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ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *PISTIA STRATIOTES* (L.)

TULIKA TYAGI[*1] AND PUNEET PARASHAR[2]

[1]Department of Botany, B.B.D. Govt. P.G. College, Chimanpura, Jaipur. University of Rajasthan, Jaipur. Rajasthan, India.
[2]Department of Zoology, University of Rajasthan, Jaipur, Rajasthan, India.

ABSTRACT

The present study was designed to evaluate the antioxidant and antimicrobial activity of various extracts of *P. stratiotes*. Antioxidant Activity is done by *in vitro* DPPH (2,2-diphenyl-1- picryl hydrazyl) using spectrophotometric method. Antimicrobial Activity is done by by well diffusion method and MIC (Minimum Inhibitory Concentration) using plate method. The methanolic extracts of *P. stratiotes* roots showed an IC_{50} values of 4.098±0.03 mg/ml highest antioxidant activity whereas leaves show IC_{50} values of 2.463±0.018 mg/ml of minimum activity in DPPH method. When assayed for Antibacterial activity leaves extract at 160 mg/ml produced zone of inhibition of 9.00 ± 0.817 against *E. coli* organism, 8.00±0.00 against *S. aureus* organism, 8.33± 0.47 against *P. aeruginosa* organism and 9.33± 0.47 against *B. subtilis* organism. While root extract at 160 mg/ml against *S. aureus*, *P. aeruginosa*, *B. subtilis* show zone of inhibition of 9.67± 0.47 mg/ml, 8.67 ± 0.47mg/ml and 8.33± 0.47mg/ml respectively. Antifungal activity for *C. albicans* and *A. niger* at 300 mg/ml for leave extract produce zone of inhibition of 10.00±0.00 mg/ml and 9.67±0.47 mg/ml while for root extract produce zone of inhibition of 8.33±0.47 mg/ml and 9.66±0.47mg/ml respectively. The result of this study reveals that *Pistia stratiotes* could be considered as potential source of natural antioxidant and antimicrobial plant for an alternative topical choice in the treatment of infections.

KEYWORDS: ***Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeuroginosa, Aspergillus niger, Candida albicans, MIC and DPPH.***

TULIKA TYAGI

Department of Botany, B.B.D. Govt. P.G. College, Chimanpura,Jaipur. University of Rajasthan, Jaipur. Rajasthan, India.

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INTRODUCTION

Herbal plants produce and contain a variety of chemical substances, of these substances certain isolated compounds serve as models for new synthetic compounds and can be used as taxonomic markers for the discovery of new compounds. *Pistia stratiotes* (Araceae) is commonly known as water cabbage or water lettuce. It floats on the surface of the water and its roots hanging submersed beneath floating leaves. The leaves can be up to 14 cm long and have no stem. The plant is distributed throughout the tropical and sub-tropical regions of the world. Water lettuce used to produce Selenium-enriched plant for animal nutrition.[1] *Pistia stratiotes* leaves are used for the healing of ringworm infection of the scalp, syphiltic eruptions, skin infections, boils and wounds.[2-3] The oil extract of *Pistia stratiotes* is used for the treatment of worm infestations, tuberculosis, asthma and dysentery and applied externally to treat skin diseases, inflammation, piles, ulcer and burns.[4] The plant is used as an anodyne for eye-wash in Gambia. The plant's juice is used by Mundas (tribal people in India) in ear complains.[5] Antihelmintic,[6] antidermatophytic, antifungal,[3] diuretic,[7] antiprotease,[8] antitubercular, emollient,[9] antidiabetic[10] and antimicrobial properties[11] has been found. Antioxidants are the compounds which possess the ability to protect the cell organelles from damage caused by free radicals induced oxidative stress either by inhibiting the initiation or propagation of oxidative chain reactions.[12-13] Antioxidants exhibit their antioxidant activity either by inhibiting lipid peroxidation, by scavenging free radicals and active oxygen species, preventing the decomposition of hydrogen peroxides into free radicals or by chelating heavy metal ions.[14,15] In the present study, we evaluated the antimicrobial and antioxidant activity of of *Pistia stratiotes* (Araceae) in leaves and roots of the plant.

MATERIALS AND METHODS

Pistia stratiotes L. (Leaves and Root) were collected from Kishor Sagar lake, Kota city, Rajasthan, India. It is situated between 25°11'0"N latitude and 75°50'0"E longitude. The plant samples were authenticated by Dr. Mala Agarwal, Senior Lecturer, Department of Botany, B.B.D. Govt. P.G College, University of Rajasthan, Jaipur.

Sources of Test Organisms

Bacteria and Fungi

The bacterial strains *Escherichia coli* (MTCC No. 1687) (Gram –ve), *Bacillus subtilis* (MTCC 0121)(Gram–ve), *Staphylococcus aureus* (MTCC 0737) (Gram+ve) and *Pseudomonas aeruginosa* (MTCC 7925) (Gram +ve). The fungal strains *Candida albicans* (MTCC 0227) and *Aspergillus niger* (MTCC 1344) are procured from The Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology (IMTECH), Chandigarh, India.

Culture of test microbes

For the cultivation of bacteria, Nutrient Broth Medium (NB) was prepared using 8% Nutrient Broth (Difco) in distilled water and agar-agar and sterilized at 121^0C for 20 min. A peptone saline solution was prepared (by mixing 3.56gm KH_2PO_4 + 7.23gm NaH_2PO_4+ 4.30 gm NaCl +1gm peptone in 1000 ml of distilled water, followed by autoclaving) and the bacterial cultures were maintained on this medium by regular sub-culturing and test bacteria were incubated at 37°C for 24 hrs. However, for the cultivation of fungi, Potato dextrose agar (PDA) medium was prepared (by mixing 100ml potato infusion + 20gm agar + 2gm glucose, followed by autoclaving) and the fungal cultures were maintained on this medium by regular subculturings and the test fungi were incubated at 27°C for 48 hrs.

Preparation of test extracts

Powdered different plant parts of *P. stratiotes* L. were soxhlet extracted with ethanol. Similarly, 10gm of different plant parts were homogenized separately with ethanol and left overnight at the room temperature. Later, each of the homogenates was filtered and the residue was re-extracted twice for complete exhaustion, the extracts were pooled and dried in vacuum. All these fractions were stored at 4°C in a refrigerator until screened and fresh suspension of test organism in saline solution was prepared from a freshly grown agar slant before every antimicrobial assay[16].

Determination of Antibacterial Assay

In vitro antibacterial activity of the crude ethanol extract was studied against gram positive and gram negative bacterial strains by the agar well diffusion method.[17] Mueller Hinton agar no. 2 (Hi Media, India) was used as the bacteriological medium. The extracts were diluted in 100% Dimethylsulphoxide (DMSO) at the concentrations of 5mg/ml. The Mueller Hinton agar was melted and cooled to 48 - 50ºC and a standardized inoculum (1.5×108 CFU/ml, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petri dishes to give a solid plate. Wells were prepared in the seeded agar plates. The test extract (160mg/ml), control solvent, Ciprofloxacin (5mg/ml) as standard separately was introduced in the well (6mm). The plates were incubated overnight at 37ºC. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well. Three replicates of each test extract were examined and the mean values were then referred.

Determination of Antifungal Assay

Anti fungal activity of the experimental plant was investigated by agar well diffusion method.[18]The yeasts and saprophytic fungi were subcultured onto Sabouraud Dextose Agar SDA (Merck, Germany) and respectively incubated at 37°C for 24 hrs and 25°C for 2-5 days. Suspensions of fungal spores were prepared in sterile PBS and adjusted to a concentration of 106 cells/ml. Dipping a sterile swab into the fungal suspension and rolled on the surface of the agar medium. The plates were dried at room temperature for 15 min. Wells of 6 mm in diameter and about 7 mm apart were punctured in the culture media using sterile glass tube. 300mg/ml of fresh extracts was administered to fullness for each well as test sample, Flucanozol (5mg/ml) as standard, control solvent. Plates were incubated at 37°C. After incubation of 24 hrs bioactivities were determined by

measuring the diameter of inhibition zone (in mm). All experiments were made in triplicate and mean were calculated.

Minimum Inhibitory Concentration (MIC)

In vitro antimicrobial activity of formulations was carried out by using the Agar cup plate method or Agar well diffusion method.[19]This classic method yields a zone of inhibition in mm result for the amount of antibacterial or antifungal agents that is needed to inhibit growth of specific microorganisms. It was carried out in petriplates. Each purified formulation was dissolved in DMSO and Tween 80, sterilized by steam sterilization. For the determination Muller Hinton agar was used and MIC plates were seeded with liquid suspension culture and different concentrations (20, 40, 60, 80, 100, 120, 160, 200, 250, 300mg/ml) were applied to check the minimum inhibitory sensitivity of sample. The MIC plates were incubated for 24hrs at 37^0C in case of bacteria and 72hrs at 28^0C in case of fungi. Zone of inhibition around the concentration measured in mm to detect the inhibition concentration. The lowest concentrations without visible growth were defined as concentrations that completely inhibited bacterial growth (MICs).

Quantitative determination

Plant Extraction

Two gm each of the dry material (leaves and root) was extracted with 50ml of methanol at room temperature for 48 hrs, filtered through whatmann paper no. 1 filter paper, stored and used for quantification.

Determination of Total Phenolic Content: *Total phenolic compound contents were determined by the Folin-* Ciocalteau method. [20-23] The extract samples (0.5 ml; 1:10 diluted) were mixed with Folin Ciocalteu reagent (1.5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na_2CO_3 (4 ml, 1M) was then added. The mixture was allowed to stand for 30 min and the total phenols were determined by colorimetric method at 765 nm. The standard curve was prepared using the standard solution of Gallic acid in methanol in the range 0.2-1mg/ml.Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is a common reference compound. Total phenolic content can be calculated from the formula:

$$T = \frac{C.V}{M}$$

Where,
T = Total Phenolic concentration
C = Concentration of gallic acid from caliberation curve (mg/ml)
V = Volume of extract (ml)
M = Wt. of methanol plant extract

Determination of Total Flavonoid Content

Total flavonoid content was determined by using aluminium chloride colorimetric method ($AlCl_3$) according to the known method.[24,25] with slight modifications using quercetin as standard. 0.5ml of test material was added to 50ml volumetric flask containing 3ml of methanol. To above mixture, 2ml of 10% $AlCl_3$ was added. After 5min, the total volume was made up to 5ml with methanol. Then the solutions were mixed well and absorbance was measured against blank at 420nm. The standard curve was prepared using the standard solution of Quercetin in methanol in the range 0.2-1mg/ml (R^2=0.991). Total flavonoid content of the extracts was expressed in milligram of quercetin equivalents/gdw. Total flavonoid content can be calculated from the formula:

$$T = \frac{C.V}{M}$$

Where,
T = Total flavonoid concentration
C = Concentration of quercetin from caliberation curve (mg/ml)
V = Volume of extract (ml)
M = Wt of methanol plant extract

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DPPH radical scavenging assay

Antioxidant activity of the plant extracts and standard was assessed on the basis of the radical scavenging effect of the stable DPPH free radical. The diluted working solutions of the test extracts were prepared in methanol. Gallic acid was used as standard in solutions ranging from 0.5 to 4.0 µg/ml. 0.135mM DPPH solution in methanol was prepared. Then 2 ml of this solution was mixed with 2 ml sample solutions of *P. stratiotes* leaves extract 0.8 to 3.4 mg/ml and *P. stratiotes* root extract 1.15 to 4.6 mg/ml and the standard solution to be tested separately. These solution mixtures were kept in the dark for 30 min and optical density was measured at 517 nm using a UV-Vis spectrophotometer against methanol as blank. The control was used is 2 ml of methanol with 2 ml of DPPH solution. The optical density was recorded and percentage of inhibition was calculated using the formula given below:

% of inhibition of DPPH activity = (A–B/A) × 100
Where, A is optical density of the control and B is optical density of the sample.

Linear graph of concentration Vs percentage inhibition was prepared and IC_{50} values were calculated. The antioxidant activity of each sample was expressed in terms of IC_{50} (micromolar concentration required to inhibit DPPH radical formation by 50%), calculated from the inhibition curve.[26]

STATISTICAL ANALYSIS

Experimental results are expressed as Mean value ± standard Error Mean (SEM). All measurements were replicated three times. IC_{50} values were also calculated by linear regression analysis.

RESULTS AND DISCUSSION

ANTIMICROBIAL ACTIVITY

In the present study antibacterial and antifungal activity of both the selected plant species were observed. Sequential extracts of different plant parts in ethanol and along with the methanolic extracts were checked for their antibacterial and antifungal activity (Table 1). Antibacterial activity of 160mg/ml ethanol leaves extract was highest against *B. subtilis* while minimum activity was seen against *S. aureus* (Figure 1 A, B, D). Antibacterial activity of 160 mg/ml ethanol root extract was highest against *S. aureus* while no activity was seen against *E. coli* (Figure 1 C, E, F). Antifungal activity of 300 mg/ml ethanol leaves extract was seen (Figure 2 G, I). Antifungal activity of 300 mg/ml ethanol root extract was seen higher against *A. niger* than *C. albigans* (Figure 2 H, J). MIC values of the ethanolic extract of leaves and root of *P. stratiotes* extract against all the tested bacteria and fungi (Table 1).

Table 1
Bactericidal and fungicidal efficacy of
Ethanolic plant part extract of Pistia stratiotes

Microorganism			Plant Parts	
Bacteria			**Leaves**	**Root**
Escherichia coli	**MTCC No. 1687**	**IZ**	9.00 ± 0.817	0
		AI	0.61	0
MIC(mg/ml)			160	160
Staphylococcus aureus	**MTCC No. 0737**	**IZ**	8.00±0.00	9.67± 0.47
		AI	0.52	0.62
MIC(mg/ml)			160	160
Pseudomonas aeruginosa	**MTCC 7925**	**IZ**	8.33± 0.47	8.67 ± 0.47
		AI	0.56	0.54
MIC(mg/ml)			160	160
Bacillus subtilis	**MTCC 0121**	**IZ**	9.33± 0.47	8.33± 0.47
		AI	0.6	0.53
MIC(mg/ml)			160	160
Fungi				
Candida albicans	**MTCC 0227**	**IZ**	10.00±0.00	8.33±0.47
		AI	1.2	1.36
MIC(mg/ml)			300	300
Aspergillus niger	**MTCC 1344**	**IZ**	9.67±0.47	9.66±0.47
		AI	1.34	1.27
MIC(mg/ml)			300	300

IZ = Inhibition zone (in mm) including the diameter of well (6 mm)

$$\text{Activity index} = \frac{\text{Inhibition area of the Standard}}{\text{Inhibition area of the test sample}}$$

Standards: Ciprofloxacin = 5.0 mg/ml; Fluconazole = 5.0 mg/ml
Results are mean value SEM from at least three experiment
Results are expressed as mean ± SEM (Standard Error Mean) (n=3)

$$SEM = \frac{SD}{\sqrt{3}}$$

SD = Standard Deviation
SEM = Standard Error Mean
n = no. of set

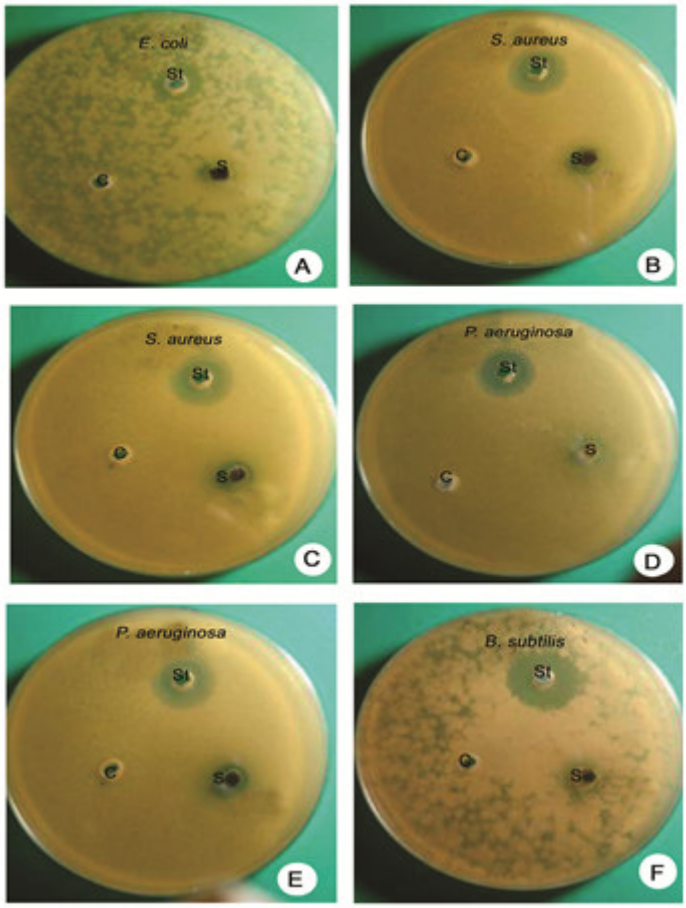

Figure 1
(A-F) Antibacterial activity of crude ethanolic extracts of different plant parts of Pistia stratiotes

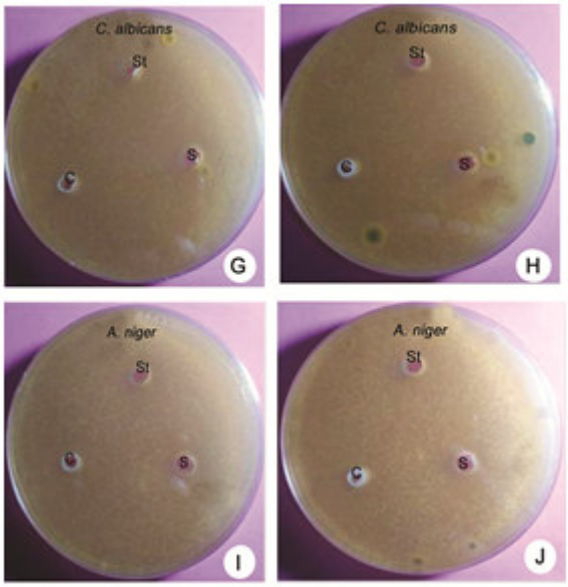

Figure 2
(G-J) Antifungal activity of crude ethanolic extracts of different plant parts of Pistia stratiotes

In the present study, ethanolic extracts were analysed for their antimicrobial activity. Roots and leaves of *P. stratiotes* species have shown significant results. No antibacterial activity was observed in 20, 40, 60, 100mg/ml methanolic extract and 20, 40, 60, 100, 120, 140mg/ml ethanolic extract while no antifungal activity was observed in 20, 40, 60, 100, 120, 140, 160, 200, 250 mg/ml ethanolic extract. Antimicrobial activities of the plants extracts (ethanolic and water solutions) *in vitro* showed inhibitory activities against some Gram positive bacteria, Gram negative bacteria, and yeast with significant effects depending not only on the tested

microorganisms but also on the extraction solvent. Some research reported that *S. aureus* and *Bacillus* were the most sensitive isolates to the estuarine submersed aquatic plants extracts, due to the cell wall structure of Gram positive bacteria that consists of a single layer, while Gram negative bacteria cell wall have a double layer membrane surrounding the bacterial cell, that make cell membrane not permeabilized to the antimicrobial agents and delay the osmotic lysis of a bacterial cell and membrane permeabilization led to resistance the ethanolic effect.[27,28] For water extracts of *P. stratiotes* and *N. lotus* no inhibitory activity were found against all the tested microorganisms due to the relatively low capacity of most bioactive compounds to dissolve in water. The ethanol appeared better as an extractant, judging from the wider activity spectrum and the resultant effect on the tested isolates, this is due to the great ability of alcohol to solublize and extract some active compounds such as: phenolic compounds, saponins, tannins, and flavonoids is greater than water some compounds of these groups are known to be either bactreiostatic or bactericidal materials, based on their concentration.[29] The antibacterial activity of ethanolic leaf extract of *P. stratiotes* against *Streptococcus species, Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumoniae* was studied by the agar diffusion and broth dilution methods. *Streptococcus species* was the most sensitive to the plant material while *E. coli* was the most resistant. The extract of *P. stratiotes* could be useful as a source of antibiotics against some disease causing bacteria.[30] Antimicrobial activity of *P. stratiotes* leaves against eight clinical pathogens showed no inhibition zone was observed for chloroform and hexane extracts against eight test pathogens. The methanol extract showed characteristic zone of inhibition against five pathogens including *Pseudomonas aeruginosa*, *Shigella sp*., *Serretia sp*., *Salmonella sp*. and *Klebsiella sp*. among eight test pathogens. Methanol extract of *P. stratiotes* leaves showed antimicrobial activity against both Gram positive and Gram negative.[31]

ANTIOXIDANT ACTIVITY

In the present study, we have investigated the antioxidant activity of *Pistia stratiotes* by DPPH assay and found their scavenging activity. Table 2 show total phenolic content and total flavonoidal content in different plant parts of *P. stratiotes.* According to this, total phenolic content was reported to be more in leaves than root (leaves; 0.596± 0.015mg/gdw>root; 0.173±0.022mg/gdw). Similarly the total flavonoid content was observed more in leaves than roots (leaves; 0.519±0.020mg/gdw> root;0.418±0.040 mg/gdw).

Table 2
Total Phenolic and Flavanoid content in different plant parts of Pistia stratiotes

Plant Part	Total Phenolic Content (mg GAE/gdw)	Total Flavonoidal Content (mg QE/gdw)
Leaves	0.596±0.015	0.519±0.020
Root	0.173±0.022	0.418±0.040

Table 3 show the IC_{50} values of methanolic extracts of different plant parts, which shows roots have highest antioxidant activity whereas leaves show minimum activity (root; 4.098±0.03 mg/ml> leaves; 2.463±0.018 mg/ml). The graph for % inhibition at different concentrations of different plant parts of *P. stratiotes* are shown in Figure 3.

Table 3
The IC_{50} values of different plant parts of Pistia stratiotes of DPPH radical scavenging assay (mg/ml)

Plant Parts	IC_{50} values (mg/ml)
Leaves	2.463±0.018
Root	4.098±0.03

Each value is expressed as mean ± SEM (Standard Error Mean) (n=3)

$$SEM = \frac{SD}{\sqrt{3}}$$

SD = Standard Deviation
SEM = Standard Error Mean
n = no. of set

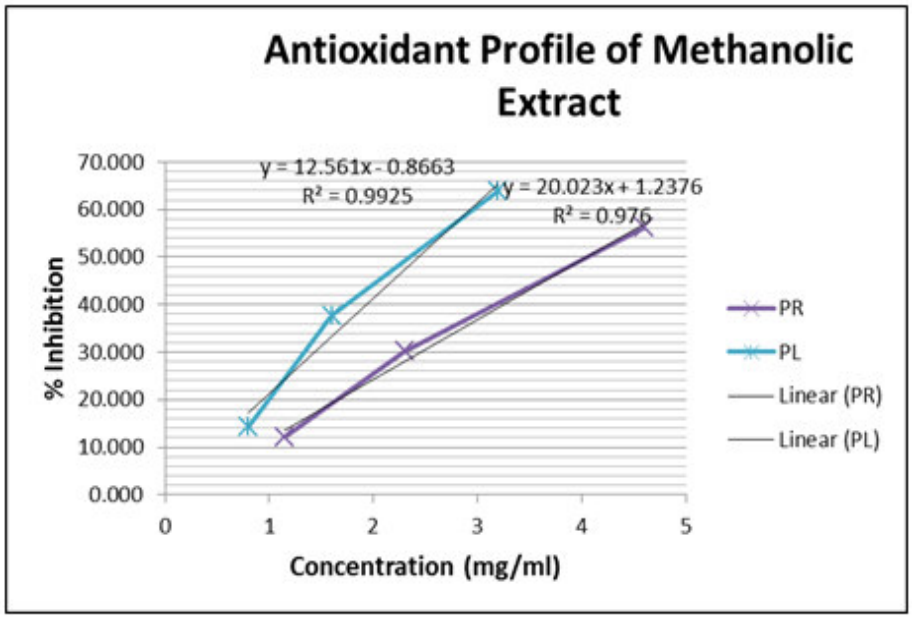

Figure 3
Graphical representation of Antioxidant activity of different parts of P. stratiotes

An IC_{50} value in mg/ml of each extract was found correlated with the higher concentration of polyphenol and flavonoids. Leaves of *P. stratiotes* has less IC_{50} concentration confirms better antioxidant activity while root of *P. stratiotes* show higher concentration of polyphenol and flavonoids has high IC_{50} concentration confirms lesser antioxidant activity.The therapeutic potential of natural medicinal plants as an antioxidant in reducing free radical induced tissue injury.[32] Flavonoids are polyphenolic compounds and widely reported for vasoprotective, anti-inflammatory as well as antioxidants properties. The antioxidants plays significant role in maintaining integrity of the cell membrane by prevention of lipid peroxidation and DNA damage caused by a cascade of free radical reaction.[33] The study shows leaf extracts appeared pharmacologically effective as an antidiarrheal and both leaf and root extracts may be effective against helminth by inducing paralysis and death.[34] Antimicrobial activities of the plants extracts (ethanolic and water solutions) *in vitro* showed inhibitory activities against some Gram positive bacteria, Gram negative bacteria, and yeast with significant effects depending not only on the tested microorganisms but also on the extraction solvent. Ethanolic extracts of *P. stratiotes* show inhibitory effects for both *B. subtilis* and *E. coli* without any significant difference with inhibition zones[35]. The antibacterial activity of ethanolic leaf extract of *P. stratiotes* against *Streptococcus species, Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumoniae* was studied by the agar diffusion and broth dilution methods. *Streptococcus species* was the most sensitive to the plant material while *E. coli* was the most resistant. The extract of *P. stratiotes* could be useful as a source of antibiotics against some disease causing bacteria[36].

CONCLUSION

The results of present study indicates extract of *P. stratiotes* act as useful source of antibiotics against some disease causing bacteria as *Streptococcus species* was the most sensitive and *E. coli* was the most resistant. Leaves confirm better antioxidant activity while roots show higher concentration of polyphenol and flavonoids confirms lesser antioxidant activity. The phytochemical compounds may contribute as useful source of drug against bacteria as seen from the current antibacterial activity studies. The plant extracts and fractions are active against microorganisms act as good antioxidant and antimicrobial agents from the analysis. The study can be further extended for determining the major single constituent that is responsible for these activities for developing as a herbal product for the sake of humanity.

CONFLICT OF INTERESTS

Conflict of interest declared none.

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Reviewers of this article

Prof.Dr. M.Ranga Priya, M.Pharm., Ph.D., R.Ph.

Professor, Dept of Pharmaceutics,Sun Institute Of Pharmaceutical Education & Research, Kakupall, Nellore Rural, Nellore, Andhra Pradesh 524346

Ritu Jain, MSc (Botany), PhD

Assistant Professor, Department of Botany,Kanoria PG Mahila Mahavidyalya Near Gandhi Circle, Jawahar Lal Nehru Marg, Jaipur (Rajasthan), India

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

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