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ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF TWO PLANTS FROM NILGIRIS OF INDIA

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ABSTRACT

Our objective is to evaluate the antibacterial and antioxidant activity of ethanolic extracts of *Plectranthus mollis (P.mollis)* and *Salvia officinalis* (*S. officinalis*) leaves locally used for treatment of microbial infection in Nilgiris of India. Antibacterial activity was determined by using agar diffusion and broth dilution methods against seven microorganisms. The antioxidant potential was assessed by using 2,2 diphenyl -1- picryl hydrazine (DPPH) scavenging assay. Ethanolic extract of *P. mollis* showed antibacterial activity in the following order viz *Pseudomonas aerugenosa* (20 mm, MIC of 12.5 µg/ml), *Staphylococcus aureus* (19 mm, MIC of 6.3µg/ml), *Bacillus cereus* (18 mm, MIC of 25µg/ml), *Salmonella typhimurium* (16 mm, MIC of 25µg/ml) and *Bacillus subtilis* (14 mm, MIC of 12.5µg/ml). *Escherichia coli* and *Klebsiella pneumoniae* were resistant to ethanolic extract of P.mollis, whereas the ethanolic extract of *S. officinalis* and control (DMSO) did not show any inhibition against all the bacteria tested. Both the extracts possess marked DPPH radical scavenging activities compared to the reference antioxidant ascorbic acid in a dose dependent manner with the IC_{50} values being 73.26µg/ml, 107µg/ml and 40.17µg/ml for *P. mollis*, *S. officinalis* and ascorbic acid respectively. Based on the results, it may be confirmed that the antioxidants might have enhanced the antibacterial activities of *P.mollis*. But *S. officinalis* did not show any antibacterial activities even though it has good antioxidant potential than *P.mollis*. Further studies on isolation and purification of compounds that are responsible for their antibacterial and antioxidant activities are needed.

KEYWORDS: ***P. mollis*, *S. officinalis,* Ethanolic extract, Antibacterial activity, Minimum inhibitory concentration, Antioxidant activity**.

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INTRODUCTION

For various reasons, the search for new antibiotics has been most intensive. Certain disease entities however remain serious problem and some of the major antibiotics have considerable drawbacks in terms of limited antibacterial spectrum or serious side effects.These factors impel a continuing search for new agents and hence the present work was undertaken with the objective of screening the selected medicinal plants for antibacterial and antioxidant activities.The genus *Plectranthus* contains more than 300 species and found in tropical Africa, Asia and Australia. *Plectranthus mollis* (Aiton) Spreng (*P.mollis*) belongs to Lamiaceae and is used in the traditional medicine to cure ear infections, inflammations, respiratory diseases and burns or as an antiseptic and antimicrobial[1].Vernolic acid and cyclopropenoid fatty acids were present in *P.mollis*[2,3] It was found to exhibit smooth and skeletal muscle relaxant activity[4]. Leaves of *Plectranthus* species contains essential oils, flavanoids and terpenes, which have shown inhibitory effects on several microorganisms[5] *Salvia officinalis* L.(*S.officinalis*) is also a Lamiaceae member and is used as nerve tonic, anti-spasmodic and anti-inflammatory[6] in Indian traditional medicine. Rosmarinic acid, chlorogenic acid, steroids and flavonoid glycosides were present in *S. officinalis*[7] The present study is aimed at evaluating the antibacterial and antioxidant activities of *P. mollis* and *S. Officinalisthe* grown in Nilgiris of India and used in traditional medicine to treat microbial infection or general ailments.

MATERIALS AND METHODS

Collection of plant material

Leaves of the plants (*P. mollis* and *S. officinalis*) were collected from Nilgiris region, Tamilnadu, India and authenticated by Botanist Dr.Rajan, Medicinal Plant Collection Unit, Nilgiris. They were compared with the voucher specimens (accession no. Pharmacog./1052 and Pharmacog./1053) previously deposited for reference in the Herbarium of Department of Pharmacognosy, Sri Adichunchanagiri College of Pharmacy, B.G.Nagara, Karnataka, India.

Extraction of plant material

Shade dried and pulverized leaves (100 g each) were exhaustively defatted with petroleum ether (60-80º C) followed by extraction with 500 ml of 70% ethanol using soxhlet apparatus for 5-6 hr. The extracts were collected and the solvents were evaporated to dryness in a rotary evaporator at 45º C to get 6.2 g and 6.8 g for *P.mollis* and *S.officinalis* respectively. The extracts were stored at 4º C until tested.

Demonstration of Antibacterial activity

Test Microorganisms

The bacterial cultures employed to test the antibacterial activity were obtained from the Department of Microbiology, Adichunchanagiri Institute of Medical Sciences (AIMS), B.G. Nagara, Karnataka, India. Three gram positive bacteria, viz. *Staphylococcus aureus*, *Bacillus subtilis, Bacillus cereus* and four gram negative bacilli , viz. *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae and Salmonella typhimurium* were used for the studies.

Demonstration of antibacterial activity and determination of minimum inhibitory concentration

The antibacterial activity was determined by agar well diffusion method[8]. 40 ml of Muller-Hinton Agar medium was seeded with suitable quantity of bacterial inoculums by pour plate method. Subsequently, the seeded medium was poured into prelabelled petriplates and allowed to solidify. Wells of 6 mm of diameter were made with 6 mm sterile cork borer and 25 µg/ml concentration of each extract with one positive control ciprofloxacin (5 µg/ml) in DMSO and one negative control DMSO (dimethyl sulfoxide)was individually loaded in the well with the help of micropipette. The plates were incubated at 37º C for 24 hr and the zone of inhibition was measured. Minimum inhibitory concentration (MIC) of the extracts to inhibit the bacterial growth in liquid media was assessed with different concentrations of extract by broth dilution method[9]. Five concentrations of each extract (3.125, 6.25, 12.5, 25, 50 µg/ml) were tested. The lowest concentration of the plant extract inhibiting the growth of bacteria was considered as MIC.

Antioxidant testing

Scavenging Activity of DPPH Free Radical

Qualitative analysis

This method is generally used for the screening of potent antioxidant activity of crude extracts. It involves the chromatographic separation of the plant extracts, after which the developed chromatogram is sprayed with a colored radical solution and the presence of antioxidant compounds indicated by the disappearance of radical color. To measure antioxidant activity, DPPH free radical scavenging assay by TLC was performed[10]One mg of each extract was diluted with 1 ml of ethanol (1 mg/ml) and 10µl aliquot of each dilution of ethanolic extracts was applied to a chromatographic plate (E Merck, Silica gel $60F_{254}$). Chromatography was conducted using ethyl acetate: acetic acid: formic acid : water (100 : 11 : 11 : 27) as mobile phase. The plate was sprayed using a DPPH solution (2mg/ml in ethanol). The colour changes (yellowish colour development on a purplish background on TLC plate after 30 minutes due to the discoloration of DPPH) were noted as an indicator of the presence of antioxidant substances.

Quantitative analysis

The effects of extracts on DPPH radicals were measured by standard method[11]with minor modifications as reported by Badami et al[12]Aliquot (10 µl) of 80% methanolic solution at various concentrations (50, 100, 200, 400, 800 and 1000 µg/ml of ethanolic extract) were mixed with 200 µl of DPPH in methanol solution (150 µM) in a 96-well microtitre plate. After incubation at 37º C for 20 minutes, the absorbance of each solution was determined at 517nm using an ELISA reader. A corresponding blank reading was also taken and the remaining DPPH was calculated. Ascorbic acid was used as a positive control. The IC_{50}, the half maximal inhibitory concentration was obtained by plotting the percentage of free radicals scavenged versus the putative antioxidant concentration. All determinations were performed thrice.

Table1

Antibacterial activity of ethanolic extracts of P.mollis, S. officinalis and ciprofloxacin (in mm)

S.No	Microorganisms	*P.mollis*	*S.officinalis*	Ciprofloxacin	Dmso
1	*Staphylococcus aureus*	19	--	20	--
2	*Bacillus subtilis*	14	--	21	--
3	*Bacillus cereus*	18	--	21	--
4	*Escherichia coli*	--	--	20	--
5	*Pseudomonas aeruginosa*	20	--	20	--
6	*Klebsiella pneumoniae*	--	--	19	--
7	*Salmonella typhimurium*	16	--	21	--

(--) Represents absence of measurable inhibition

Table 2

Minimum inhibitory concentration (MIC) of ethanolic extracts of P. mollis and S. officinalis (concentration: µg /ml)

S.No	Microorganisms	*P.mollis*	*S.officinalis*
1	*Staphylococcus aureus*	6.3	--
2	*Bacillus subtilis*	12.5	--
3	*Bacillus cereus*	25.0	--
4	*Escherichia coli*	--	--
5	*Pseudomonas aeruginosa*	12.5	--
6	*Klebsiellapneumoniae*	--	--
7	*Salmonella typhimurium*	25.0	--

Table 3
DPPH scavenging activity of ethanolic extracts of P. mollis and S. officinalis (%)

S.No	Concentration (µg/ml)	*P.mollis* (% Inhibition)	*S.officinalis* (% Inhibition)	Ascorbic acid (% Inhibition)
1	50	35.21	42.27	62.46
2	100	44.29	51.45	72.24
3	200	67.58	64.10	78.54
4	400	78.92	80.15	83.92
5	800	86.26	83.58	89.21
6	1000	91.21	92.89	93.78
7	IC_{50}	73.26	107.21	40.17

RESULTS AND DISCUSSION

Demonstration of antibacterial activity and determination of minimum inhibition concentration

The zone of inhibition of extracts and ciprofloxacin by agar well diffusion method was shown in table 1. The minimum inhibitory concentration (MIC) of the extracts is shown in table 2.In the present study, the ethanolic extract of *P. mollis* showed antibacterial activity in the following order viz *Pseudomonas aerugenosa* (20 mm, MIC of12.5µg/ml),*Staphylococcus aureus*(19 mm, MIC of 6.3µg/ml),*Bacillus cereus*(18 mm, MIC of 25µg/ml),*Salmonella typhimurium*(16 mm, MIC of 25µg/ml), *Bacillus subtilis* (14 mm, MIC of 12.5µg/ml), and no inhibition was observed against *Escherichia coli* and *Klebsiella pneumoniae*, whereas the ethanolic extract of *S. officinalis* and control (DMSO) did not show any inhibition.Highest antibacterial activity observed in the extract of *P. mollis* was against *S.aureus* with the lowest MIC of 6.3 µg/ml and diameter of zone of inhibition as 19 mm respectively. It is well known that *S. aureus* has developed resistance to many antibiotics[13] in the past decade. In the present study, *P.mollis* showed activity against *S. aureus* and can be used in the field of phytotheraphy. Steroids and terpenoids could be responsible for the antibacterial activity against *S. aureus*. Tannins present in the plant material react with proteins to form stable water soluble compounds which leads to bactericidal activity by damaging its cell membrane[14] . Our results provide the scientific evidence for the traditional use of the plant in treating microbial infection. Hence *P.mollis* could be exploited as a new potent antimicrobial agent. The results of the present work is closely related to that of Ndhlala et al.[15]who reported antimicrobial potential of extracts of *Aloe barberae.* Scavenging Activity of DPPH Free Radical Qualitative analysis DPPH (2,2diphenyl -1-picryl hydrazine) is a stable free radical and has strong electron or hydrogen radical accepting ability to become a stable diamagnetic molecule. The extracts are able to reduce the stable free radical to the yellow coloured diphenylpicrylhydrazine. The TLC-DPPH chromatogram reveals the presence of potent antioxidant compounds in both the extracts, which were detected as yellow spots against a purple background on TLC plates sprayed with DPPH (2 mg/mlin ethanol).

Quantitative analysis

The scavenging activities of the ethanolic extracts are shown in table 3. the table showed that the concentration of both extracts were directly proportional to the % scavenging. The minimum scavenging activity of both extracts were shown at lowest concentration of 50 µg/ml which were 35.21 % and 42.21 % for *P. mollis* and *S. officinalis* respectively.The order of scavenging activity is 50< 100<200<400<800 <1000 µg/ml for both the extracts.The activity increased by increasing the concentration of both the extracts and ascorbic acid were found to be 91.21%, 92.89% and 93.78% respectively at higher doses with theIC_{50}values being 73.26 µg/ml, 107 µg/ml and 40.17 µg/ml respectively. Both the extracts possess marked DPPH radical scavenging activities. Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. We

reportedterpenoids, tannins, β-sitosterol,α-amirin, quercetin, caffeic acid, luteolin and chlorogenicacid by TLC in both the extracts of *P. mollis* and *S. officinalis*[16] in our earlier studies. A positive correlation has been found between antioxidant activity and total phenolics& total flavonoid content.Terpenoids have anti-inflammatory, antioxidant and neuroprotective activities[17] which may contribute directly to their antioxidant activity. The reports of Duenaset al[18]and Kilani et al[19] were found to be similar to the present work. An considerable increase in the antibacterial activities of plant extracts in combination with antioxidants was demonstrated by Belofsky et.al[20] So it may be confirmed that the antioxidants might have enhanced the antibacterial activities of *P.mollis*. But *S. officinalis* did not show any antibacterial activities eventhough it has good antioxidant potential than *P.mollis*. Nonetheless, further studies on isolation and purification of compounds that are responsible for their antibacterial and antioxidant activities are needed.

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