ORIGINAL ARTICLE

Phytochemical analysis, antimicrobial and antioxidant activities of different parts of *Pleocaulus sessilis* (Nees) Bremek (Acanthaceae)

H. L. Raghavendra[1], T. R. Prashith Kekuda[2], S. Akarsh[2], M. C. Ranjitha[2] H. S. Ashwini[3]

[1]Department of Biochemistry, School of Medicine, Wollega University, Nekemte, Ethiopia, [2]Department of Microbiology, S.R.N.M.N College of Applied Sciences, N.E.S Campus, Shivamogha, Karnataka, India, [3]Department of PG Studies and Research in Applied Botany, Jnana Sahyadri, Kuvempu University, Shankaraghatta, Karnataka, India

Abstract

Aim: This study aims to investigate antimicrobial and antioxidant activity of leaf, stem, and inflorescence of *Pleocaulus sessilis* (Nees) Bremek belonging to Acanthaceae. **Materials and Methods**: The leaves, inflorescences, and stems were separated, dried under shade, powdered, and extracted using methanol by maceration process. Preliminary phytochemical analysis was carried out by standard phytochemical tests. Antibacterial and antifungal activity was carried out by agar well diffusion and poisoned food technique, respectively. Antioxidant activity was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, 2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate (ABTS) radical scavenging and ferric reducing assays. Folin-Ciocalteu reagent method was employed to estimate the total phenolic content of extracts. **Results and Discussion**: Flavonoids, saponins, steroids, and phenols were present in all three extracts. Extracts were inhibitory to all test bacteria with maximum activity against *Klebsiella pneumoniae*. Overall, inflorescence extract exhibited high inhibition of test bacteria when compared to other extracts. Extracts were effective in reducing mycelial growth of test fungi. Leaf extract was more effective against test fungi followed by inflorescence and stem extracts. All extracts exhibited dose-dependent radical scavenging and ferric reducing activity. Leaf extract exhibited marked antioxidant activity when compared to other two extracts. The leaf extract scavenged DPPH and ABTS radicals with an inhibitory concentration value of 27.16 µg/ml and 9.16 µg/ml, respectively. Total phenolic content was high in leaf extract (112.13 mg gallic acid equivalents [GAE]/g) followed by inflorescence (85.65 mg GAE/g) and stem (42.42 mg GAE/g) extracts. **Conclusion**: The plant can be used to treat diseases caused by pathogenic bacteria, prevention, and control of phytopathogens and oxidative damage caused by free radicals. Further studies are to be carried out to isolate and characterize active principles from the plant and to determine their biological activities.

Key words: Antimicrobial, antioxidant, phytochemical, *Pleocaulus sessilis, Strobilanthes sessilis*

INTRODUCTION

Plants are an integral part of daily life as humans depend on many plants for food, shelter, cloth, timber, dyes, and medicine. The use of herbal medicine for therapy is as old as humanity itself, and it is estimated that 80% of world's population depend on plant based formulations for healthcare needs. The traditional medicine that involves the utilization of plants plays a significant protective role in humans and animals particularly in developing and under-developing countries. Worldwide, the traditional medicinal practitioners use the plants for treatment of several diseases and disorders. Traditional medicine is commonly practiced in various countries such

Address for correspondence:
T. R. Prashith Kekuda, Department of Microbiology, S.R.N.M.N College of Applied Sciences, N.E.S Campus, Balraj Urs Road, Shivamogha - 577 201, Karnataka, India. Phone: +91-9739864365.
E-mail: p.kekuda@gmail.com

Received: 27-02-2017
Revised: 23-03-2017
Accepted: 05-04-2017

as China, India, Japan, Pakistan, Sri Lanka, and Thailand. Knowledge of medicinal plants and their use by indigenous culture are useful for conservation of cultural traditions and biodiversity and healthcare as well as drug development. Plants represent an integral part of several systems of medicine such as Ayurveda, Sidda, and Unani. Plants are known to be the sources lead compounds for the development of modern drugs. Several drugs such as aspirin, digoxin, quinine, vincristine, vinblastine, reserpine, and morphine have been derived from plants. Nowadays, immense interest on medicinal plants and exploration of medicinal values of plants is increased because of several complications such as the development of resistance and side effects associated with the use of modern drugs such as antibiotics and anticancer agents.[1-9]

The genus *Strobilanthes* belongs to the family Acanthaceae. The genus includes perennial flowering herbs and shrubs with about 350 species out of which at least 46 are native to India. *Strobilanthes* is the second largest genus in the family Acanthaceae. The name *Strobilanthes* is derived from the Latin words "strobilus" meaning cone and "anthos" meaning flower or shoot.[10,11] *Pleocaulus sessilis* (Nees) Bremek (synonym *Strobilanthes sessilis* Nees var. *sessilioides*) is a small perennial shrub with hardy tetragonous stem (30-45 cm) covered with brownish hairs. Leaves are up to 4-5 cm long, as broad as long, ovate, acute, coriaceous and bullate-hairy on both surfaces; crenate-serrate, rounded, or narrowed at base. Flowers blue, in terminal pedunculate, densely hairy bracteates spikes 4-6 cm long. It is distributed in peninsular India and is commonly found on rocky slopes among grasses in Baba Budangiri.[12] In the previous study, Patil *et al*.[13] screened solvent extracts of *P. sessilis* leaves for phytoconstituents, antioxidant and antimicrobial activities. Phytochemical analysis revealed constituents such as phenols, flavonoids, saponins, and tannins. Antioxidant activities and antimicrobial activities of extracts were found to be good. The present study was carried to evaluate antimicrobial and antioxidant activity of leaf, stem, and inflorescence of *P. sessilis* [Figure 1].

Figure 1: *Pleocaulus sessilis*

MATERIALS AND METHODS

Collection and Identification of Plant

The plant materials were collected at Baba Budangiri, Chikkamagalure district, Karnataka during February 2016. The plant was authenticated by referring standard flora.[12]

Extraction

The plants were washed to remove dirt and other extraneous matter. Different parts, namely, leaves, stem, and inflorescence were separated, dried under shade and were powdered separately. For extraction, we employed maceration process in which 20 g of each powder was transferred into separate conical flasks containing 100 ml of methanol. The flasks were left for 48 h (during which the flasks were stirred occasionally) followed by filtering the contents of flasks through 4-fold muslin cloth followed by Whatman No. 1 filter paper. The filtrates were evaporated to dryness at 40°C and the extracts obtained were stored in refrigerator until use.[8,14]

Phytochemical Screening of Extracts

The leaf, stem, and inflorescence extracts were subjected to preliminary phytochemical analysis. The presence of various phytoconstituents namely alkaloids, flavonoids, tannins, steroids, saponins, glycosides, terpenoids, and phenols were detected by standard phytochemical tests.[5,6,15]

Antibacterial Activity of Extracts

The potential of different extracts of *P. sessilis* to inhibit Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) was determined by Agar well diffusion method as described in our previous study.[8] In this method, the test bacteria were aseptically inoculated into sterile nutrient broth tubes and incubated at 37°C for 24 h. The broth cultures were swab inoculated on sterile nutrient agar plates. With the help of a sterile gel borer, wells of 6 mm diameter were punched in the inoculated plates. Extracts (20 mg/ml of dimethyl sulfoxide [DMSO]), reference antibiotic (chloramphenicol, 1 mg/ml of sterile distilled water), and DMSO were transferred aseptically in labeled wells. The plates were left undisturbed for 30 min and then incubated in upright position for 24 h at 37°C. Using a ruler, zones of inhibition formed around wells was measured. The presence of zone of inhibition around the wells is the indication of antibacterial activity of extracts.

Antifungal Activity of Extracts

Poisoned food technique employed in our previous studies[8,16] was used to assess the antifungal effect of extracts of *P. sessilis* against test fungi, namely, *Colletotrichum capsici*, *Fusarium oxysporum* f.sp. *Zingiberi,* and *Alternaria alternata*. In brief, control (without extract) and poisoned (0.5 mg extract/ml of medium) potato dextrose agar plates were aseptically inoculated with the test fungi followed by incubating the plates at 28°C for 96 h in upright position. Later, the colony diameter of test fungi was measured using a ruler in mutual perpendicular directions. Antifungal effect (in terms of reduction in mycelial growth of test fungi) of extracts was determined using the formula:

Inhibition of mycelial growth (%) = (A-B/A) ×100, where "A" and "B" refers to colony diameter of test fungi in control and poisoned plates, respectively. Reduction in mycelial growth of test fungi in poisoned plates is indication of antifungal potential of extracts.

1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Activity of Extracts

In this assay, 1ml of different concentrations (6.25-200 µg/ml of methanol) of extracts and ascorbic acid (reference standard) was mixed with 3 ml of DPPH radical solution (0.004% in methanol) in labeled test tubes. The tubes were then incubated in dark for 30 min at room temperature followed by measuring the absorbance of reaction mixture in spectrophotometer at 517 nm. Methanol replacing the extract/ascorbic acid served as control (i.e., 1 ml methanol + 3 ml DPPH radical solution). Inhibition of DPPH radicals (%) was calculated using the formula:

Inhibition of DPPH radicals (%) = (A-B/A) ×100, where "A" and "B" refers to the absorbance of DPPH control and absorbance of DPPH in the presence of extract/ascorbic acid. The inhibitory concentration (IC_{50}) value was calculated. IC_{50} value denotes the concentration of extract/standard required to scavenge 50% of free radicals.[16,17]

2,2-Azinobis 3-Ethylbenzothiazoline 6-Sulfonate (ABTS) Scavenging Activity of Extracts

Unlike DPPH assay, the assay that involves scavenging of ABTS radicals requires generation of the radicals. The ABTS radical was generated by mixing ABTS stock solution (7 mM) with potassium persulfate (2.45 mM). The reaction mixture was left in the dark for 16 h at room temperature and the resulting dark colored solution was diluted using distilled water to an absorbance of 0.7 at 730 nm. 1 ml of different concentrations (6.25-200 µg/ml of methanol) of extracts and ascorbic acid (reference standard) was mixed with 3 ml of ABTS radical solution in clean and labeled test tubes. The tubes were incubated in dark for 30 min at room temperature followed by measuring the absorbance of reaction mixture in spectrophotometer at 730 nm. Methanol replacing the extract/ascorbic acid served as control (i.e., 1 ml methanol + 3 ml ABTS radical solution). The ABTS radical scavenging activity of extracts was calculated using the formula:

Scavenging activity (%) = (A-B/A) ×100, where "A" is the absorbance of the ABTS solution without extract/ascorbic acid and "B" is the absorbance of ABTS solution in the presence of extract/ascorbic acid. The IC_{50} value was calculated. IC_{50} denotes the concentration of extract required to scavenge 50% of the radicals.[16]

Ferric Reducing Activity of Extracts

The reducing potential of extracts from different parts of *P. sessilis* was determined by ferric reducing assay.[17,18] The initial reaction mixture consisted of different concentrations (6.25-200 µg/ml) of extracts and ascorbic acid (reference standard) in 1 ml of methanol, 2.5 ml of phosphate buffer (pH 6.6), and 2.5 ml of potassium ferricyanide (1%). The tubes were incubated at 50°C for 20 min in a water bath. After cooling, 2.5 ml of trichloroacetic acid (10%) followed by 0.5 ml of ferric chloride (0.1%) was added to each of the tubes and the tubes were left for 10 min at room temperature. The absorbance of reaction mixture of each tube was measured at 700 nm spectrophotometrically. An increase in the absorbance with increase in concentration of extracts/standard indicated increasing reducing power.

Total Phenolic Content of Extracts

Folin-Ciocalteu reagent (FCR) method is employed to determine the content of phenolics in extracts and this method is widely used method for estimating the content of total phenolics in various samples including plant extracts. In this method, a dilute concentration of each extract (0.5 ml) was mixed with 0.5 ml of FC reagent (1:10) and 2 ml of sodium carbonate (2%) in separate tubes. The tubes were incubated at room temperature for 30 min. The absorbance of reaction mixtures of each tube was determined spectrophotometrically at 765 nm. Gallic acid was used as standard and a standard curve was plotted using different concentrations of gallic acid (0-1000 µg/ml). The total phenolic content in different extracts was estimated as mg GAE from the graph.[8,19]

RESULTS AND DISCUSSION

Phytoconstituents Detected in Extracts of *P. sessilis*

The medicinal and pharmacological properties exhibited by plants are due to the presence of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and terpenoids that are distributed in the various parts of the plants. These chemicals are studied under the concept called

phytochemistry. Most of these phytochemicals have profound physiological effects on the health. Hence, it is important to detect these phytoconstituents in medicinal plants so as to correlate the possible therapeutic role played by them.[5,6,15,19-22] There are several protocols to extract phytochemicals from plants, such as maceration, Soxhlet extraction, supercritical fluid extraction, and microwave-assisted extraction.[17] In the present study, we followed maceration process to get an extract from various parts of *P. sessilis* using methanol as the extraction solvent. It has been shown that methanol can dissolve many phytochemicals including polyphenolic compounds present in the plants.[17,20,23-25]

The yield and color of extracts of *P. sessilis* is shown in Table 1. The yield was high in case of stem followed by leaf and inflorescence. The color of inflorescence and stem extracts was light green whereas leaf extract was dark green. In the present study, we screened the presence of various phytochemicals in leaf, stem, and inflorescence extract of *P. sessilis* by standard tests. Table 2 shows the phytochemicals which were detected in extracts of *P. sessilis*. Flavonoids, saponins, steroids, and phenols were present in all three extracts. Phytoconstituents, namely, alkaloids and glycosides were not detected in all three extracts. Terpenoids and tannins were detected only in inflorescence and leaf extract, respectively. The study of Shende *et al*.[11] revealed the presence of glycosides, steroids, and flavonoids in the leaf of *S. sessilis*. The study of Patil *et al*.[13] showed the presence of phenols, flavonoids, saponins, and tannins in leaf extract of *P. sessilis*.

Table 1: Yield and color of extracts of *P. sessilis*

Extract	Yield (%)	Color
Leaf	4.33	Dark green
Inflorescence	4.10	Light green
Stem	6.41	Light green

P. sessilis: Pleocaulus sessilis

Table 2: Phytoconstituents detected in extracts of *P. sessilis*

Constituents	Leaf	Inflorescence	Stem
Alkaloids	-	-	-
Flavonoids	+	+	+
Saponins	+	+	+
Terpesnoids	-	+	-
Glycosides	-	-	-
Tannins	+	-	-
Steroids	+	+	+
Phenols	+	+	+

P. sessilis: Pleocaulus sessilis

Antibacterial Activity of Extracts of *P. sessilis*

One of the major milestones in the field of medicine is the discovery of antibiotics. The use of these wonder drugs resulted in prevention and control of huge number of deaths. However, indiscriminate use of these antibiotics resulted in the emergence of resistant pathogens. Antibiotic therapy not only affects the target pathogen but also commensal inhabitants of the human host. Moreover, the ability of these pathogens to transmit the resistance to susceptible ones created major problem in the treatment of diseases. These antibiotic-resistant pathogens are of serious concern in the community as well as hospital settings. High cost, side effects, and the resistance problems associated with these antibiotics triggered immense interest in scientific community to search alternatives for disease control. Plants, their extracts, and the purified compounds from them are shown to be effective in inhibiting pathogenic bacteria including resistant strains.[20,22,26-30]

In this study, we screened the efficacy of extracts of *P. sessilis* by agar well diffusion assay. The result of the inhibitory activity of extracts against Gram positive and Gram negative bacteria is shown in Table 3. The presence of inhibition zone around the wells was considered positive for antibacterial activity. Extracts were shown to inhibit all test bacteria. Leaf and inflorescence extracts inhibited *K. pneumoniae* to high extent while stem extract caused high inhibition of *B. subtilis* and *S. aureus*. *B. subtilis* and *K. pneumoniae* were inhibited to high extent among Gram-positive and Gram-negative bacteria, respectively. Overall, stem extract displayed least inhibitory activity. Inhibitory activity of reference antibiotic was higher than that of extracts. No

Table 3: Antibacterial activity of extracts of *P. sessilis*

Treatment	Zone of inhibition in cm			
	B. subtilis	*S. aureus*	*P. aeruginosa*	*K. pneumoniae*
Leaf extract	1.6	1.5	1.5	1.8
Inflorescence extract	1.7	1.5	1.6	1.9
Stem extract	1.4	1.4	1.2	1.4
Antibiotic	2.8	2.9	2.6	2.4
DMSO	0.0	0.0	0.0	0.0

B. subtilis: Bacillus subtilis, S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa, K. pneumonia: Klebsiella pneumonia, P. sessilis: Pleocaulus sessilis

inhibition of test bacteria was observed in case of DMSO. Extracts and purified compounds from *Strobilanthes* species have shown to possess antibacterial activity. Taraxerol, isolated from *Strobilanthes callosus* exhibited high reduction of edema but the lower antimicrobial effect at doses employed.[31] A compound 4-acetyl-2,7-dihydroxy-1,4,8-triphenyloctane-3,5-dione isolated from dichloromethane extract of *Strobilanthes crispus* was shown to possess marked inhibitory activity against Gram-positive and Gram-negative bacteria.[32] Methanol extract of leaf of *Strobilanthes cusia* exhibited marked activity against *S. aureus* followed by *B. subtilis*, *Enterobacter aerogenes*, *Escherichia coli,* and *K. pneumoniae*.[33] Venkatachalapathi and Ravi[34] evaluated the antibacterial activity of the petroleum ether and methanolic extracts from the *Strobilanthes ciliatus*. Both extracts exhibited inhibitory activity against Gram-positive and Gram-negative bacteria. The leaf extract of *S. crispus* was shown to exhibit inhibitory activity against *S. aureus* and *Streptococcus pneumoniae* whereas no visible inhibition was observed against *K. pneumoniae* and *P. aeruginosa*.[35]

Antifungal Activity of Extracts of *P. sessilis*

Several biological agents such as insects, bacteria, viruses, and fungi attack plants at various stages of growth and development resulting in a reduction of productivity and economic loss to farmers. As compared to other agents, the impact of fungi on crop production losses is highest. Agrochemicals such as pesticides and fungicides have been routinely used to prevent and control plant diseases and crop loss. However, many fungicides are toxic and have undesirable effects on non-target organisms present in the environment. Some synthetic fungicides are non-biodegradable and accumulate in the soil, plants, and water and consequently affect humans through the food chain. Besides, the development of resistance that has been noticed in phytopathogenic fungi toward the synthetic fungicides is another great challenge. Hence, it is desirable to use alternative approaches that are eco-friendly for controlling of plant diseases. Plants appear to be promising alternatives for plant disease management. It is known that the use of natural products can reduce the population of pathogens and control the development of diseases. Plants have been considered as potential agents in integrated pest management programs. The use of plant-based formulations is cheap, eco-friendly, and free of toxic effect on humans. A number of plants have been reported to cause inhibition of several phytopathogenic fungi.[23,36-41]

Table 4 and Figure 2 depict the antifungal effect of extracts against test fungi. Poisoning of medium with the leaf, inflorescence, and stem extracts revealed a considerable reduction in the size of colonies of test fungi. Extracts exhibited varied inhibitory activity against test fungi (inhibitory activity ranged between 20% and 60%). Leaf extract inhibited *C. capsici* (60.52%) to higher extent

Table 4: Colony diameter of test fungi on control and poisoned plates

Treatment	Colony diameter in cm		
	C. capsici	*F. oxysporum*	*A. alternata*
Control	3.8	4.0	3.3
Leaf extract	1.5	2.4	1.8
Inflorescence extract	2.4	3.2	1.8
Stem extract	3.0	3.2	2.1

C. capsici: Colletotrichum capsici, F. oxysporum: Fusarium oxysporum, A. alternate: Alternaria alternata

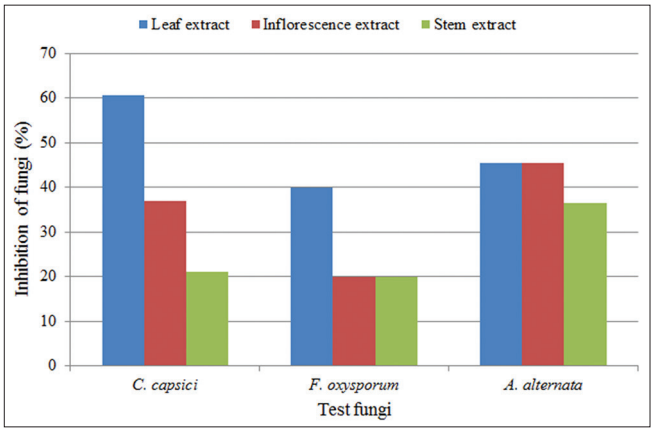

Figure 2: Inhibition of test fungi (%) by extracts of *Pleocaulus sessilis*

followed by *A. alternata* (45.45%) and *F. oxysporum* (40%). Both inflorescence and stem extracts inhibited *A. alternata* to a maximum extent followed by *C. capsici* and *F. oxysporum*. Earlier studies have shown the efficacy of extracts of *Strobilanthes* species against fungi. Inhibitory effect of petroleum ether and methanol extract of *S. ciliatus* was tested against fungi, namely, *Trichophyton rubrum*, *Microsporum gypseum*, *Monascus purpureus* by Venkatachalapathi and Ravi.[34] The petroleum ether extract exhibited significant antifungal activity against the test fungi. The leaf extract of *S. crispus* did not show any visible inhibition against *Aspergillus brasiliensis* and *Candida albicans*.[35]

DPPH Radical Scavenging Activity of Extracts of *P. sessilis*

The method of DPPH radical scavenging was first developed by Blois[42] to determine the antioxidant activity using a stable DPPH-free radical. The assay is based on the measurement of the scavenging capacity of substances termed as antioxidants. In DPPH, the odd electron of nitrogen atom is reduced when it receives a hydrogen atom from antioxidants resulting in the formation of corresponding hydrazine (DPPH). DPPH radical is a stable, organic and nitrogen-centered free radical having a strong absorption at 517 nm (in alcoholic solution). The absorption decreases as the electron pairs off. The

decolorization is stoichiometric with respect to the number of electrons taken up. The method involving scavenging of DPPH radicals is simple, rapid, inexpensive, and widely used method to measure the capacity of compounds to behave as free radical scavengers or hydrogen donors. This assay has been widely employed for investigating antioxidant properties of various kinds of samples including plant extracts. One more advantage is that the radical is stable and need not be generated as in case of ABTS radicals.[42-47]

In the present study, we evaluated the effect of extracts of *P. sessilis* to scavenge-free radicals by DPPH assay. Bleaching of color of DPPH radical solution in the presence of varying concentrations of extracts and standard was monitored at 517 nm. Extracts and ascorbic acid scavenged DPPH radicals in a dose-dependent manner [Figure 3]. Among extracts, leaf extract exhibited stronger scavenging potential with IC_{50} value 27.16 µg/ml followed by inflorescence extract (IC_{50} value of 37.15 µg/ml) and stem extract (IC_{50} value of 58.56 µg/ml). Ascorbic acid exhibited stronger scavenging effect (IC_{50} value of 6.17 µg/ml) when compared to extracts. Although scavenging effect caused by extracts was low when compared to ascorbic acid, it is evident from the study that the extracts possess hydrogen donating efficacy; and hence, the extracts can act as potent-free radical scavengers. It has been shown that *Strobilanthes* species exhibit antioxidant activity. The water soluble vitamins and catechins of *S. crispus* contributed to high antioxidant activity of leaves of *S. crispus*.[48] Solvent extracts of *Strobilanthes kunthiana* have been shown to possess scavenging effect against DPPH radicals.[49] In another study, Ghasemzadeh *et al*.[9] observed DPPH radical scavenging potential of aqueous and ethanol extract of *S. crispus*.

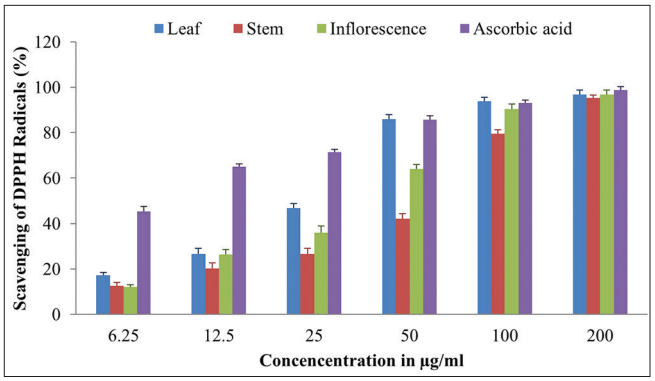

Figure 3: 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity of extracts and reference standard

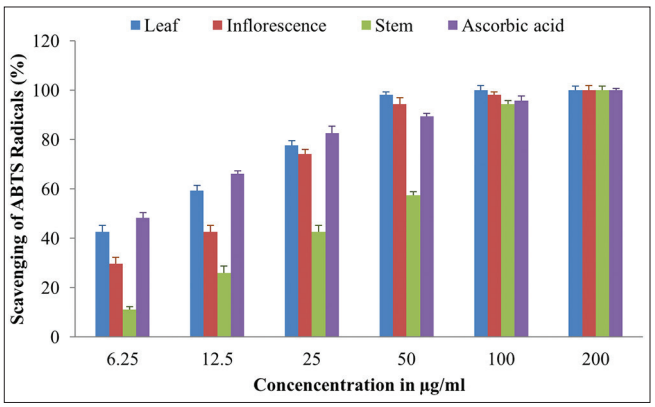

Figure 4: 2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate radical scavenging activity of extracts and reference standard

ABTS Radical Scavenging Activity of Extracts of *P. sessilis*

Similar to DPPH assay, the ABTS assay is another widely used *in vitro* radical scavenging assay. However, this method needs the generation of ABTS radicals which can be easily done by reacting ABTS salt with potassium persulfate. The ABTS radical cation is reactive towards most antioxidant compounds. ABTS radical is soluble in aqueous as well as organic solvents. The method is a useful in determining the antioxidant potential of both lipophilic and hydrophilic antioxidants in a variety of samples including plant extracts. A compound having electron donating property will reduce the blue-green ABTS radical solution to colorless neutral form. This reduction is indicated by suppression of its characteristic long wavelength absorption spectrum.[14,43,45,46,50-53]

In the present study, we evaluated the effect of extracts of *P. sessilis* to scavenge ABTS radicals and the result is shown in Figure 4. The extracts scavenged ABTS radicals in a dose-dependent manner. Among extracts, leaf extract displayed marked scavenging activity (IC_{50} value 9.16 µg/ml) followed by inflorescence extract (IC_{50} value 15.11 µg/ml) and stem extract (IC_{50} value 39.30 µg/ml). At extract concentration 100 µg/ml and higher, a scavenging activity of >90% was observed in case of all extracts. Reference compound ascorbic acid scavenged ABTS radicals more efficiently (IC_{50} value of 5.69 µg/ml) than extracts of *P. sessilis*. Extracts scavenged ABTS radicals more effectively than DPPH radicals. Although the extracts have displayed low radical scavenging effect when compared to ascorbic acid, it is evident that the extracts possess the electron donating property due to which the extracts could serve as potent scavengers of free radicals.

Ferric Reducing Activity of Extracts of *P. sessilis*

Several assays are designed and used to determine the overall antioxidant activity as an indication of total capacity to withstand adverse effect of stress induced by the formation of free radicals. The reducing potential reflects the electron donating capacity which is associated with antioxidant activity. The presence of reductants (antioxidants) in samples results in the reduction of ferric complex to ferrous form and this reducing potential of sample can be determined by the direct reduction of $Fe[(CN)_6]_3$ to $Fe[(CN)_6]_2$. The addition of free Fe^{3+} to the reduced product results in the formation of the intense Perl's Prussian blue complex, $Fe_4[Fe(CN)_6]_3$, which possess a strong absorbance at 700 nm. An increase in absorbance of the reaction mixture indicates an increase in

the reducing capacity of extract.[14,17,54-58] In the present study, we screened the extracts of *P. sessilis* for ferric reducing activity. An increase in the absorbance of reaction mixtures was observed on increasing the concentration [Figure 5]. This indicated reducing power of the extracts. Among extracts, marked potential was observed in case of leaf extract followed by inflorescence and stem extracts. Ascorbic acid displayed higher reducing potential than that of extracts. Although the reducing potential of extracts observed was low when compared to reference standard, it is evident that the extracts possess reductive ability which could serve as electron donors, terminating the radical chain reactions.

Total Phenolic Content of Extracts of *P. sessilis*

Plants are known to produce a wide array of secondary metabolites. Out of many phytochemicals, the phenolic compounds are considered to be the most important plant secondary metabolites as they are beneficial to the plants and exhibit a range of bioactivities including antioxidant activity. These compounds are shown to exhibit strong antioxidant activity due to their ability to scavenge-free radicals, break radical chain reactions, and to chelate metal ions. Consumption of foods containing phenolic compounds is proven to be associated with reduced risk of cardiovascular diseases and certain types of cancer.[56,59-63] FCR method is widely used to estimate the content of total phenolics in plants. The method is simple, oldest, and the results are reproducible. The phenolic compounds react with FCR under basic conditions and results in the formation of a blue colored complex which exhibit absorption maxima near 750 nm.[14,64] In this study, we estimated the content of total phenolics in extracts of *P. sessilis* by FCR method. The phenolic content was found to be high in leaf extract followed by inflorescence extract and stem extract [Table 5]. A positive correlation was observed between the phenolic content of extracts and antioxidant activity observed, i.e., extracts containing high phenolic content exhibited stronger antioxidant activity. Such observations were made in earlier studies of Tilak *et al*.,[59] Vivek *et al*.,[64] Coruh *et al*.[65] and Poornima *et al*.[66] where extracts containing high phenolic content exhibited marked antioxidant activity.

CONCLUSIONS

Plants have been used traditionally for the treatment of various ailments throughout the world. Extracts and purified metabolites from plants exhibit a range of bioactivities which can be exploited for drug development. In the present study, we observed antimicrobial and antioxidant activity in leaf, inflorescence, and stem extracts of *P. sessilis*. Overall, leaf extract exhibited stronger bioactivities when compared to other two extracts. The observed bioactivities could be ascribed to the presence of secondary metabolites detected in the extracts. The plant can be used against microbial infections and oxidative damage. Further studies on isolation of active principles from extracts and their bioactivity determinations are to be carried out.

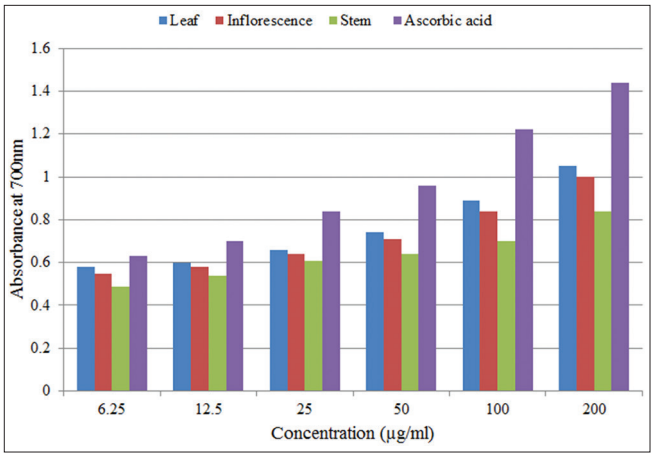

Figure 5: Ferric reducing activity of extracts and reference standard

Table 5: Content of total phenolics (mg GAE/g) in extracts

Extract	Total phenolic content
Leaf	112.13
Inflorescence	85.65
Stem	42.42

GAE: Gallic acid equivalents

ACKNOWLEDGMENTS

Authors would like to thank Principal, S.R.N.M.N College of Applied Sciences and N.E.S, Shivamogga for providing facilities and moral support to conduct research.

REFERENCES

1. Vedavathy S. Scope and importance of traditional medicine. Indian J Tradit Knowl 2003;2:236-9.
2. Williams LA. Ethnomedicine. West Indian Med J 2006;55:215-6.
3. Idu M, Erhabor JO, Efijuemue HM. Documentation on medicinal plants sold in markets in Abeokuta, Nigeria. Trop J Pharm Res 2010;9:110-8.
4. Rath S, Dubey D, Sahu MC, Debata NK, Padhy RN. Surveillance of multidrug resistance of 6 uropathogens in a teaching hospital and *in vitro* control by 25 ethnomedicinal plants used by an aborigine of India. Asian Pac J Trop Biomed 2012;2:S818-29.
5. Bhandary SK, Kumari S, Bhat VS, Sharmila KP, Bekal MP. Preliminary phytochemical screening of various extracts of *Punica granatum* peel, whole fruit and seeds. Nitte Univ J Health Sci 2012;2:34-8.

6. Mir AM, Sawhney SS, Jassal MM. Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*. Wudpecker J Pharm Pharmocol 2013;2:15.
7. Swamy SH, Asha MM, Chaithra M, Vivek MN, Kambar Y, Kekuda PT. Antibacterial activity of flower extract of *Caesalpinia pulcherrima*, *Delonix regia* and *Peltaphorum ferrugineum* against urinary tract pathogens. Int Res J Biol Sci 2014;3:80-3.
8. Kekuda PT, Akarsh S, Darshini SM, Prafulla D, Raghavendra HL. Antiradical and antimicrobial activity of *Atylosia lineata* Wt. And Arn. Sci Technol Arts Res J 2015;4:180-3.
9. Ghasemzadeh A, Jaafar HZ, Rahmat A. Phytochemical constituents and biological activities of different extracts of *Strobilanthes crispus* (L.) Bremek leaves grown in different locations of Malaysia. BMC Complement Altern Med 2015;15:422.
10. Preethi F, Suseem SR. A comprehensive study on an endemic Indian genus - *Strobilanthes*. Int J Pharmacogn Phytochem Res 2014;6:459-66.
11. Shende VS, Jadhav SD, Aloorkar NH, Kulkarni AS, Suryavanshi SV. Pharmacognostic and phytochemical evaluation of *Strobilanthes sessilis* nees. Leaves. Int J Pharmacogn 2015;2:310-4.
12. Yoganarasimhan SN, Subramanyam K, Razi BA. Flora of Chikmagalur District. Dehra Dun: International Book Distributors; 1981. p. 250.
13. Patil SV, Mane RP, Anbhule PV, Shimpale VB, Deshmukh MB. Phytochemical estimation, antioxidant and antimicrobial properties of *Pleocaulus sessilis*: An endemic plant from Western Ghats of India. J Appl Chem 2015;3:1-10.
14. Hazra B, Biswas S, Mandal N. Antioxidant and free radical scavenging activity of *Spondias pinnata*. BMC Complement Altern Med 2008;8:63.
15. Yusuf AZ, Zakir A, Shemau Z, Abdullahi M, Halima SA. Phytochemical analysis of the methanol leaves extract of *Paullinia pinnata* Linn. J Pharmacogn Phytother 2014;6:10-6.
16. Kekuda PT, Raghavendra HL, Solomon T, Duressa D. Antifungal and antiradical potential of *Moringa stenopetala* (Baker f.) Cufod (Moringaceae). J Biosci Agric Res 2016;11:923-9.
17. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ju Y. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J Food Drug Anal 2014;22:296-302.
18. Kekuda PT, Manasa M, Poornima G, Abhipsa V, Rekha C, Upashe SP, *et al*. Antibacterial, cytotoxic and antioxidant potential of *Vitex negundo* var. *negundo* and *Vitex negundo* var. *purpurascens*- A comparative study. Sci Technol Arts Res J 2013;2:59-68.
19. Zlotek U, Mikulska S, Nagajek M, Swieca M. The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (*Ocimum basilicum* L.) extracts. Saudi J Biol Sci 2016;23:628-33.
20. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12:564-82.
21. Omar S, Lemonnier B, Jones N, Ficker C, Smith ML, Neema C, *et al*. Antimicrobial activity of extracts of eastern North American hardwood trees and relation to traditional medicine. J Ethnopharmacol 2000;73:161-70.
22. Gobalakrishnan R, Kulandaivelu M, Bhuvaneswari R, Kandavelb D, Kannan L. Screening of wild plant species for antibacterial activity and phytochemical analysis of *Tragia involucrata* L. J Pharm Anal 2013;3:460-5.
23. Gurjar MS, Ali S, Akhtar M, Singh KS. Efficacy of plant extracts in plant disease management. Agric Sci 2012;3:425-33.
24. Naz F, Qamarunnisa S, Shinwari ZK, Azhar A, Ali SI. Phytochemical investigations of *Tamarix indica* Willd. and *Tamarix passernioides* Del. ex Desv. leaves from Pakistan. Pak J Bot 2013;45:1503-7.
25. Iloki-Assanga SB, Lewis-Luján LM, Lara-Espinoza CL, Gil-Salido AA, Fernandez-Angulo D, Rubio-Pino JL, *et al*. Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*. BMC Res Notes 2015;8:396.
26. Lenski RE. Bacterial evolution and the cost of antibiotic resistance. Int Microbiol 1998;1:265-70.
27. Jernberg C, Löfmark S, Edlund C, Jansson JK. Long-term impacts of antibiotic exposure on the human intestinal microbiota. Microbiology 2010;156:3216-23.
28. Shakeri A, Hazeri N, Vlizadeh J, Ghasemi A, Tavallaei FZ. Phytochemical screening, antimicrobial and antioxidant activities of *Anabasis aphylla* L. Extracts. Kragujevac J Sci 2012;34:71-8.
29. Dahiya P, Purkayastha S. Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates. Indian J Pharm Sci 2012;74:443-50.
30. Naz R, Bano A. Phytochemical screening, antioxidants and antimicrobial potential of *Lantana camara* in different solvents. Asian Pac J Trop Dis 2013;3:480-6.
31. Singh B, Sahu PM, Sharma MK. Anti-inflammatory and antimicrobial activities of triterpenoids from *Strobilanthes callosus* nees. Phytomedicine 2002;9:355-9.
32. Koay YC, Wong KC, Osman H, Eldeen IM, Asmawi MZ. Chemical constituents and biological activities of *Strobilanthes crispus* L. Rec Nat Prod 2013;7:59-64.
33. Shahni R, Handique PJ. Antibacterial properties of leaf extracts of *Strobilanthes cusia* (Nees) Kuntze, a rare ethno-medicinal plant of Manipur, India. Int J PharmTech Res 2013;5:1281-5.
34. Venkatachalapathi S, Ravi S. Antimicrobial activity of *Strobilanthes ciliatus* nees. Indo Am J Pharm Res 2013;3:3124-8.
35. Lim V, Yap CS, Chong HW, Shukkoor MS, Priya M. Antimicrobial evaluation and GC-MS analysis of *Strobilanthes crispus* ethanolic leaf extract. Eur J Med

Plants 2015;10:1-8.
36. Tapwal A, Nisha, Garg S, Gautam N, Kumar R. *In vitro* antifungal potency of plant extracts against five phytopathogens. Braz Arch Biol Technol 2011;54:1093-8.
37. Nashwa SM, Abo-Elyousr KA. Evaluation of various plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. Plant Prot Sci 2012;48:74-9.
38. Al-Aksar AA. *In vitro* antifungal activity of three Saudi plant extracts against some phytopathogenic fungi. J Plant Prot Res 2012;52:458-62.
39. Kambar Y, Manasa M, Vivek MN, Kekuda PT. Inhibitory effect of some plants of Western Ghats of Karnataka against *Colletotrichum capsici*. Sci Technol Arts Res J 2014;3:76-82.
40. Neela FA, Sonia IA, Shamsi S. Antifungal activity of selected medicinal plant extract on *Fusarium oxysporum* Schlechtthe causal agent of fusarium wilt disease in tomato. Am J Plant Sci 2014;5:2665-71.
41. Sales MD, Costa HB, Fernandes PM, Ventura JA, Meira DD. Antifungal activity of plant extracts with potential to control plant pathogens in pineapple. Asian Pac J Trop Biomed 2016;6:26-31.
42. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958;181:1199-200.
43. Antolovich M, Prenzler PD, Patsalides E, McDonald S, Robards K. Methods for testing antioxidant activity. Analyst 2002;127:183-98.
44. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 2004;26:211-9.
45. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem 2005;53:4290-302.
46. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Comp Anal 2006;19:669-75.
47. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol 2011;48:412-22.
48. Ismail M, Manickam E, Danial AM, Rahmat A, Yahaya A. Chemical composition and antioxidant activity of *Strobilanthes crispus* leaf extract. J Nutr Biochem 2000;11:536-42.
49. Brijyog, Singh B, Das S, Maithi A. Antioxidant property for lipophilic extract of *Strobilanthes kunthiana* flowers. Indian J Res Pharm Biotechnol 2014;2:1005-9.
50. Wan C, Yu Y, Zhou S, Liu W, Tian S, Cao S. Antioxidant activity and free radical-scavenging capacity of *Gynura divaricata* leaf extracts at different temperatures. Pharmacogn Mag 2011;7:40-5.
51. Martysiak-Zurowska D, Wenta W. A comparison of ABTS and DPPH methods for assessing the total antioxidant capacity of human milk. Acta Sci Pol Technol Aliment 2012;11:83-9.
52. Rakesh KN, Junaid S, Dileep N, Vinayaka KS, Kekuda PT, Raghavendra HL. Antibacterial and antioxidant activity of *Fahrenheitia zeylanica* (Thw.) Airy. Sci Technol Arts Res J 2013;2:27-33.
53. Shalaby EA, Shanab SM. Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of *Spirulina platensis*. Indian J Geo Mar Sci 2013;42:556-64.
54. Gulcin I, Topal F, Sarikaya SB, Bursal E, Bilsel G, Goren AC. Polyphenol contents and antioxidant properties of Medlar (*Mespilus germanica* L.). Rec Nat Prod 2011;5:158-75.
55. Meir S, Kanner J, Akiri B, Hadas SP. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J Agric Food Chem 1995;43:1813-7.
56. Chung Y, Chien C, Teng K, Chou S. Antioxidative and mutagenic properties of *Zanthoxylum ailanthoides* Sieb and Zucc. Food Chem 2006;97:418-25.
57. Kekuda PT, Vinayaka KS, Swathi D, Suchitha Y, Venugopal TM, Mallikarjun N. Mineral composition, total phenol content and antioxidant activity of a macrolichen *Everniastrum cirrhatum* (Fr.) Hale (*Parmeliaceae*). E J Chem 2011;8:1886-94.
58. Kiran R, Kekuda PT, Kumar PH, Hosetti BB, Krishnaswamy K. Biological activities of *Sarcanthus pauciflorus*. J Appl Pharm Sci 2013;3:105-10.
59. Tilak JC, Adhikari S, Devasagayam TP. Antioxidant properties of *Plumbago zeylanica,* an Indian medicinal plant and its active ingredient, plumbagin. Redox Rep 2004;9:219-27.
60. Kusirisin W, Jaikang C, Chaiyasut C, Narongchai P. Effect of polyphenolic compounds from *Solanum torvum* on plasma lipid peroxidation, superoxide anion and cytochrome P450 2E1 in human liver microsomes. Med Chem 2009;5:583-8.
61. Sudharshan SJ, Valleesha NC, Chinmaya A, Kekuda PT, Murthuza S, Rajeshwara AN. Radical scavenging activity, phenol and flavonoid content of selected traditionally used Indian medicinal plants. Asian J Exp Sci 2010;24:11-5.
62. Pavithra GM, Siddiqua S, Naik AS, Kekuda PT, Vinayaka KS. Antioxidant and antimicrobial activity of flowers of *Wendlandia thyrsoidea*, *Olea dioica*, *Lagerstroemia speciosa* and *Bombax malabaricum*. J Appl Pharm Sci 2013;3:114-20.
63. Sayari N, Saidi MN, Sila A, Ellouz-Chaabouni S, Bougatef A. Chemical composition, angiotensin I-converting enzyme (ACE) inhibitory, antioxydant and antimicrobial activities of *Ononis natrix* leaves extracts. Free Radic Antioxid 2016;6:23-33.
64. Vivek MN, Kambar Y, Manasa M, Kekuda PT, Vinayaka KS. Radical scavenging and antibacterial activity of three *Parmotrema* species from Western Ghats

of Karnataka, India. J Appl Pharm Sci 2014;4:86-91.

65. Coruh N, Celep AG, Ozgokce F, Iscan M. Antioxidant capacities of *Gundelia tournefortii* L. Extracts and inhibition on glutathione-S-transferase activity. Food Chem 2007;100:1249-53.
66. Poornima G, Kekuda PT, Vinayaka KS. Antioxidant efficacy of *Olea dioica* Roxb (*Oleaceae*) leaves. Biomedicine 2012;32:506-10.

Source of Support: Nil. **Conflict of Interest:** None declared.