 1). Components of *Haplophyllum tuberculatum* essential oil were borneol (25.73%), bornyl acetate (18.07%) and α-pinene (14.00%); containing: 23.99% monoterpenes hydrocarbons, 50.99% Oxygen containing monoterpenes, 7.43% sesquiterpene hydrocarbons and 1.74% Oxygen containing sesquiterpenes. Main components of the *Haplophyllum tuberculatum* in other reports are β-phellandrene, limonene, (2)-β-ocimene, β-caryophyllene, myrcene and α-phellandrene[7] or γ-3-carene, linalyl acetate and α-terpineol.[34] Table 2 shows that *H. tuberculatum* has mild antimicrobial activity on microorganisms. MIC and MBC tests showed that the best result was for *Streptococcus pneumoniae* and *Staphylococcus aureus*, respectively (Table 3). *H. tuberculatum* inhibits the growth of *Escherichia coli, Salmonella coleraesuis, Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa*. [7]

Yield for *Teucrium polium* volatile oil was 0.34% (v/w). Major components of essential oil were limonene (37.70%), 2,4-di-tetra-Butylphenol (10.81%) and p-Cymene (8.20%) including Monoterpene hydrocarbons (51.00%), Oxygen containing monoterpenes (1.5%), sesquiterpene hydrocarbons (6.10%) and Oxygen-containing sesquiterpenes
Major reported components of essential oils of *Teucrium polium* are α-pinene, linalool, cariophylene oxide, β-pinene, β-cariophylene [35] or myrcene, germacrene D and α-cadinol [36] or 3β-hydroxy-α-murolene [37] or ρ-cymene [38]. Table 2 shows that *Teucrium polium* has mild antimicrobial activity on microorganisms. MIC and MBC tests indicated that the best result was for *Streptococcus pneumoniae* and *Staphylococcus aureus*, respectively (Table 3). The extract of *Teucrium polium* was effective on *Bacillus anthracis*, *Bordetella bronchiseptica* and *Salmonella typhi* [39]. Another study showed better effectiveness of this plant against *Staphylococcus epidermidis* when compared with gentamicin [40].

Mixture of all these essential oils has antimicrobial activity against all microorganisms and the best result was for *Streptococcus pneumoniae* and *Staphylococcus aureus*, respectively. The best antimicrobial activity by agar diffusion method was respectively belonged to *Salvia macrosiphon, Artemisia sieberi* and the mixture that had more activity than positive standard.

Agar dilution method was used to identify MIC and MBC for each essential oil and the mixture. Best results were obtained for *Haplophyllum tuberculatum, Cymbopogon olivieri* and *Teucrium polium* that showed MIC and MBC in 5 μL concentration on *Streptococcus pneumoniae* and MIC in similar concentration on *Staphylococcus aureus*. *Salvia macrosiphon* and *Artemisia sieberi* showed MIC in 5 μL concentration on *Streptococcus pneumoniae*. The mixture has good effect on *Streptococcus pneumoniae* and *Staphylococcus aureus*.

Differences between our results and previous works may be due to differences between position and time of plant collecting that can lead to changes in constitutes. But overall, our results and previous works indicate that these essential oils have good antimicrobial activity and have potentials for future works in this field.

### Table 3: Antimicrobial activity of essential oil by agar dilution method

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<tr>
<th>Bacteria</th>
<th>Plant</th>
<th>MIC (μL)</th>
<th>MBC (μL)</th>
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## REFERENCES