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SHORT COMMUNICATION

CHEMICAL CONSTITUENTS AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OF *SATUREJA BACHTIARICA* (LAMIACEAE)

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The genus *Satureja* L. (Lamiaceae) consists of more than 200 species of herbaceous perennials worldwide. The Mediterranean region can be described as the center of the genus (1). This genius in flora of Iran is represented by fourteen species distributed commonly in rocky mountains (2, 3). Nine *Satureja* species has been reported in "Flora Iranica" (4), two of which, *S. kallarica* Jamzad and *S. bachtiarica* Bunge are distributed in Chaharmahal va Bakhtiari province, Southwest Iran (4). The areal parts and volatile constituents of savory are used as a medicinal herb. *Satureja* species are commonly used for herbal tea, flavoring agents (condiment and spice) and medicinal purposes (5). Infusion and decoction of aerial parts of *Satureja* species are used to produce a tonic, carminative, digestive and expectorant and for the treatment of colds in Iranian traditional medicine (5, 6). The essential oils composition, antimicrobial and antioxidant activities of some *Satureja* species have been studied (7-17). The previous study showed that essential oil and extract of *S. bachtiarica* exhibited antimicrobial activities against *E. coli* O157:H7, *Bacillus cereus* and *Listeria monocytogenes* (18), *Bacillus subtilis, Bacillus cereus, Micrococcus luteus, Staphylococcus* sp. and *Staphylococcus aureus, Klebsiella pneumoniae, Klebsiella oxytoca* and *Pseudomonas aeruginosa* (19), *Vibrio parahaemolyticus* and *Vibrio harveyi* isolated from infected fishes (20), *Candida albicans* (21) and *Streptococcus iniae* (22). Previous results (23) showed that the essential oil of *S. bachtiarica* affected on immune system and growth of rainbow trout (*Oncorhynchus mykiss*). The essential oil of *S. bachtiarica* strong aromatic odor is due to the presence of volatile oils especially carvacrol, γ-terpinene and thymol (19). To our knowledge, there are no published reports on the chemical composition, and antibacterial activity of the essential oil of *S. bachtiarica* against *Pseudomonas aeruginosa* isolated meat here described. For this reason, the chemical composition of this oil was analyzed by gas chromatography-mass spectroscopy.

EXPERIMENTAL

Plant material

The aerial parts (up to ~ 5 cm, 200 g) of wild population of *Satureja bachtiarica* were collected from Chaharmahal va Bakhtiari province, Southwest of Iran in July 2011. The sample of the plant was identified by regional floras and authors with floristic and taxonomic references (2), and voucher specimen was deposited at the Herbarium of Research Center of Agricultural of Chaharmahal va Bakhtiari province, Iran (No. 1999).

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Sample preparation

Harvested aerial parts (leaves and stems) were dried at room temperature for one week. Dried plant materials were powered (100 g) and subjected to hydro-distillation (1000 mL distilled water) for 3 h using a Clevenger-type apparatus according to the method recommended by British Pharmacopoeia Commission (24).

Bacteria strain

Clinical isolates of *Pseudomonas aeruginosa* (Gram-negative) bacteria strain were obtained from Food Microbiology Laboratory, Food Medicine Faculty, (I.A.U.) Iran. Bacteria strain was identified using polymerase chain reaction – restriction fragment length polymorphism (PCR–RFLP) and conventional morphological as well as biochemical tests. Stock cultures of bacteria were kept in 20% glycerol PBS (phosphate buffered saline) at -70°C. Active cultures were generated by inoculating 100 µL of the thawed microbial stock suspensions into 5 mL nutrient broth (Merck, Germany) followed by overnight incubation at 37°C. The density of bacteria culture required for the test was adjusted to 1.0 McFarland standards, (1.0×10^7 cfu/mL) measured using the spectrophotometer (Eppendorf AG, Germany).

Antibacterial activity with disc diffusion assay

These experiments were performed by the disc diffusion method (25, 26) with some modification. Sterile paper discs (6 mm in diameter) were impregnated with 60 µL of dilutions of known EO concentrations (0.08-0.50 mg/µL) and incubated at 37°C for 24 h. Bacterial growth inhibition was determined as the diameter of the inhibition zones (DIZ) around the discs (mm). The growth inhibition diameter was an average of three measurements, taken at three different directions.

Determination of minimum inhibitory concentration (MIC)

The MIC values were evaluated using the broth serial dilution method according to standard methods (27). Bacterial strains were cultured overnight at 37°C in Mueller Hinton broth (MHB, Oxoid). Stock solution of the essential oil was prepared in 5.0% (v/v) dimethyl sulfoxide (DMSO). Dilution series, using MHB, were prepared from 1 to 0.016 mg/mL. After incubation at 37°C for 24 h, the microorganism growth inhibition was evaluated by measuring absorbance at 630 nm, using a spectrophotometer. Experiments were performed in triplicate but at three different times.

Food sample preparation

Minced beef sirloin was purchased at husbandry farm of university, and brought to laboratory. Beef grounded contains 7% protein, 8% fat, 1% ash and 73% moisture. The minced beef was exposed to UV light ($\lambda = 260$ nm) for 10 min to kill surface contaminants.

Treatments of meat samples

The inhibitory effect of essential oil (0.1, 0.05 and 0.025% w/w) of *S. bachtiarica* applied to the minced beef (10 g) was determined after *P. aeruginosa* inculcation (10^3 cfu/g 10 g meat), and stored for 14 days at 5°C. A control consisted of cow meat inoculated with *P. aeruginosa* with no essential oil. Inoculated samples in Petri dishes were left undisturbed for 30 min to allow residual moisture to be absorbed. The number of *P. aeruginosa* on the cow meat was determined with plate count method at 1, 3, 5, 7, 10 and 14 days (5°C) of storage. Thus the samples were serially diluted (1 : 10), and 100 µL was spread plated onto BHI agar (Merck, Germany). The plates were incubated at 37°C for 24 h to determine the population of *P. aeruginosa*. Selected presumptive colonies of *P. aeruginosa* were confirmed by biochemical tests (28) and polymerase chain reaction – restriction fragment length polymorphism (PCR–RFLP). Three replications of the treatments were performed.

Gas chromatography/mass spectrometry (GC/MS) analysis

The essential oil was analyzed using an Agilent 7890 A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HP-5MS 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm, 0.25 µm film thickness). Oven temperature was kept at 60°C for 4 min initially, and then raised at the rate of 4°C/min to 260°C. Injector and detector temperatures were set at 290 and 300°C, respectively. Helium was used as carrier gas at a flow rate of 0.8 mL/min, and 0.5 µL samples were injected manually in the splitless mode. Peaks area percents were used for obtaining quantitative data. The GC/MS analyses were carried out using an Agilent 5975 C apparatus (Agilent Technologies, Palo Alto, CA, USA) with a HP-5MS 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm, 0.25 µm). Mass range was from *m*/*z* 50 to 550 amu. Retention indices were calculated for all components using a homologous series of *n*-alkanes (C_5-C_{24}) injected in conditions equal to those for samples. Identification of oil components was accomplished based on com-

parison of their retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (WILEY /ChemStation data system) (29, 30).

Statistical analyses

The data were statistically analyzed using one-way ANOVA by the program SPSS (17.0), and comparison of the means of the main constituents of essential oils were evaluated by Duncan's multiple range test at $p < 0.05$ level.

RESULTS AND DISCUSSION

EO yield and chemical composition

The yellow oil of *S. bachtiarica* was obtained by hydro-distillation in the yield of 1.55% based on dry weight. Yield of essential oil of *S. bachtiarica* (1.55%) in this study was similar to these reported by Sefidkon et al. (31) for oil yields of *S. bachtiarica* (2.15% for Yazd sample and 1.65% for Fars sample). Also, the yield of the oils extracted from other species was: 2% from *S. laxiflora* C. Koch (15), 2-3% from *S. khuzestanica* Jamzad (11, 19, 32) and 3.3-1.7% from *S. sahendica* Bornm. (33). Yield and chemical composition were expected as they are affected by several factors, such as genotype, ecological conditions and cycle stage (34). The chemical constituents identified by GC and GC/MS as the results concerning the qualitative and quantitative analysis of the essential oil are presented in Table 1. Twenty-one compounds were identified, representing 98% of the essential oil, in which the major components of the essential oil of *S. bachtiarica* were: carvacrol (7.7%), γ-terpinene (6.9%), *p*-cymene (40.4%), thymol (17.9%), geraniol (4.6%), linalool (5.7%) and borneol (4.6 %); in most of essential oils monoterpenes and sesquiter-

Table 1. Chemical composition of essential oil of *Satureja bachtiarica*

No.	Components	R.I[a] (det.)	R.I[b] (lit.)	Peak area[c] (%)
1	α-Thujene	931	929	0.27
2	α-Pinene	934	939	1.70
3	Comphene	953	953	1.58
4	β-Pinene	979	980	0.21
5	β-Myrcene	994	991	0.85
6	α-Terpinene	1019	1018	1.16
7	***p*-Cymene**	1025	1026	**40.46**
8	Limonene	1030	1031	0.62
9	γ-Terpinene	1054	1062	**6.89**
10	Linalool	1095	1098	**5.65**
11	Borneol	1161	1165	4.63
12	Terpinen-4-ol	1175	1177	0.89
13	β-Fenchyl acetate (endo)	1219	1220	0.31
14	Neral	1238	1240	0.24
15	Geraniol	1258	1255	**4.60**
16	**Thymol**	1283	1290	**17.93**
17	**Carvacrol**	1294	1298	**7.75**
18	Thymol acetate	1359	1355	0.19
19	Caryophyllene <(*E*)->	1398	1404	1.27
20	Caryophyllene oxide	1573	1581	0.37
	Total	**97.57**		

[a] RI: Retention indices determined on HP-5MS capillary column. [b] Retention index (literature). [c] Calculated from TIC data.

Table 2. Antibacterial activity of essential oil of *Satureja bachtiarica* and antibiotic against *Pseudomonas aeruginosa*.

Treatments	Concentration (% w/w)		
	0.1	0.05	0.025
S. bachtiarica	30.00 ± 1.01†	20.33 ± 3.21	16.3 ± 1.15
Erythromycin	15 ± 4.35		
Ethanol 70% (as solvent)	11.33 ± 0.58		

†: Mean ± SD in mm

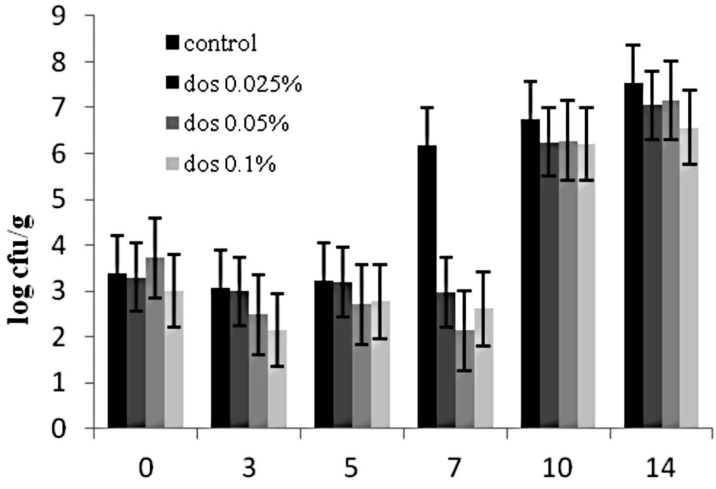

Figure 1. *Pseudomonas aeruginosa* population count (count ± S.D.) (log10 cfu/g) for various times when inoculated by *P. aeruginosa* on beef meat (control: no essential oil; dos: essential oils at concentrations 0.025, 0.05 and 0.1%, w/w)

penes were the main constituent groups (Table 1). The following components have been identified in the *S. bachtiarica* EO: thymol (44.5%), γ-terpinene (23.9%), *p*-cymene (7.3%), *(E)*-caryophyllene (5.3%) and borneol (4.2%) (10). Accession of *S. bachtiarica* from presented carvacrol (49.3%), *p*-cymene (12.7%) and (*E*)-α-bergamotene (5.8%) for Fars population and, carvacrol (66.5%), *p*-cymene (15.2%) and linalool (4.6%) for Yazd population (31). Sefidkon et al. (19) reported that main components of essential oil of *S. bachtiarica* were: *p*-cymene (36.5%), carvacrol (20%), thymol (19%) and γ-terpinene (9.1%) before flowering and *p*-cymene (25.2%), carvacrol (25.8%), p-menth-3-en-8-ol (18.5%), borneol (6%) and thymol (5%) at full flowering stage. The major compounds characterized in *S. bachtiarica* essential oil grown in central Zagros Mountains, Iran, were thymol, carvacrol and *p*-cymene.

Antibacterial activity with disc diffusion assay

P. aeruginosa isolated from beef meat was tested for its sensitivity to essential oil of *S. bachtiarica*. The potency was initially determined by the agar diffusion method. Diameter of inhibition zones (clear zones around discs) exerted by the different concentrations of the essential oil and standard antibiotic discs towards *P. aeruginosa* is presented in Table 2. The antibacterial activity of essential oil of *S. bachtiarica* against *P. aeruginosa* have inhibition zone ranging from 16 to 30 mm. The diameters of inhibition zones were significant (p = 0.05) as compared to standard antibiotic discs. Crude *S. bachtiarica* essential oil inhibited the growth of test bacteria (*P. aeruginosa*) isolated from cow meat. Percentage inhibition increased with the concentration. At a concentration of 5 mg/mL, bacteria were moderately inhibited, while at a concentration of 10 mg/mL, the crude essential oil completely inhibited the

growth of the bacteria. Antibacterial effect observed in this study is in accordance with other studies (19, 21). The result of identification of phenolic compounds using GC-MS showed that the major components of *S. bachtiarica* were carvacrol and thymol (10). Some studies claim that the phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects (35). Previous studies (36) showed that a highly positive linear relationship exists between antioxidant and antimicrobial activity and total phenolic content in some spices and herbs. Many aromatic plants essential oils, for example *S. bachtiarica,* contained high levels of phenolics and exhibited antimicrobial activity (10).

Determination of minimum inhibitory concentration (MIC)

The results of serial dilution showed that *P. aeruginosa* strain was sensitive to the essential oil of *S. bachtiarica* and MIC value was 31 µg/mL. Integrated results of both assays, indicate high antibacterial activity of essential oil of *S. bachtiarica*. Probably, this is correlated with the differences found in chemical composition from essential oil, especially carvacrol, thymol and *p*-cymene, which are known as good phytoalexins (37). Phenolic compounds are widely known for their beneficial effects, such as preventing hormone-related cancers, potent antioxidant, and antibacterial agents (38).

Growth of *P. aeruginosa* populations in meat

In one day after inculcation, there was no significant difference in the populations of *P. aeruginosa* (3 to 3.4 $\log_{10}$ cfu/g) (Fig. 1). In three days, treatments had significant difference ($p \leq 0.05$) in *P. aeruginosa* population (2.1 to 3.1 $\log_{10}$ cfu/g). In seven days, oil treatments were able to reduce significantly ($p \leq 0.05$) the *P. aeruginosa* populations by 2.1 $\log_{10}$ cfu/g to 6.1 $\log_{10}$ cfu/g as compared to control (Fig. 1). In five and ten days, there was no significant difference in the populations of *P. aeruginosa*. 0.1% Bakhtiari savory oil treatment was able to reduce significantly the *P. aeruginosa* populations by 6.5-7.6 $\log_{10}$ cfu/g as compared to control after 14 days of storage at 5°C. During 14 days of storage of control (without oil) meat at 5°C, *P. aeruginosa* populations increased by 3.4-7.6 $\log_{10}$ cfu/g (Fig. 1). Mytle et al. (39) reported that the application of 1% clove oil (v/w) to frankfurter surfaces or the inclusion of cloves or clove oil in the frankfurters, coupled with low temperature storage, can reduce the potential of *L. monocytogenes* contamination and growth without significantly changing flavor. Also, the results of other study (40) showed that application of 1% (v/w) essential oil of *S. bachtiarica* to frankfurter surfaces can reduce population's *L. monocytogenes* as compared to control after 14 days of storage at 4°C.

CONCLUSION

The results of GC-MS analysis of *S. bachtiarica* essential oil showed major compounds as *p*-cymene, thymol, carvacrol, γ-terpinene, geraniol, linalool and borneol. The essential oil of *S. bachtiarica* possesses good antibacterial activity. This study can be considered as the first report on the *in vitro* antibacterial activity against *P. aeruginosa* isolated from meat properties of the essential oil prepared from *S. bachtiarica*. We hope that our results introduce a unique natural source which possesses strong antimicrobial substances. However, essential oil of *S. bachtiarica* is accessible and with optimization of the concentration can be used in meat and meat industry as a natural preservative.

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