Innovare Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 7, Issue 4, 2015

Original Article

A COMPARATIVE STUDY ON *IN VITRO* ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF METHANOL EXTRACT FROM THE LEAVES OF *STACHYTARPHETA INDICA (*L) VAHL AND *PREMNA CORYMBOSA* ROTTL

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Received: 20 Jul 2015, Revised and Accepted: 03 Sep 2015

ABSTRACT

Objective: The objective of this research was to investigate the *in vitro* antioxidant and antibacterial activities of methanol extract from the leaves of S*tachytarpheta indica* (L) vahl. and *Premna corymbosa* Rottl.

Methods: The various photochemicals were analyzed according to the methods described by Allen and Harbone. The total phenolic contents were estimated by Folin–Ciocalteu method. Antioxidant activities of these plants were tested on the basis of ABTS (2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical scavenging assay, inhibition of lipid peroxidation, Super oxide radical scavenging activity, Nitric oxide radical scavenging activity and Metal chelating activity. Similarly antibacterial activity was performed by Disc diffusion method against *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pnemoniae, Escherichia coli* and *Enterococcus faecalis.*

Results: The phytochemical analysis showed the presence of Tannins, Flavonoids, Alkaloids and Anthraquinones. The total phenolic contents were more in *P. corymbosa* (381.10±13.00 mg/g) than *S. indica* (195.72±8.75 mg/g). The methanol extract of the leaves of *P. cormbosa* showed significant antioxidant activity, while it was comparatively less in *S. indica*. Similarly the study on antibacterial activity of these two plant leaves extracts revealed inhibitory activity. However *P. corymbosa* showed higher inhibitory zone against *E. coli, K. pneumonia, P. aeroginosa* (18, 17, 16 mm) respectively compared with S*. indica*.

Conclusion: This research work has made it clear that *P. corymbosa* possess excellent antibacterial and antioxidant activity and the extracts can be more widely used in developing countries for the prevention and treatment of ageing related diseases and may be considered as good source for drug discovery.

Keywords: S*tachytarpheta indica, Premna corymbosa,* Antioxidant, Antibacterial.

INTRODUCTION

Medicinal plants grip decisive liability in enhancement of individuals around planet. Hi-tech explorations of medicinal plants have been instigated in copious parts of our nation as rich hand-outs to health care. Indian traditional system of medicine particularly in Ayurveda and Siddha, herbs are used in rejuvenation therapy as they contain many types of polyphenols that could control ageing related diseases. Natural compounds from medicinal plants have aroused much attention and increasing efforts have been made to search for plant derived antioxidants and antimicrobial agents [1, 2]. The plants develop several antioxidants that aid in antioxidant defense system, protecting plants against damage caused by active O_2 formed due to exposure to UV radiation. Furthermore, medicinal plants are used as 'antioxidants' in traditional medicine for their therapeutic properties in scavenging oxygen free radicals. Reactive oxygen species (ROS) including free radicals such as superoxide anion radicals, hydroxyl radicals, singlet oxygen and non-free radical species such as hydrogen peroxide are various forms of activated oxygen and often generated by oxidation product of biological reactions or exogenous factors.

ROS have aroused significant interest among scientists in the past decade. Their broad range of effects in biological and medicinal systems had drawn the attention of many experimental works. In living organism, various ROS can be formed by different ways leading to ageing. Many antioxidant compounds, naturally occurring from plant sources, have been identified as free radical or active oxygen scavengers [3, 4]. Recently, interest has increased considerably in finding naturally occurring antioxidant for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects, such as carcinogenicity. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as retard lipid oxidative rancidity in foods [5]. Similarly there are several reports of antibiotic resistance of human pathogens to available antibiotics [6, 7]. Hence there is an urgent need to identify novel substances that are active towards highly resistant pathogens [8]. However high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Thus there has been continuous search for new and more potent antibiotic [9]. Due to the cost effectiveness, safety, increasing failure of chemotherapy and antibiotic resistance exhibited by pathogenic microbial agents, search for plant products has increased for their potential antimicrobial activity [10].

S*tachytarpheta indica* (L) vahl. commonly known as snake weed belong to the family Verbenaceae. A well branched herb, 2-3 feet height with very long narrow spikes; flowers deep blue with white center; a weed. Drought tolerant. The plant has been used locally as an abortifacient and in the management of headache, bronchitis, asthma, bruise, chest cold, constipation, itch, dysentery, fever, venereal disease, cataract, sedative, antifertility and rheumatism. *Premna corymbosa* Rottl. popularly known as"Munney" in Tamil and "Agnimantha" in Ayurvedic system of medicine. A small tree or large shrub, Flower small, greenish yellow, in terminal, pubescent, paniculate corymbose cymes, fruit 4 mm long, and pear shaped. Whole plant possesses medicinal properties, useful in treatment of cardiovascular diseases, Skin diseases, inflammatory diseases, arthritis, gonorrhea, rheumatism, anorexia and jaundice. Though there were many scientific validations attempted and reported on S*. indica* and *P. corymbosa*, very less study highlighting its antioxidant and antibacterial activities have been reported in their leaves. So there was a need to evaluate their antioxidant and antibacterial activities. In the present study, invitro antioxidant and antibacterial activities of methanol extract from the leaves of S*. indica* and *P. corymbosa* have been evaluated using various *in vitro* models.

MATERIALS AND METHODS

Materials

Leaves of *Premna corymbosa* and *Stachytarpheta indica* were collected from Sri Sairam Siddha Medical College and Research Centre, Chennai, Tami Nadu, India. Plants were authenticated by Dr. S. Sankaranarayanan, Head, Department of Medicinal Botany, Sri Sairam Siddha Medical College and Research Centre, Chennai.

Phytochemical analysis of *Stachytarpheta indica* and *Premna corymbosa*

The aqueous methanol extract of *S. indica* and *P. corymbosa* was freshly prepared and various chemical constituents were analyzed according to methods described by Allen [11] and Harbone [12]. The different chemical constituents were tested for included tannins, saponins, glycosides, alkaloids, terpenoids, anthocynins, polyphenols and flavonoids.

Extraction of plant materials

The plant materials (leaves of *S. indica* and *P. corymbosa*) were dried in hot air oven at (40 °C) for 1 w, after which it was ground to uniform powder with house hold mixer grinder. The methanol extracts were prepared by soaking 100 g each of the dry powdered plant materials in 500 L of methanol at room temperature for 48 h. The extracts were filtered first through a Whatmann filter paper No. 42 (125 mm) and then centrifuged at 5000 rpm for 10 min (Remi-R-8C, India). The clear solution was collected and concentrated using a rotary evaporator with the water bath set at 40 °C. The percentage yield of extracts ranged from 7–19% w/w.

Determination of total Phenolics

The concentration of total phenolics in the methanol extract of *S. indica* and *P. corymbosa* were determined by using Folin-Ciocalteu reagent and calibrated externally with gallic acid. Briefly, about 0.2 ml of methanol extract and 0.2 ml of Folin-Ciocalteu reagent were added and mixed vigorously. After shaking for 4 min, 1 ml of 15% Na_2CO_3 was added, and finally the mixture was allowed to stand for 2 h at room temperature. The absorbance was measured at 760 nm using Deep Vision 1371 spectrophotometer. The concentration of the total phenolics was estimated as mg of gallic acid equivalent by using an equation obtained from gallic acid calibration curve. The quantification of phenolic compounds in all the fractions was carried out in triplicate and the results were averaged [13].

Invitro antioxidants properties

ABTS (2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical scavenging assay

ABTS radical scavenging activity of Methanol extract of *S. indica* and *P. corymbosa* were determined according to Re *et al.* [14]. ABTS radical was freshly prepared by adding 5 ml of 4.9 mM potassium persulfate solution to 5 ml of 14 mM ABTS solution and kept for 16 h in dark. This solution was diluted with distilled water to yield an absorbance of 0.70 at 734 nm and the same was used for the antioxidant assay. The final reaction mixture of standard group was made up to 1 ml with 950 µl of ABTS solution and 50 µl of Vitamin-C. Similarly, in the test group, 1 ml reaction mixture comprised 950 µl of ABTS solution and 50 µl of the extract solution. The reaction mixture was vortexed for 10 s and after 6 min, absorbance was recorded at 734 nm against distilled water by using a Deep Vision (1371) UV–Vis Spectrophotometer and compared with the control ABTS solution. Ascorbic acid was used as reference antioxidant compound.

Inhibition of lipid peroxidation activity

Lipid peroxidation induced by Fe_{2+}-ascarbate system in egg yolk by the method of Bishayee and Balasubramaniyam1971, was estimated as thiobarbituric acid reacting substances (TBARS) by the method of Ohkawa *et al.* [15]. The reaction mixture contained 0.1 ml of egg yolk (25% w/v) in Tris-HCl buffer (20 mM, pH 7.0); KCl (30 mM); $FeSO_4$ $(NH_4)_2SO_4.7H_2O$ (0.06 mM); and various concentrations of methanol extract of *S. indica* and *P. corymbosa* in a final volume of 0.5 ml. The reaction mixture was incubated at 37°C for 1 h. After the incubation period, 0.4 ml was removed and treated with 0.2 ml sodium dodecyl sulphate (SDS) (1.1%); 1.5 ml thiobarbituric acid (TBA) (0.8%); and 1.5 ml acetic acid (20%, pH 3.5). The total volume was made up to 4.0 ml with distilled water and then kept in a water bath at 95 to 100°C for 1 h. After cooling, 1.0 ml of distilled water and 5.0 ml of n-butanol and pyridine mixture (15:1 v/v) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 min. The butanol-pyridine layer was removed and its absorbance at 532 nm Deep Vision (1371) UV–Vis Spectrophotometer) was measured to quantify TBARS. Inhibition of lipid peroxidation was determined by comparing the optical density (OD) of test sample with control. Ascorbic acid was used as standard.

Inhibition of lipid peroxidation (%) by the extract was calculated according to 1-(E/C) X 100, where C is the absorbance value of the fully oxidized control and E is absorbance of the test sample ($Abs_{532+TBA}$–$Abs_{532+TBA}$).

Superoxide radical scavenging assay

This assay was based on the capacity of the extract to inhibit the photochemical reduction of Nitroblue tetrazolium (NBT) [16] in the presence of the riboflavin-light-NBT system, as described earlier Tripathi and Pandey [17] and Tripathi and Sharma [18]. In brief, each 3 ml reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 100 µM Ethylene diamine tetra acetic acid (EDTA), NBT (75 µM) and different concentration of test sample solution. It was kept in front of fluorescent light and absorbance was taken after 6 min at 560 nm by using a Deep Vision (1371) UV–Vis Spectrophotometer. Identical tubes with reaction mixture were kept in the dark and served as blanks. The percentage inhibition of superoxide generation was measured by comparing the absorbance of the control and those of the reaction mixture containing test sample solution.

$$\% \text{ Super oxide radical scavenging capacity} = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of control and A_1 was the absorbance of organic solvent extract or standard.

Nitric oxide radical scavenging activity

Nitric oxide scavenging capacity of methanol extract of *S. indica* and *P. corymbosa* were measured according to the method described by Olabinri *et al.* [19]. 0.1 ml of sodium nitroprusside (10 mM) in phosphate buffer (0.2 M, pH 7.8) was mixed with different concentration of three different solvent extract and incubated at room temperature for 150 min. After incubation period, 0.2 ml of Griess reagent (1% Sulfanilamide, 2% Phosphoric acid and 0.1% N-(1-Naphthyl) ethylene diamine dihydrochloride) was added. The absorbance of the reaction mixture was read at 546 nm against blank. All readings were taken in triplicate and Vitamin C was used as the standard. The % inhibition was calculated by following equation.

$$\% \text{ Nitric oxide radical scavenging capacity} = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of control and A_1 was the absorbance of different solvent extract.

Metal chelating activity

Metal chelating capacity of methanol extract of *S. indica* and *P. corymbosa* were measured according to the method described by Iihami *et al.* [20]. 1 ml of different concentrations of methanol extract was added to 0.05 ml of 2 mM ferric chloride solution. The reaction was initiated by the addition of 0.2 ml of 5 mM Ferrozine and the mixture was shaken vigorously. After 10 min, the absorbance of the solution was measured at 562 nm against blank. All readings were taken in triplicate and Vitamin C was used as the standard. The % inhibition of ferrozoine-Fe2+complex was calculated by following equation.

$$\% \text{ Inhibition of ferrozoine-Fe}^{2+}\text{complex} = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of control and A_1 was the absorbance of different solvent extract.

Antibacterial Properties

Bacterial strains

Bacteria used for the determination of antibacterial activities were Gram positive viz; *Staphylococcus aureus* MTCC 29213, *Klebsiella*

pneumoniae MTCC 1771 and *Enterococcus faecalis* MTCC 439 and gram negative viz; *Pseudomonas aeruginosa* MTCC 2488, and *Escherichia coli* MTCC 25922. The bacterial strains were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology Sector 39-A, Chandigarh-160036, India. All bacterial strains were sub cultured on nutrient agar medium, incubated at 37 °C for 24 h and stored at 4 °C in refrigerator to maintain stock culture.

Antibacterial assay

Antibacterial activity was carried out using disc diffusion method [21]. Petriplates were prepared with 20 ml of sterile nutrient agar (HIMEDIA). The tested cultures were swabbed on top of the solidified media and allowed to dry for 10 min. The crude extract impregnated discs (Whatman No.1 filter paper was used to prepare discs) were prepared and air dried well. The test was conducted at four different concentrations of the crude extract (5, 10, 15 & 20 µl/ml) with 3 replicates. The loaded discs were placed on the surface of the medium and incubated at room temperature for 24 h. The relative susceptibility of the organisms to the crude extract was indicated by the clear zone of inhibition around the discs. It was then observed, measured and recorded in millimeters.

Statistical analysis

The influence of the methanol, extract of *P. corymbosa* and *S. indica* on its antioxidant activity was measured by the ABTS assay reducing power, lipid peroxidation, superoxide scavenging, metal chelating and Nitric oxide radical were ascertained using one way analysis of variance (ANOVA). Furthermore, Duncan's post hoc test was applied, so as to determine the statistically significant different values. All statistical handling was performed using SPSS software, v. 14.0 (SPSS, Chicago, Ill., U. S. A.).

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemicals screening of methanol extract from the leaves of *P. corymbosa* and *S. indica* showed the presence of Tannins, Flavonoids, Alkaloids, Anthraquinones. But in these two samples Saponins, cardioactive glycosides & glycosides were not found.

Table 1: Phytochemical screening of *P. corymbosa* and *S. indica*

Phytochemical constituents	Result indicated	Aqueous methanol leaves extract of two different medicinal plants	
		P. corymbosa	*S. indica*
Alkaloids Dragendroffs reagent-	Brown precipitation	+	+
Mayers reagent	Yellow precipitation	+	+
Flavonoids Alkalaine test	Yellow coloration	+	+
Lead acetate	Immediate precipitation	+	+
Polyphenols Ferrozine Test	Blue Coloration	+	+
Terpenoids Salkowski test	Brown ring	+	+
Tannins	Dark green blue	+	+
Glycosides Keller-Killani test	Reddish brown ring	--	--
Bronbagers Test	Pink colour in ammonia layer	--	--
Saponins Froth Test	Foam	--	--
Anthocynin Ammonia Test	Yellow colour in ammonia layer	+	+

--Negative result; +Positive result

Total phenolic content

The total phenolic contents of methanol extract from the leaves of *P. corymbosa* and *S. indica* were determined by Folin-Ciocalteu method and reported as gallic acid equivalents. Comparatively *P. corymbosa* showed the highest (381.10±13.00 mg/g) amount of phenolic compounds than *S. indica* (195.72±8.75 mg/g).

In recent reports, it has been reported that the yield of extractable compounds was highest in methanol extract from the peel and seeds of pomegranate in comparison with the solvents such as chloroform, butanol, ethyl acetate and hexane. Furthermore, the extraction of phenolic compounds from the fruits or vegetables is commonly achieved with methanol [22].

Free radical-scavenging ability using ABTS assay

The radical scavenging ability was measured by ABTS assay as given in table 2. The Percentage inhibition of the ABTS radical activity assayed on average, and higher free radical-scavenging values were found in leaves of *P. corymbosa* than *S. indica*. In ABTS assays, the EC_{50} of the pure ascorbic acid was lower than *P. corymbosa* and *S. indica* plants, being 58.28 and 13.78 µg/ml, respectively (Table-2). However, the agreement between this assays, in our study, probably indicate that these activities were mainly due to phenolics and flavonoids. It is known that vitamin C (ascorbic acid) and carotenoids are major source of discrepancy of antioxidant/ antiradical activities in plant materials.

Although these constituents were not investigated, their contributions toward antioxidant/ antiradical activities of these studied medicinal plants may be very minimal. Antioxidant assay is based on electron transfer reaction, whereas ABTS assays are based on electron and H atom transfer [23]. Furthermore, the Vitamin C equivalent antioxidant capacity method was used to express the antioxidant activity which provides a meaningful method for direct comparison with known potent antioxidant widely distributed in plant materials [24].

Table 2: Free radical-scavenging ability ABTS assay

Methanol extracts	ABTS radical Scavenging				
	Different concentrations of extract µl/ml				
	5 µl/ml	10 µl/ml	15 µl/ml	20 µl/ml	EC_{50} Value µl/ml
P. corymbosa	25.41±0.55	41.43±0.69	58.74±0.62	73.85±0.70	58.28
S. indica	22.29±0.75	38.77±0.57	54.18±1.01	70.82±0.62	13.78
Positive Control Vitamin C	23.57±0.6	44.56±0.58	36.33±0.41	82.45±0.32	20.32

Data are presented as the means±standard error of the mean of triplicates.

Inhibition of lipid peroxidation

Methanol extract of *P. corymbosa* and *S. indica* also inhibited the lipid peroxidation induced by ferrous sulfate in egg yolk homogenates. Maximum inhibition was observed in methanol extract of *P. corymbosa* with EC_{50} value at 174.67 µg/ml. As it is known that lipid peroxidation is the net result of any free radical attack on membrane and other lipid constituents present in the system, the lipid peroxidation may be enzymatic (Fe/NADPH) or non-enzymatic (Fe/ascorbic acid). Here we have used egg yolk as a substrate for FR mediated lipid peroxidation, which is a non-enzymatic method. *P. corymbosa* significantly inhibited the degree of lipid peroxidation than *S. indica* (Table-3). This study was correlated with the previous report which resulted pasteurization of blackberry juice preserves polyphenol-dependent inhibition for lipid peroxidation and intracellular radical. No significant reductions were observed for the peroxidation inhibitory capacity of the pasteurized juices for any of the oxidation substrates tested: liposomes, liver homogenates and erythrocytes [25].

Superoxide scavenging assay activity

The methanol extract of *P. corymbosa* exhibited potent scavenging activity for superoxide radicals in a concentration dependent manner than *S. indica*. The methanol extract of P. *corymbosa* had EC_{50} values of 19.39 µg/ml and the methanol extract of *S. indica* least potent with an EC_{50} value of 20.50 µg/ml (Table-4). Superoxide radicals generated by photochemical reduction of nitro blue tetrazolium (NBT) in the presence of a riboflavin-light-NBT system, which is one of the standard methods. These superoxide radicals are highly toxic and may be generated either through xanthine activity or through mitochondrial reaction. Although it is a relatively weak oxidant, it may decompose to form stronger reactive oxidative species, such as singlet oxygen and hydroxyl radicals [26]. Plant phenols may exert protective effects by scavenging superoxide, which is implicated in tissue damage and accelerated inactivation of vasorelaxing nitric oxide. Preventing the interaction of superoxide with tissue biomolecules depends not only on the extent of superoxide scavenging but also on scavenging velocity [27].

Table 3: Inhibition of lipid peroxidation induced by $FeSO_4$ using egg yolk homogenates

Methanol extracts	Different concentrations of extract µl/ml				
	Inhibition percentage of lipid peroxidation				
	5 µl/ml	10 µl/ml	15 µl/ml	20 µl/ml	EC_{50} Value µl/ml
P. corymbosa	22.66±1.52	41.33±4.16	55.66±1.52	71.00±2.00	174.67
S. indica	19.33±1.52	34.66±2.08	51.00±2.00	63.00±2.00	14.99
Positive Control Vitamin C	18.35±1.42	36.44±1.62	54.78±1.85	66.89±1.79	13.47

Data are presented as the means±standard error of the mean of triplicates.

Table 4: Superoxide anion scavenging activity

Methanol extracts	Different concentrations of extract µl/ml				
	Inhibition percentage of lipid peroxidation				
	5 µl/ml	10 µl/ml	15 µl/ml	20 µl/ml	EC_{50} Value µl/ml
P. corymbosa	22.76±0.81	34.96±1.06	51.97±1.16	67.82±0.87	19.39
S. indica	18.10±0.58	31.93±0.92	48.4±1.05	63.23±0.81	20.50
Positive Control Vitamin C	18.58±1.34	38.29±1.26	66.33±1.75	79.24±1.89	12.19

Data are presented as the means±standard error of the mean of triplicates.

Table 5: Nitric oxide radical scavenging activity

Different Medicinal plant methanol extracts	Nitric oxide radical scavenging activity percentage				
	Different concentrations of extract µl/ml				
	5 µl/ml	10 µl/ml	15 µl/ml	20 µl/ml	EC_{50} Value µg/ml
P. corymbosa	19.16±0.50	34.47±0.71	52.09±0.91	68.62±0.91	15.67
S. indica	16.19±0.91	32.51±1.12	47.83±0.91	63.75±0.62	174
Positive Control Vitamin C	25.47±0.85	44.56±0.79	58.12±1.27	71.29±1.68	217.71

Data are presented as the means±standard error of the mean of triplicates.

Table 6: Metal chelating activity

Methanol extracts	Different concentrations of extract µl/ml				
	Metal chelating activity percentage				
	5 µl/ml	10 µl/ml	15 µl/ml	20 µl/ml	EC_{50} Value µg/ml
P. corymbosa	18.73±1.28	36.02±1.3	50.84±1.27	63.98±1.10	144.51
S. indica	15.56±0.92	30.16±0.92	45.98±1.19	58.99±1.27	17.87
Positive Control Vitamin C	27.89±0.55	44.87±0.48	63.23±1.48	82.29±1.57	196.58

Data are presented as the means±standard error of the mean of triplicates.

Nitric oxide radical scavenging assay

In the present study, the nitric oxide radical quenching activity of the methanol extract from the leaves of *P. corymbosa* and *S. indica* were detected and compared with the standard ascorbic acid. The methanol extract of *P. corymbosa* exhibited the maximum inhibition of 68% at a concentration of 20 µg/ml with an EC_{50} value of 15.67 µg/ml, in a concentration-dependent manner when compared to *S. indica* that showed lowest activity against nitric oxide EC_{50} value of 174 µg/ml (Table-5). The scavenging activity of the extract against nitric oxide was detected by its ability to inhibit the formation of nitrite through direct competition with oxygen and oxides of

nitrogen in the reaction mixture [28]. The decrease in the concentration of the nitric oxide radical activity was more significant in these two plants than standard. This may be due to the presence of antioxidant polyphenolic molecules.

Metal chelating activity

The metal chelating activity of methanol extract from the leaves of *P. corymbosa* and *S. indica* were shown in Table-6. The methanol extract was assessed for their ability to compete with ferrozine for ferrous iron in the solution. In this assay, the leaf extract of both the plants interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and are capable of capturing ferrous iron before ferrozine. The methanol extract of *P. corymbosa* reduced the red color complex immediately and showed the highest chelating activity (EC_{50} 144.51µg/ml) than *S. indica*. Iron, in nature, can be found as either ferrous (Fe^{2+}) or ferric ion (Fe^{3+}), with ferric ion predominating in foods. Ferrous ions (Fe^{2+}) chelation may render important antioxidative effects by retarding metal-catalyzed oxidation [29].

Table 7: Antibacterial activities of Methanol extract from the leaves of *Premna corymbosa* and *Stachytarpheta indica* against pathogenic bacteria

Methanol extracts	Different concentrations of methanol extract µl/ml											
	[a]Inhibition zone of the *S. aureus* (mm)				[a]Inhibition zone of the *E. Coli* (mm)				[a]Inhibition zone of the *P. aeruginosa* mm)			
	5 µl/ml	10 µl/ml	15 µl/ml	20 µl/ml	5 µl/ml	10 µl/ml	15 µl/ml	20 µl/ml	5 µl/ml	10 µl/ml	15 µl/ml	20 µl/ml
P. corymbosa	8.66±0.77	10.5±1.11	12.7±0.69	15.9±0.79	9.2±0.65	11.3±0.35	14.83±0.65	18.2±0.55	7.81±0.91	10.73±0.40	12.16±0.60	16.0±0.17
S. indica	7.63±0.56	9.86±0.35	11.73±0.85	14.03±0.40	8.2±0.25	10.8±0.75	12.8±0.81	15.7±0.55	7.73±0.50	10.0±0.3	12.33±0.55	13.66±0.65

Methanol extracts	Different concentrations of extract µl/ml							
	[a]Inhibition zone of the *K. pneumoniae* measured (mm)				[a]Inhibition zone of the *E. faeclais* measured (mm)			
	5 µl/ml	10 µl/ml	15 µl/ml	20 µl/ml	5 µl/ml	10 µl/ml	15 µl/ml	20 µl/ml
P. corymbosa	8.6±0.45	11.83±0.41	13.76±0.83	17.23±0.40	7.73±0.56	10.66±0.35	13.83±0.35	15.93±0.70
S. indica	7.66±0.65	10.33±0.51	12.4±0.88	15.7±0.79	7.63±0.40	10.16±0.60	12.13±0.35	14.16±0.50

*The antibacterial activity was determined by measuring the diameter of zone of inhibition that is the mean of triplicates±standard error of the mean of three replicates

Anti-bacterial activity of methanol extract from the leaves of *Stachytarpheta indica* and *premna corymbosa* tested against pathogenic bacteria

Antibacterial activities of the methanol extracts obtained from the leaves of *Stachytarpheta indica* and *Premna corymbosa,* against the tested organisms were shown in Table-7. The plants differ in their activities against the micro-organisms tested. Methanol extracts of *P. corymbosa* and *S. indica* showed maximum antimicrobial activity against *Klebsiella pneumoniae, Escherichia coli, Enterococcus faecalis, Staphylococcus aureus* than *Pseudomonas aeruginosa.* Highest antibacterial activity was observed with methanol extract of *P. corymbosa* against *E. coli, K. pneumoniae, P. aeruginosa* (18, 17, 16 mm) respectively while lowest activity was observed with methanol extract of *S. indica* against *P. aeruginosa* and *S. aureus* with the inhibition zone of 13 and 14 mm respectively. Results obtained in the current investigation revealed that both herbal methanol extracts possess potential antibacterial activity against entire tested organisms which was similar to pervious data in which methanol extract was found to have strongest and broadest spectrum [30]. According to Sathyabama *et al.* [31], the methanol extract has high capacity to dissolve more organic and active antimicrobial compounds. These results confirmed the substantiation of previous studies which reported that methanol is a better solvent for more consistent extraction of antimicrobial substances from medical plants compared to other solvents, such as water and other organic solvents [32, 33].

CONCLUSION

The methanol extract of *P. corymbosa* and *S. indica* can protect the body from oxidative stress from ROS and bacterial infection, which may be due to the phytochemicals in the form of polyphenols that occur in the plant. These may be used in nutraceuticals and in the food industry. However, additional studies are necessary to develop a method for the fractionation and identification of most active antioxidant and antibacterial molecules and thereby can be used in the prevention and treatment of ageing related diseases and may be considered as good source for drug discovery.

ACKNOWLEDGEMENT

I take great pleasure in expressing my heartfelt thanks to the Managing Director, Dr. P. Bama M. Sc., Ph. D., and Dr. S. Sankaranarayanan M. Sc., Ph. D., Gloris Biomed Research Centre (P) Ltd, No. 171, Fourth st, Kannigapuram, K. K. Nagar Chennai-78 for suggesting the topic, for the invaluable guidance and supervision throughout my work and permitting me to use the facilities available in the laboratory. I am deeply indebted to the Principal, Stella Maris College and all the staff in the Department of Botany for their constant support and guidance to carry out my work.

CONFLICT OF INTERESTS

Declared None

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