Evaluation of Antioxidant and Antimicrobial Activity of Satureja mutica Fisch. & C.A.Mey. Collected from North Khorasan Province, Iran

Masoumeh Mazandarani (PhD)
Department of Biology, Islamic Azad University, Gorgan Branch, Gorgan, Iran

Leili Monfaredi (PhD)
Department of Biology, Islamic Azad University, Gorgan Branch, Gorgan, Iran

Corresponding author: Masoumeh Mazandarani

Email: Mazandarani.M@gorganiau.ac.ir

Tel: +989111719477

Address: Islamic Azad University, Gorgan Branch, Gorgan, Iran

Received : 05 Jul 2015
Revised: 01 Oct 2015
Accepted: 04 Oct 2015

ABSTRACT

Background and Objectives: Many aromatic plants from the genus Satureja have been used in traditional medicine in north of Iran. This study aimed to determine the ecological requirements for the growth of Satureja mutica Fisch. & C.A. Mey, and evaluate antioxidant and antibacterial activity of ethanolic extract of S. mutica collected from North Khorasan Province, Iran.

Methods: Aerial parts of S. mutica were collected in blooming stage. Ecological requirements and the traditional uses of the plant were recorded. Ethanol extract of the plant was prepared by maceration. Antioxidant capacity of the extract was measured by three methods of total antioxidant capacity, reducing power and 2,2-Diphenyl-1-picrylhydrazyl, and then compared with standard antioxidants (butylated hydroxyanisole and butylated hydroxytoluene). Antibacterial activity of the extract was studied against nine Gram-positive and Gram-negative bacteria by agar dilution method and determining the minimum inhibitory concentrations (MICs).

Results: S. mutica is the most common wild aromatic annual herb in north slob and sunny areas around mountains of Bojnord (1020-1300 m). The ecological features of this region are as follows: annual rainfall 308 mm, average temperature 11.5 oC, semi dry cold climate in the sandy clay loam soil, Ec=0.7 desizimence, and pH= 7.30. Ethnopharmacological data showed that this plant has been widely used by rural people as an anti-infective, antispasm and sedative agent that could treat rheumatic pain, migraine, toothache and diarrhea. The ethanol extract of S.mutica had relatively high antioxidant activity with IC50 value of 11.2 mg/ml. The extract also had high antibacterial activity against Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus and Enterococcus faecalis, with inhibition zone diameters ranging between 15.1±0.5 and 27.7±0.8 mm and MIC values of 60, 68, 53 and 83 mg/ml, respectively.

Conclusion: It can be concluded that the extract of S. mutica has favorable antibacterial and antioxidant activity, which could be used as natural anti-microbial agent for treatment of some infection diseases.

Keywords: Antioxidant, Antimicrobial, Bojnord, Ecological Requirements, North Khorasan, BHT, BHA.

This paper should be cited as: Mazandarani M, Monfaredi L [Evaluation of Antioxidant and Antimicrobial Activity of Satureja mutica Fisch. & C.A.Mey. Collected from North Khorasan Province, Iran] . mljgoums. 2017; 11(1): 23-27

INTRODUCTION

Aromatic plants have been used for centuries as food and natural drugs in traditional medicine. Recently, researchers have been interested in antioxidantand antimicrobial properties of extracts from these plants (1-3). The genus Satureja L. from the Lamiaceae family has more than 200 species that are mainly distributed in the Mediterranean region. Eight of these species are endemic in mountainous regions of Iran (3-5). Phytochemical, antibacterial, antioxidant and antifungal properties of essential oils from this genus of plants have been investigated in previous studies (3,6). It is suggested that phenolic constituents of these plants such as thymol and carvacrol have antioxidant and antimicrobial activities against several human pathogens (3,7). Many studies reported that the essential oil of Satureja species are rich in monoterpenoides and phenolic compounds such as carvacrol, γ-terpinene, thymol and p-cymene (8,9-11). Although the antimicrobial activity of the essential oil and extract of some Satureja species has been reported (7,12-14), no study has investigated the antimicrobial and cytotoxic activity of Satureja mutica extract. Therefore, this study aimed to determine the ecological requirements for the growth of S. mutica Fisch.&C.A.Mey in mountains of Bojnord, and evaluate the antioxidant and antibacterial activity of S. mutica extract.

MATERIAL AND METHODS

The main ecological requirements of the plant and its pharmaceutical propetries were determined form field observations at its natural habitat (Mamalje village, 1020m above sea level), 70 km far from Bojnord (northeast of Iran, latitudes of 55°57' 55" to 52°57' 55" and longitudes of 25° 46' 37" to 15° 42' 37").

A voucher specimen of the plant (No.HRCMP:219) was identified and preserved at the Herbarium of Research Center of Medicinal plants, Islamic Azad University of Gorgan, Iran. Aerial parts of the plant were shade-dried in blooming time. The dried parts were powdered and stored at 4ºC until in vitro testing. One gram of the powder was macerated in 100 ml methanol 80%. The extract was filtered with Whatman No. 1 filter paper. The filtrates were evaporated in a rotary evaporator at 40°C and then stored at 4 °C (15). 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co. (St., Louis, USA), and butylated hydroxytoloane (BHT), butylated hydroxyanisole (BHA) and methanol were purchased from Merck Co. (Germany).

The testing was done based on the method described by Arabshahi-Delouee. First, the dried extract (12.5–1000 µg) in 1 ml of corresponding solvent was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (K_3 $Fe(CN)_6$; 10 g l-1). After the mixture was incubated at 50°C for 30 min, 2.5 ml of trichloroacetic acid (100 g l-1) was added and the mixture was centrifuged at 1650g for 10 min. Then, 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of FeCl3 (1 g l-1). The absorbance of the mixture was read at 700 nm (16). Free radical scavenging activity of the extract was assessed using a method previously described (16). Briefly, 1ml of 1mM methanolic DPPH solution was mixed with 3ml of extract in methanol (containing 12.5–1000 µg of dried extract). The mixture was then vortexed vigorously and placed for 30 min at room temperature in the dark. The absorbance was read at 517 nm, and the activity was expressed as percentage DPPH scavenging compared to control using the following equation:

DPPH scavenging activity (%) =)] absorbance of control – absorbance of sample) / absorbance of control] ×100

This experimental procedure is based on the reduction of Mo (VI) to Mo (V) by the sample and observation of a green phosphate/Mo (V) complex at acidic pH. Then, 0.1 ml of sample containing 12.5-1000µg of dried extract in the solvent was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in a tube. The tube was incubated at 95 °C for 90 min. After cooling the samples, their absorbance was measured at 695 nm. A typical blank solution containing 1 ml of the reagent solution and the appropriate volume of the solvent was used as the negative control. The negative control was incubated under the same conditions (16).

Bacterial strains were obtained from the Microbiology Laboratory of Golestan University of Medical Sciences, Iran. The ethanolic extract of S. mutica root was tested separately against nine strains of Gram-positive and Gram-negative bacteria including

Shigella dysenteriae (PTCC1188), Pseudomonas aeruginosa (PTCC1430), Escherichia coli (PTCC1399), Staphylococcus aureus (PTCC1431), Bacillus cereus (PTCC1015), Salmonella typhimurium (ATCC1596), Staphylococcus epidermidis (PTCC1114), Enterococcus faecalis (PTCC1393) and Klebsiella pneumoniae (PTCC1291). Minimum inhibitory concentration (MIC) of the extract was determined against each bacterium using the agar dilution method at concentrations ranging from 0.93 to 60 µg/mL. Twofold serial dilutions were prepared from the extract in molten Mueller Hinton (MH) agar (Pronadisa-Madrid). After placing the sample in water bath at 45-50 oC, the extract was dispersed in the mixture using dimethyl sulfoxide. Then, 0.01 mL of each bacterial suspension equivalent to half McFarland standard (108 CFU/mL) was inoculated onto the MH agar. The culture plates were then incubated at 37 oC for 24 h. MIC was defined as the lowest concentration at which no visible growth was observed (17). The MH agar containingdimethyl sulfoxide without the essential oil was used negative control, while gentamicin was used as positive control.

ANOVA was used to compare the anti-Candida activities of the extract and controls. P-value less than 0.05 was considered statistically significant.

RESULTS

S. mutica is a common aromatic annual herb, which often grows wild in north slob and sunny areas around mountains of Bojnord (1020-1300m). The ecological feutures of this region are as follows: annual rainfall 308 mm, average temperature11.5 oC, semi dry cold climate in the sandy clay loam soil, Ec=0.7 desizimence, and pH= 7.30.

Table 1 shows the antioxidant activity of the plant. IC50 values varied in the three methods. However, the highest antioxidant and radical scavenging effects of the ethanolic extract was observed in the DPPH method with IC50 of 11.20±0.03 µg/ml.

Table 1- Antioxidant activity of *S.mutica* collected from Mamlaje, North Khorasan Province

Part Antioxidant Activity IC50 (µg/ml)	Mamlaje (1020m) North Khorasan Province	BHA	BHT
	Aerial parts		
TAC	21.9±0.18	3.85±0.351	3.13±0.404
RP	26.6±0.21		
DPPH	11.20±0.03		

Table 2- MIC values of the ethanolic extract of *S.mutica* against the tested bacteria

Microorganisms	Sarvelayt region (2020m)		
	Inhibition zone(mm) ±SD	MIC of the extract (µg/mL)	MIC of Gentamicin (µg/mL)
S. aureus	27.7±0.8	60.1	16.7
S. epidermidis	22.3±0.7	68.6	14.7
B. cereus	16.4±0.1	53.2	16.5
E. faecalis	15.1±0.5	83.1	9.6
E. coli	14.1±0.3	92.5	11
P. aeroginosa	13.8±0.1	118.1	9
K. pneumonia	12.7±0.6	129.7	--
S. typhimurium	12.1±0.8	172	11
S. dysenteriae	10.7±0.1	212.3	11

Table 2 compares the inhibition zone (IZ) diameters and MIC values of the ethanolic extract of S.mutica and standard antioxidants (BHT and BHA). The ethanolic extract showed moderate to high antibacterial activity against the tested bacteria except for S. dycentria. S. aureus, S. epidermidis, B.cereus and E. faecalis were the most sensitive bacteria to the ethanolic extract with MIC of 60, 68, 53 and 83 mg/ml, respectively.

DISCUSSION

The results revealed that all tested bacteria are absolutely insensitive to the ethanolic extract of S. mutica, despite the relatively favorable antibacterial and antioxidant activity. The main phenolic compositions of the Satureja species are thymol and carvacrol, which might be responsible for the antimicrobial and antioxidant activity. Similar studies have reported the antioxidant and antimicrobial activity of the Satureja species (S.laxiflora, S. montana, S. subspicata, S. spicigera, S. biflora, S. masukensis and S. pseudosimensis). Most of these studies reported that the antimicrobial activity of essential oil and extracts of these species is related to their phenolic content (thymol, carvacrol,γ-terpinene and p-cymene), which show high inhibitory effects against a wide range of microorganisms (3,18-20). According to previous studies, the ethanolic extract of S. mutica has great potential antibacterial, antioxidant and hypoglycemic properties (21). Several studies have reported that the antibacterial and antioxidant activity of Satureja species essential oils or extracts depends on their terpenoid and flavonoid components (thymole and carvacrol) (12, 22, 23). A number of studies reported that the antimicrobial properties of S. bachtiarica, S. atropatana, S. mutica and S. hortensis could be attributed to their main constituents; monoterpenoides and phenolic compounds (24-26). Another study reported the favorable antioxidant activity of extract from aerial parts of S. sahendica, which is mainly composed of thymol (32.5%), γ-terpinen (29.3%) and p-cymene (23.5%) (27).

The use of oral antioxidants could improve sperm quality and increase the chance of pregnancy. In this regard, Safarnavadeh et al. reported that S. khuzestanicahas favorable antioxidative properties that could enhance fertility potential (28). Other studies have shown that the essential oil of S. montana L. has relatively good antimicrobial activity against five Gram-negative bacteria (E. coli , K. pneumoniae, P. mirabilis, P. aeruginosa, S. typhi), four Gram-positive bacteria (B. subtilis, B. cereus, S. aureus, S. faecalis) and five pathogenic fungi (Aspergillus fumigatus, Aspergillus niger, Candida albicans, Candida rugosa and Saccharomyces cerevisiae) (29).Another study reported that Satureja thymbra, S. abyssinica ssp. and S. paradoxa which grow wild in Libya, have strong antioxidant activity (IC50 = 0.0967 mg/mL) and significant antimicrobial activity against fungi and bacteria at concentration of 0.001-0.1 mg/mL and 0.002-0.2 mg/mL, respectively (17).

Another study also showed that the extracts from Satureja species especially S.mutica, have antibacterial effects against some Gram-positive and Gram-negative bacteria with MIC values ranging from 150 to 2300 μg/ml (11).

CONCLUSION

Our study is the first to report the antispasmoic, sedative and anti-infective properties of S. mutica. This plant has been used in traditional medicine for treatment of rheumatic pain, migraine, toothache and diarrhea. Similar to other Satureja species, the ethanolic extract of S.mutica has favorable antimicrobial and antioxidant potential. The results indicate that S. mutica extract and its constituents could be used as natural antibacterial agents or food additives.

ACKNOWLEDGMENTS

The authors wish to thank the Research Center of Medicinal plants at Islamic Azad University of Gorgan for providing practical assistance in antibacterial testing.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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