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Original Research

Analysis of Essential Oil Composition and Antimicrobial Effect of *Stachys discolor* subsp. *mazandarana*

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Abstract

Research on the antimicrobial effects of herbal extracts and natural compounds has proven that herbs have valuable sources of anti-infective agents, and numerous new compounds have been introduced to the market in this regard. The aim of the present study was to investigate the antimicrobial effects of *Stachys discolor* subsp. *mazandarana* and to analyze the essential oil constituents of this herb. Antimicrobial effects of ethanol (80%) extract and n-hexane, and chloroform fractions were investigated against three gram-positive bacteria (*Staphlococus aureus, Streptococus mutans,* and *Bacillus subtilis*), one gram-negative bacterium (*Escherichia coli*), and also a fungus (*Candida albicans*) by well plate and agar dilution methods and diameter of the zone of inhibition was determined. The essential oil of the herb aerial parts was extracted by water distillation method and the essential oil compounds were analyzed by GC-Mass. The results of the present study showed that total extract of *S. discolor subsp. mazandarana* and its various fractions had almost no antimicrobial activity and their minimum inhibitory concentration (MIC) was more than 100 mg / ml. Analysis of essential oils showed that anymol (alpha-bisabolol) (18.10%) and curcumene (13.20%) were the most abundant components. The results revealed that the studied herb had no antibacterial and antifungal effects. However, given the compounds in the essential oil of this herb, it can be supposed that it may have significant analgesic and anti-inflammatory effects that can be investigated in future studies.

Keywords: Antimicrobial; Alpha-bisabolol; Curcumene; Essential oil; *Stachys*

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Introduction

Diseases caused by pathogenic microorganisms cause a lot of damage to human society every year. In response to these microorganisms, humans are trying to reduce the complications caused by their pathogenicity by using antibiotics. However, the incidence of microbial resistance over the years has led to the need to find new antimicrobial compounds, among which natural compounds are important sources of antimicrobial agents. *Stachys* (Lamiaceae family) are widely distributed worldwide, and more than 300 species of this herb grow around the world. The herbs of this genus have a relatively high distribution in Iran, with about 34 species of this perennial herb being identified in the country [1]. Different species of this plant are important in Iranian traditional medicine: *Stachys lavandulifolia* (mountain tea) is used as a sedative for gastrointestinal pains [2]. *Stachys officinalis* is also used as herbal incense in a complex with other herbs [3]. Its aerial parts are used to treat headaches, nervous disorders, indigestion, and to develop moderate diuresis. In addition, its infusion has disinfectant, anti-diarrhea, anti-inflammatory, antispasmodic, expectorant effects and is also used in the treatment of colds according to local documentation and reports [4-6]. Previous studies have also reported the anti-helicobacter effect of different species of this genus [7].

Numerous studies have also reported significant anti-inflammatory and analgesic effects, as well as significant antimicrobial effects of different species of herbs of this genus [8,9].

Stachys discolor subsp. *mazandarana* is an endemic subspecies of Iran and there has been no study on this herb so far. Studies on other species of this genus have shown that hydrocarbon compounds of sesquiterpens constitute the highest amount of essential oils [10-12]. Since these compounds can also have antimicrobial property, *S. discolor* subsp. *mazandarana* was studied for its antimicrobial effects and the essential oil of this herb was also analyzed by GC-Mass to determine its main compounds.

Methods

Stachys discolor subsp. *mazandarana* was collected from Sangedeh Region of Mazandaran Province in June 2015 and was identified and deposited in the Herbarium *Ministrii Iranici Agriculturae* (Iran) (Code No. IRAN70089).

Chemicals and Media

The microbial organisms including *Staphylococcus aureus* ATCC 6538, *Streptococcus mutans* ATCC 35668, *Bacillus subtilis* ATCC 6633 as Gram positive bacteria; *Escherichia coli* ATCC 8739 as Gram negative bacteria; *Candida albicans* ATCC 10231 as fungi were obtained from Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences. Mueller-Hinton agar (MHA), Caso agar, Caso broth, Mannitol salt agar (Merck, Germany) was used as medium for the growth of bacterial and fungal strains.

Extraction and Preparation of Fractions

A total of 80 grams of the studied herb was powdered and extracted by maceration method using 80% ethanol 3 times with a 3-day interval. After extracting and condensing the extract by rotary evaporation, part of the total extract was kept for antimicrobial testing and the remaining part was fractioned with n-hexane and chloroform solvents. The resulting fractions were then dried at temperatures below 40 °C and stored in the freezer until microbial studies.

Essential Oil Extraction

A total of 100 g of flowering aerial parts of the herb were transferred to a balloon and 1 liter of distilled water was added. Clevenger was used for distillation and the essential oil was collected after 4 hours. The essential oil was dehydrated by anhydrous sodium sulfate and analyzed by GC-Mass.

Analysis and Identification of Essential Oil Components

The resulting essential oil was analyzed by GC-Mass. We used a GC-Mass gas chromatograph equipped with a non-polar DB1 column (30 m in length, 0.25 µm in diameter, and static layer thickness of 0.25 µm) with helium gas at a flow rate of 1 mm / min. The process started at 50°C and ended at 30°C.

To identify the compounds, the mass spectra of the sample compounds were compared with the standard spectra of the Wiley software library. To ensure the similarity, the standard mass spectra in the Adams book were also checked [13]. Moreover, Relative inhibition value (Kovats index) was also used along with the mass spectrum. The standard inhibition index was calculated based on C9-C20 series of alkanes injected under the same conditions and compared with standard reference numbers.

Antimicrobial Effect

The antimicrobial property of total extract and resulting fractions were studied using agar dilution method. Consecutive concentrations of the prepared fractions (0.78, 1.5, 3.125, 6.25, 12.5, 25, 50, and 100 mg / ml) were prepared in a solid medium. Then different strains were cultured on the solid medium. After incubation, the bacteria were incubated at 37 ° C for 24 hours and the fungus at 25 ° C for 48 hours. The lowest concentration that inhibited the growth of the microorganism was reported as MIC [14].

Results

The essential oil obtained from flowering aerial parts of *Stachys discolor* subsp. *mazandarana*, a pale yellow with a lemon-like aroma and with a yield of 0.5% V / W, was analyzed. Table 1 shows the essential oil compounds and their percentages.

Thirty-eight compounds (98.2%) of all essential oil components were identified. The main compounds included bisabolane-type sesquiterpene, anymol (alpha-bisabolol) (18.10%) and curcumene (13.20%). Other important compounds, which were less abundant included 8-epi-alpha-bisabolol (9.86%), alpha-caryophyllene (5.95%), and caryophyllene (4.81%) (Figure1). The total extract and its fractions were used to evaluate the antimicrobial effect using well plate method and to ensure that the solubility of the extract in the culture medium did not affect the final result, agar dilution method was used. The results of antimicrobial effect analysis using both well plate and agar dilution methods showed that MIC of the total extract and fractions of *Stachys discolor* subsp. *mazandarana* on 5 important pathogens (including E. *coli, S. aureus, S. mutans, B. subtilis,* and *C. albicans*) is more than 100 mg / ml and almost all of these extracts lack good antimicrobial activity.

Discussion and Conclusion

Several studies have reported that various species of the *Stachys* genus have significant antimicrobial effects [10-12]. The phytochemicals of *Stachys* herbs include many compounds such as flavonoids, iridoids, phenylethanoid glycosides, and terpenoids, including monoterpenes, diterpenes and sesquiterpene terpenes [9]. The main components of essential oils of *Stachys* have been reported in several studies. The essential oils of *Stachys racta* include linalool, beta-pinene and *Stachys balansae* include β-caryophyllene, α-pinene, and β-pinene [15]. Bayat et al. (2015) reported the antibacterial effects of methanolic and aqueous extract of *Stachys schtschegleevii* against UTI- causing bacteria [16]. Another study also investigated volatile compounds of 8 different *Stachys* species and their antimicrobial effects on 6 bacteria and 5 fungi. Analysis of volatile compounds showed

Table1. Essential oil composition of *Stachys discolor* subsp. *mazandarana*

RI[a]	Components	Retention Time (min)	Percentage (%)
1097	limonene	8.626	0.44
1164	Myrtenil	17.506	0.96
1177	Carvacrol	20.31	0.92
1884	Isodurol	20.573	0.50
1189	Myrtenyl	21.191	1.56
1230	alpha-linalool	22.811	0.48
1253	Caryophyllene	23.715	4.81
1265	Aromadendrene	24.207	0.63
1290	alpha-caryophyllene	24.584	5.95
1299	beta-Gurjnene	24.733	0.77
1313	Isolongifolene	25.134	1.73
1338	Germacrene	25.185	1.14
1349	Curcumene	25.265	13.20
1419	Epiprezizene	25.322	1.70
1443	Alloaromadedrene	25.403	0.83
1445	alpha-Farnesene	25.528	0.96
1481	beta-Gurjunene	25.586	0.28
1483	beta-Bisabolene	25.895	0.87
1494	alpha-Cedrene	25.975	1.27
1494	Germacrene-D	26.066	0.96
1500	7-epi-alpha-Selinene	26.169	1.0
1506	delta-Cadinene	26.278	3.81
1512	alpha-Bergamotene	26.759	2.05
1523	cis-Carvyl acetate	27.039	0.70
1563	Bergamotol	27.165	0.74
1579	trans-Carane	27.543	0.26
1583	Spathulenol	27.611	2.93
1593	Caryophllene-oxide	27.749	4.23
1685	Selina-3,7(11) diene	28.241	1.46
1756	Humulene-6,7 epoxide	28.355	0.19
1764	beta Elemene	28.819	0.09
1789	Bisabolene	28.887	1.70
1791	shyobunol acetate	29.076	1.0
1801	beta-Panasisene	29.379	1.36
1832	g-Seliene	29.482	2.76
1846	beta-Bisabolene	29.705	5.42
1849	Anymol (alpha-Bisabolol)	29.980	18.10
1851	8-epi-alpha-Bisabolol	30.192	9.68

[a] RI: Retention Indices

that sesquiterpene hydrocarbons were the main constituents of all species studied. The results of antimicrobial study showed that the essential oils of these herbs showed better effect on different bacterial strains and *S. scardica* essential oil has been effective on both bacteria and fungi [8].

The study of essential oils constituents of *Stachys* discolor subsp. *mazandarana* showed that the major constituents in the essential oil belongs to oxygenated sesquiterpenes with anymol (18.10%) and curcumene (13.20%) and caryophyllene being the most abundant components of the essential oil. The percentage of oxygenated sesquiterpenes in this essential oil is similar to the essential oil of *S. acerosa* Boiss., which has been studied previously [12].

Anymol, which is the most abundant component of the essential oil, is one of the constituents of sesquiterpenes and is found abundantly in the rosemary herb and is one of the main compounds for identification the above herb. This compound has potent anti-inflammatory, analgesic, and antiprolifrative properties [17]. Another compound that accounts for a significant percentage in the essential oil analysis is curcumene, which belongs to sesquiterpenes [18]. This compound has also been identified in ginger and sandalwood oil whereas anti-inflammatory property of sesquiterpens has been reported in some studies [19,20]. Another compound that comprises a relatively high percentage of herb essential oils is caryophyllene, which is a bicyclic sesquiterpene. Considering its anti-inflammatory, analgesic, and antipyretic properties, this compound can be considered in the production of non-steroidal analgesics through inhibition of prostaglandin production by inhibiting cyclooxygenase [21,22].

Considering the results of investigating essential oil compounds and the identification of significant percentages of anymol, curcumene, caryophyllene, as well as the common interest in using indigenous herb growth in treating inflammatory diseases, it can be concluded this herb may be of interest considering its analgesic and anti-inflammatory effects, and therefore, its usability must be explored in further studies. After comparing the results on the one hand, and since carvacrol has been considered as a compound responsible for the antimicrobial effects in other species according to most reports and the percentage of the above compound in the essen-

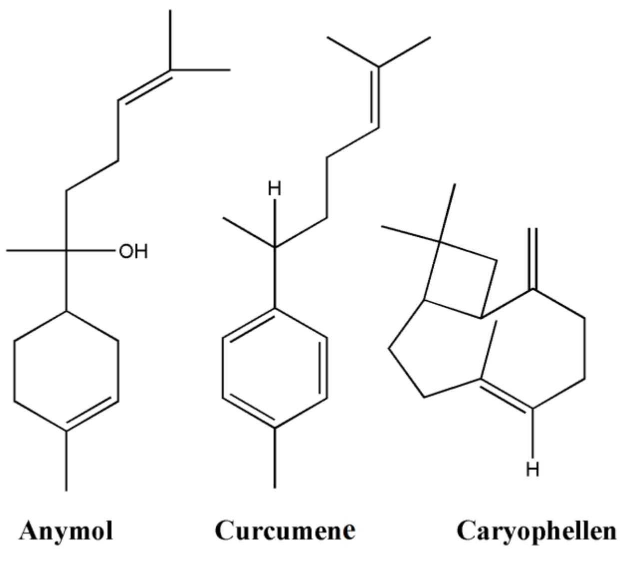

Figure1. Chemical structures of the main components of *Stachys discolor* subsp. *mazandarana* essential oil.

tial oil of this species was 0.92% on the other, we can justify the weak antimicrobial effect in this species as compared to other ones [23].

Results of studying of essential oil of *Stachys discolor* subsp. *mazandarana* and identifying its components showed that the herb with a low percentage of important antimicrobial agents such as carvacrol and phenol displayed minor antimicrobial effects in this study. On the other hand, the presence of analgesic and inflammatory effects of the major substances in this essential oil may justify its use in inflammatory diseases.

Conflict of Interest

None.

Acknowledgment

None.

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