

Full Length Research Paper

Antibacterial and antioxidant activities of crude aerial part extracts of *Satureja Punctata* (Benth) Briq

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This study evaluates the antimicrobial and antioxidant activities of the crude extract of an aerial part of *Satureja punctata* (Benth.) Briq. The dried aerial part of *S. punctata* (Benth.) Briq was extracted separately using aqueous, ethanol and n-hexane. The antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* was assessed using disc diffusion method, and the minimum inhibitory concentration (MIC) and bactericidal concentration (MBC) of the extracts were determined by the agar dilution method. The radical scavenging activity of the extracts was evaluated by DPPH assay. The result indicated that the aqueous and ethanol extracts exhibited significant antibacterial activities on the test microorganisms. The antibacterial activity of 600 mg/ml aqueous extract as measured by inhibition zones against *S. aureus* was 10.33±0.25 mm, *E. coli* 9.83±0.19 mm and *P. aeruginosa* 8.53±0.12 mm. The 600 mg/ml ethanol extract inhibited through zone of inhibitions *S. aureus* (10.33±0.41 mm), *E. coli* (9.37±0.18 mm) and *P. aeruginosa* (10.17±0.46 mm). Furthermore, the MIC values for both aqueous and ethanol extracts were from 75 to 100 mg/ml; whereas the MBC values for same extracts were from 100 to 150 mg/ml. The *in vitro* free radical scavenging activity showed that the aqueous, ethanol and n-hexane extracts showed maximum antioxidant activity of 87.89, 78.68 and 47.18 % at 25 mg/ml concentration, respectively. The results showed that the aerial part of *S. punctata* contains components that have antibacterial and antioxidant properties that substantiate the medicinal importance of the plant.

Key words: *Satureja punctata*, antibacterial activity, antioxidant activity, zone of inhibition.

INTRODUCTION

The overuse and abuse of antibiotics in the treatment of bacterial infections has led to the emergence of multiple

drug resistant bacteria (MDR) and has become a major cause of failure in the treatment of infectious diseases

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(Basak et al., 2015). Antioxidants can delay, inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress (Reuter et al., 2010). Natural antioxidants have been studied extensively in order to find compounds protecting against a number of diseases related to oxidative stress and free radical-induced damage.

Ethnobotanical studies revealed that a wider range of Ethiopian plants are being used in the treatment of various diseases in the traditional health care system of the country (Dawit, 2001; Tilahun and Mirutse, 2010).

Satureja punctata (Benth) Briq (Lamiaceae), locally known as “Lomishet” in the Amharic language, is an erect perennial herb having purple or violet flowers with a pleasant fragrance and grows in different parts of Ethiopia (Sebsebe, 1993). In the traditional health care system of Ethiopia, the aerial parts of *S. punctata* (Benth.) Briq are used for the treatment of diseases such as diabetes mellitus, various other ailments (Tsegaye et al., 2010) and liver disorders (Wolde et al., 2010). The essential oil composition of the leaves of *S. punctata* has been recorded (Chagonda and Chalchat, 2005; Tariku et al., 2010). *In vitro* propagation protocol for *S. punctata* has also been developed, showing the importance of this plant (Teshome et al., 2016).

The preliminary phytochemical analysis of the methanol fraction of *S. punctata* showed the presence of flavonoids, alkaloids, tannins and polyphenols (Wolde et al., 2010). In this study the antibacterial and antioxidant activities of the crude extract of the aerial parts of *S. punctata* was evaluated.

MATERIALS AND METHODS

Chemicals

Ethanol (Reagent chemical Services Ltd., United Kingdom), n-Hexane (Fisher Scientific UK Limited, UK), Sulfuric Acid (SDFCL Fine Chemical Ltd., Mumbai, India), Muller Hinton Agar (Oxoid Ltd, Basingstoke, Hampshire, England), Nutrient Agar (Oxoid Ltd, Basingstoke, Hampshire, England), Nutrient Broth (Oxoid Ltd, Basingstoke, Hampshire, England), Mannitol Salt Agar (Oxoid Ltd, Basingstoke, Hampshire, England), MacConkey Agar (Oxoid Ltd, Basingstoke, Hampshire, England), Barium Chloride (Griffin, UK), NaCl (Labmerk chemicals (India) PVT Ltd), Tetracycline 30 µg/disc (Oxoid Ltd, Basingstoke, Hampshire, England), 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, Germany) Barium Chloride Dehydrate (BaCl₂·2H₂O) (BDH Chemicals Ltd, Poole, England), Methanol (Reagent chemical Services Ltd, United Kingdom) were used for the study

Plant material collection and authentication

The aerial part of *S. punctata* was collected from a nearby hilly place called Entoto in Addis Ababa, which is located at an altitude of 2720 m in December, 2014. The plant was authenticated by a botanist and a representative sample with the specimen number

MM01/14 was kept at the Natural Herbarium of Addis Ababa University (AAU), Addis Ababa, Ethiopia

Preparation of the plant material

The collected aerial part was washed thoroughly with tap water to remove dust particles, spread over clean cloth and then kept in an open shady area for 14 days in order to avoid loss of volatile compounds by direct sun light.

After complete dryness, plant sample was ground using a coffee grinder to a fine powder and then sieved using a mesh of 0.5 mm size. The powder was stored in an airtight closed bottle for further use.

Preparation of the extracts

The crude extracts were prepared by cold maceration technique (O'Neill et al., 1985). The dried powder (100 gm) of *S. punctata* was extracted by, soaking separately 1 L each of ethanol, n-hexane and aqueous (1/10 w/v) in conical flasks. The flasks were plugged with cotton wool and wrapped with aluminum foil and put on orbital shaker at 120 rpm for 72 h at room temperature. The extracts were filtered through a cotton plug followed by a qualitative filter paper.

After filtration, the n-hexane and ethanol extracts were subjected to partial concentration using rotary evaporator, attached to a vacuum pump set in a water bath at 45°C. The partially concentrated extracts in screw capped bottle were placed in an oven at 40°C for complete drying. The aqueous extracts were placed in deep-freeze at -20°C for 24 h and allowed for lyophilization to obtain fine crude extract. The dry residues of n-hexane, ethanol and aqueous extracts were weighed and, the yield of the extracted samples was calculated using the following formula:

$$\text{Percent of yield of the extract} = \frac{\text{final weight (gm)}}{\text{initial weight (gm)}} \times 100$$

All extracts were reconstituted with their respective solvent for antibacterial test and were dissolved in methanol for antioxidant activity test.

Test microorganisms

Standard bacteria culture of *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922) were obtained from the Microbiology Department, Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia.

Preparation of inoculums

All test strains were transferred into sterile nutrient broth and incubated at 37°C for 24 h, then streaked on 90 mm Petri dishes using sterilized loop, which contained sterilized selective media and incubated at 37°C for 24 h. Four to five well-isolated colony of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a loop, and the growth was transferred into a tube containing 10 ml of 0.85% sterile normal saline to dilute the suspension.

The turbidity of the 0.5 McFarland standard was prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dehydrate with 99.5 ml 1% (v/v) sulfuric acid (Lalitha, 2009). The turbidity of the actively growing bacteria suspension was adjusted with sterile saline to

obtain turbidity comparable to that of the 0.5 McFarland standards. This resulted in a suspension containing approximately 1.5×10^8 CFU/ml.

Determination of antibacterial activity

The antibacterial activity of the ethanol and aqueous extracts was evaluated using disc diffusion method (NCCLS, 1997). Sterilized Muller Hinton Agar (25 ml) was poured in 90 mm petri dishes which were allowed to solidify. The plates were seeded with suspension of test bacteria adjusted to 10^8 cells/ml using sterile cotton swab and allowed to settle for 10 min. Sterile, 6 mm diameter filter paper discs were soaked each in plant extracts at different concentrations (300, 400 and 600 mg/ml) and placed on the surface of the inoculated media agar plates using sterile forceps, gently pressed down onto the agar surface.

Disk soaked with solvents and tetracycline 30 µg/disc was used as negative and positive controls, respectively. All plates were incubated at 35 to 37°C for 24 h. Clear inhibition zones around the discs, indicated the presence of antibacterial activity. Diameter of inhibition zones was measured in millimeters. Each experiment was carried out in triplicates and the mean diameter of the inhibition zones was recorded for each test organism.

Determination of minimum inhibitory concentration (MIC) values

The MIC of the ethanol and aqueous extracts of plant was determined by agar dilution technique (NCCLS, 2002). Series of two folds dilution of ethanol and aqueous extracts (300 to 37.5 mg/ml) each was mixed with 19 ml of sterilized and molten nutrient agar medium and 1 ml each concentration was poured into pre-labeled sterile petri dishes.

Plates were dried at 35°C for 30 min prior to spot inoculation with bacterial suspension (adjusted to 0.5 McFarland standards), containing approximately 1.5×10^8 CFU/spot using a sterilized inoculating loop. Nutrient agar with solvent was used as a positive control. The inocula spots were allowed to dry at room temperature and plates were incubated at 35 to 37°C for 24 h. Each test was done in triplicate. Growth inhibition was judged by comparison with growth in the control plates.

Determination of MBC (Minimum bactericidal concentration) values

The MBC of the extracts on the test isolates was determined according to Mathur (2013). Fresh nutrient agar medium was poured into petri dishes and allowed to solidify. Inocula from the different plates of MIC experiment that did not show any growth was subcultured on freshly prepared plates. The lowest concentration at which test bacteria did not recover on fresh medium was considered to be MBC.

In vitro antioxidant activity

The radical scavenging activity of plant extracts was determined on the basis of the radical scavenging effect of DPPH (Blois, 1958), which is a known compound for test. Dose dependent

concentrations of 1.56, 3.12, 6.25, 12.5 and 25 mg/ml; 1 ml each of the extract and the standard control ascorbic acid was mixed with 2 ml each of 0.1 mM solution DPPH in methanol in labeled tubes.

The tubes were incubated in dark for 30 min at room temperature and then the absorbance was measured at 517 nm using UV-Vis spectrophotometer 201215 (Single Beam, India). The control was prepared by mixing 2 ml of DPPH solution with 2 ml methanol. Experiment was done in triplicates. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\% \text{DPPH radical scavenging capacity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where;

A control is the absorbance of DPPH radical + methanol reaction
A sample is the absorbance of DPPH radical + sample extract /standard
The antioxidant activity of the different extract was expressed as % inhibition.

Data analysis

The data obtained for antibacterial and antioxidant tests were analyzed with Microsoft office Excel 2007. Results were expressed as mean \pm SEM. The statistical analysis was performed by one-way analysis of variance (ANOVA) followed by *Post Hoc* Multiple Comparison Tests using statistical software (SPSS) package version 20.0 for windows. P-value < 0.05 was considered as significant.

RESULTS AND DISCUSSION

Yield of extraction

The yields in grams and percentage (w/w) of n-hexane, ethanol and aqueous extracts of the aerial parts of *S. punctata* were 2.11 (4.63), 6.28 (12.56) and 5.10 gm (10.19%), respectively. The ethanol extracts give relatively higher yield.

Antibacterial activity

The aqueous and ethanol extracts showed dose dependent antibacterial activity against *S. aureus*, *E. coli* and *P. aeruginosa* at concentrations 600, 400 and 300 mg/ml (Table 1). For example the inhibition zones at the highest concentration of 600 mg/ml were 10.33 ± 0.41 mm for *S. aureus*, 9.37 ± 0.18 mm for *E. coli* and 10.17 ± 0.46 mm for *P. aeruginosa*. The standard drug tetracycline (Tet) showed inhibition zone of 25.00 ± 0.00 , 22.33 ± 1.4 and 10.17 ± 0.17 mm for *S. aureus*, *E. coli* and *P. aeruginosa*, respectively.

Inhibition of test bacteria by tetracycline is 2 to 3 times higher when compared to ethanol and aqueous extracts as shown in Table 1. The n-hexane extract of *S. punctata*

Table 1. Antibacterial activity of *S. punctata* at different concentrations

Plants	Types of solvent/extracts	Concentration (mg/ml)	Zone of inhibition (mm)		
			<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
<i>Satureja punctata</i>	Aqueous	300	8.01±0.29 ^c	7.79±0.23 ^c	7.27±0.27 ^c
		400	9.07±0.13 ^b	8.67±0.11 ^b	7.83±0.15 ^b
		600	10.33±0.25 ^a	9.83±0.19 ^a	8.53±0.12 ^a
	Ethanol	300	8.17±0.39 ^c	7.93±0.17 ^c	7.73±0.32 ^c
		400	9.04±0.22 ^b	8.54±0.29 ^b	8.43±0.28 ^b
		600	10.33±0.41 ^a	9.37±0.18 ^a	10.17±0.46 ^e
	n-hexane	300	-	-	-
		400	-	-	-
		600	-	-	-
+ve control (Tet)	-	30 µg/disc	25.00±0.00 ^d	22.33±1.45 ^f	10.17±0.17 ^e
-ve Control	-	-	-	-	-

Data is represented as mean ± SEM (N=3). Values followed by different letters indicate statistical significance (-) in the table which showed no

Table 2. MIC and MBC (mg/ml) of ethanol and aqueous extract of *S. punctata*

Test organisms	Ethanol Extract (mg/ml)		Aqueous Extract(mg/ml)	
	MIC	MBC	MIC	MBC
<i>S. aureus</i>	100	150	75	100
<i>E. coli</i>	75	100	75	100
<i>P. aeruginosa</i>	100	150	100	150

did not show any antibacterial activity against the tested pathogenic microorganisms at the given doses.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC values of the different bacteria are shown in Table 2. The ethanol extract of *S. punctata* was 100, 75, 100 and 150, 100 and 150 mg/ml, against *S. aureus*, *E. coli* and *P. aeruginosa*, respectively (Table 2). Other workers have reported the antibacterial effect of *Satureja*. The methanol and hexane extracts of aerial part of *Satureja hortensis* L. was tested against *E. coli* (Sahin et al., 2003). The authors found that, the MIC values of the methanol extract were 0.25 to 0.5 mg/ml but the hexane extract did not show any inhibition on *E. coli*, *P. aeruginosa* and *S. aureus* (Sahin et al., 2003). This agrees with the present study, in which n-hexane extract of aerial part *S. punctata* did not show any zone of inhibition against the above microorganisms.

Furthermore, the ethanol and aqueous extracts of *Satureja bachtiarica* at 40 mg/ml has shown zones of inhibitions of 8.8±0.28 and 6.4±0.5 mm against *P. aeruginosa*, respectively (Sureshjani et al., 2013). The results of Sureshjani and co-workers (2013) showed better activity at a lower concentration than results obtained in the present study. The difference could be due to the species difference, extraction procedure of the plant parts or any other factor that contributes to the plant biology. In addition, other workers tested the antimicrobial activity of the methanol extract of the aerial part of *S. kitaibelii* Wierzb Ex heuff (Stanojkovic et al., 2013). Therefore, the present study is an important addition to the antibacterial property of genus *Satureja*.

The antioxidant test

The result of the antioxidant activity is presented in Figure 1. Data showed that, all extracts demonstrated

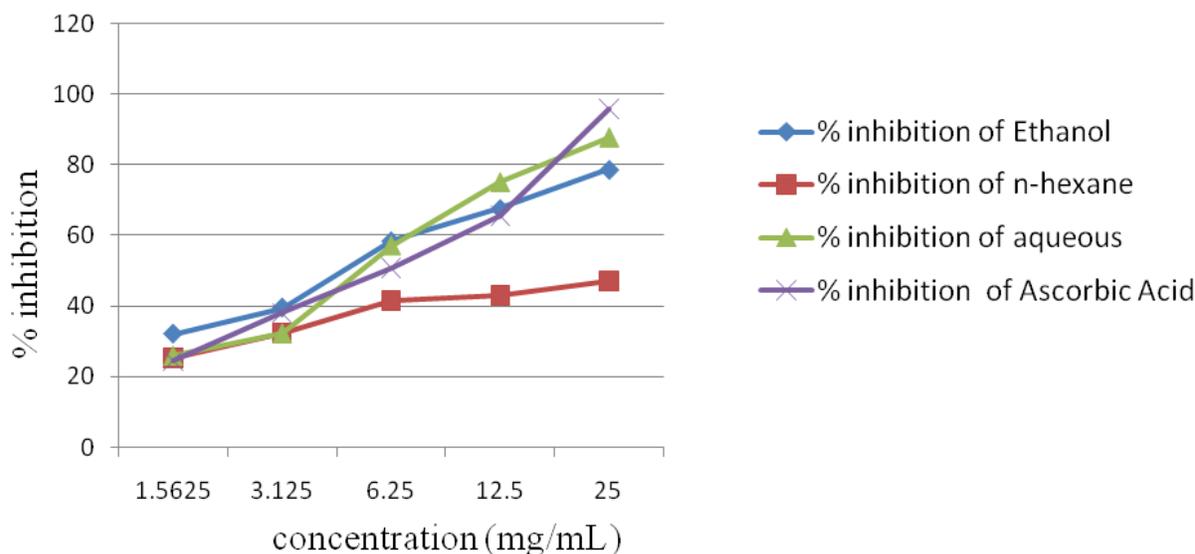


Figure 1. Antioxidant activity of aqueous, ethanol and n-hexane extract of *S. punctata*

dose dependent percentage inhibition. For example, at a concentration of 1.5625 and 25 mg/ml the percentage inhibition was 26.05 and 87.89%, respectively.

However, significant inhibitions are only observed for aqueous and ethanol extracts. The n-hexane extract exhibited limited inhibition (Figure 1). Highest radical scavenging effect was found in aqueous extract, followed by ethanol extract and the lowest by n-hexane at concentration 25 mg/ml is 87.89, 78.67 and 47.18%, respectively. A similar result was reported by Wolde and co-workers (2010), substantiating the antioxidant activity of *S. punctata*.

Conclusion

The results of the present study confirmed that, *S. punctata* aerial part has an antimicrobial and antioxidant constituents. Further fractionation of the crude extract and purification of the active compounds is recommended.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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