ejpmr, 2016,3(3), 409-412

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *ALTERNANTHERA SESSILIS* (L.) R.BR. EX DC AND *ALTERNANTHERA PHILOXEROIDES* (MART.) GRISEB

Dr. R. Sivakumar*[1] and D. Sunmathi[2]

[1]Assistant Professor, Department of Botany, L.R.G Government Arts College, Tiruppur.
[2]Research Scholar, Department of Biotechnology, SNR Sons College (Autonomous), Coimbatore.

***Author for Correspondence: Dr. R. Sivakumar**
Assistant Professor, Department of Botany, L.R.G Government Arts College, Tiruppur.

Article Received on 04/01/2016 **Article Revised on 25/01/2016** **Article Accepted on 16/02/2016**

ABSTRACT

Objective: The aim of the present study was to evaluate the phytochemical screening and antimicrobial activity of ethanolic leaf extracts of *Alternanthera sessilis* and *Alternanthera philoxeroides*. Method: Ethanol extracts of both the plants were evaluated against four gram positive bacterial species (*Staphylococcus aureus* MTCC 96, *Staphylococcus heamolyticus* MTCC 3383, *Enterococcus faecaalis* MTCC 439, *Bacillus subtilis* MTCC 10619) and four gram negative bacterial species (*Klebsiella pneumoniae* MTCC 3384 , *Escherichia coli* MTCC 443 , *Proteus vulgaris* MTCC 1771 and *Proteus mirabilis* MTCC 442) and fungi (*Candida albicans* MTCC 227) using well diffusion method. Result: Phytochemical studies revealed the presence of alkaloids, flavonoids, aminoacids, carbohydrates, phenols, steroids, terpenoids, saponins and glycosides in both the plant extracts. The results of the study revealed that all the bacterial strains and fungi (*Candida albicans*) were more sensitive to *A.sessilis* than *A.philoxeroides*. The extracts were compared with standards like Gentamycin and Nystatin for antibacterial and antifungal activity respectively. The extracts showed remarkable inhibition of zone of bacterial and fungal growth and results were comparable with that of standard drugs against the organism tested. Conclusion: In conclusion, leaf extract of *A.sessilis* and *A.philoxeroides* showed significant antimicrobial activity. It may be due to the phytochemicals present in them.

KEYWORDS: *Alternanthera sessilis*, *Alternanthera philoxeroides* leaves, antibacterial, antifungal, Gentamycin and Nystatin.

INTRODUCTION

Infectious diseases are a serious problem Worldwide.[1] Although pharmaceutical industries have produced number of new antibiotics in last few decades, resistance to these drugs by microorganisms has increased. In general, microorganisms have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents.[2] Historically, plants have provided a good source of anti-infective agents; emetine, quinine, berbine etc. remain highly effective instruments to combat against microbial infections.[3] Plants have a great potential for producing new drugs of great benefit to mankind. There are many approaches to the search for new biologically active principles in higher plants.[4] Many efforts have been done to discover new antimicrobial compounds from various sources such as soil, microorganisms, animals and plants. One of such resources is folk medicine and systemic screening of them may result in the discovery of novel effective compounds.[5] The genus Alternanthera, a medicinally important member of family Amaranthaceae is reported to contain volatile constituents, essential amino acids, flavonoids, glycosides and steroids.[6] Green leafy vegetables namely *Alternanthera sessilis* Linn and *Alternanthera philoxeroides*(Mart.) Griseb plays a vital role in human health care. Young shoots and leaves of A.sessils and A.philoxeroides are eaten as a vegetable in Southeast Asia.[7] The purpose of this study was to screen the ethanolic leaves extract of *Alternanthera sessilis* and *Alternanthera philoxeroides* that could be useful for the development of new tools as antimicrobial agent for the control of infectious diseases.

MATERIALS AND METHODS

1.Plant material

Fresh plant leaves of *Alternanthera sessilis* and *Alternanthera philoxeroides* were harvested from Coimbatore district, the Western Ghats and were identified by Botanist, Arignar Anna Government Arts College, Mussiri. Fresh plants were washed thoroughly 3-4 times with running tap water then finally with sterile water followed by shade drying at room temperature for 15-20 days. The dried plant material was made into coarse powder and passed through sieve and then used

for crude extraction. Fine powder (20gm) was extracted in 100 ml of ethanol at 50-55°C for 24 hours in rotary shaker. The extract was filtered through Whattman filter paper No.1 and then concentrated by using a rotary evaporator at low temperature (40-50°C) and reduced pressure. Further, the dried residue was preserved in airtight container and kept at 4-5°C until further use.

1.2. Phytochemical screening

Phytochemical tests were carried out to determine the presence of chemical constituents using the methods as described by Sofowora[8] and Trease and Evans.[9]

1.3.Antimicrobial assay

Test Organisms

Four gram positive (*Staphylococcus aureus* MTCC 96, *Staphylococcus heamolyticus* MTCC 3383, *Enterococcus faecaalis* MTCC 439, *Bacillus subtilis* MTCC 10619) and four gram negative bacterial species (*Klebsiella pneumoniae* MTCC 3384 , *Escherichia coli* MTCC 443 , *Proteus vulgaris* MTCC 1771 and *Proteus mirabilis* MTCC 442) and fungi (*Candida albicans* MTCC 227) were used for this study. All the stock cultures were obtained from KMCH, Coimbatore. They were maintained on Sabouraud Dextrose Agar and Mueller Hinton Agar for fungi and bacterial slants respectively at 4^{o}C prior to use.

1.4. Well diffusion assay

The ethanolic extract of *Alternanthera sessilis* and *Alternanthera philoxeroides* were screened for their *in vitro* antibacterial activity in comparison with standard antibiotic Gentamycin (100mg/ml) for bacteria and Nystatin for fungi by well diffusion method.[10] Lawn culture were used using the test organism on Mueller Hinton Agar(MHA).The inoculated plates were kept aside for few minutes using well cutter, five wells were made in those plates at required distance. In each step of well cutting the well cutter was thoroughly wiped with alcohol. Using sterilized micropipettes 10ml of different concentrations (5µg, 10µg, 15µg) with selected extract was added into the well. The plates were incubated at 37˚c for overnight. The activity of the extracts was determined by measuring the diameters of zone of inhibition (mm). For each bacterial and fungal strains, controls were maintained in which pure solvent (ethanol) without extracts were used.

RESULTS AND DISCUSSION

1.Phytochemical analysis

The results of phytochemical analysis of ethanolic leaf extract of *A.sessilis* and *A.philoxeroides* were tabulated in table 1. Phytochemical studies revealed the presence of alkaloids, flavonoids, aminoacids, carbohydrates, phenols, steroids, terpenoids, saponins and glycosides in both the plant extracts. In addition to these, *A.philoxeroides* revealed the presence of tannins and in *A.sessilis* tannins were absent.

Table 1. Results of phytochemical analysis

S.No	Phytochemicals	*Alternanthera sessilis*	*Alternanthera philoxeroides*
1	Alkaloids	+	+
2	Tannins	-	+
3	Flavonids	+	+
4	Aminoacids	+	+
5	Carbohydrates	+	+
6	Phenols	+	+
7	Steroids	+	+
8	Terpenoids	+	+
9	Saponins	+	+
10	Glycosides	+	+

+ - Present - - Absent

Phytochemical constituents such as flavonoids, tannins, phenols, saponins and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanism against prediction by many microorganisms, insects and other herbivores.[11-12] The young shoots of *A.sessilis* contains carotenoids, triterpene,[13] saponins,[14] flavonoids, steroids, stigmasterol, β- sitosterol.[15] Phytochemical studies yielded β-carotene, ricinoleic acid, myristic, palmitic, stearic, oleic and linoleic acids, α-spiraterol and uronic acid.[15] Herb contains 2,4-methylene cycloartanol and cyclo eucalenol, choline, oleanolic acid. Saponins have been isolated from the leaves. Roots contain lupeol.[16] Young shoots contain protein.[17] It also contains 5-a - stigmasta-7-enol.[18-19] Nayak *et al*.[20] reported the isolation of flavonoids, triterpenoids, steroids and β-sitosterol, stigmasterol, campesterol, lupeol.

The presence of carbohydrates, aminoacids, proteins, cardiac glycosides, steroids, alkaloids, flavonoids, total phenolics and tannin contents were reported in *A.philoxeroides*.[21] The reported phytoconstituents of the plant include phaeophytin a, phaeophytin a', oleanolic acid, β- sitosterol, 3β-hydroxystigmast-5-en-7-one, α-spinasterol, 24-methylene cycloartanol, cycloeucalenol and phytol.[22]

2. Antimicrobial Assay

The results of the antibacterial activity (Table 2) revealed that all the bacterial strains were more sensitive to *A.sessilis* and *A.philoxeroides*. The maximum zone of inhibition is achieved with ethanolic extract of *A.sessilis* for *Staphylococcus aureus* and *Staphylococcus haemolyticus* species (1.8mm). *A.philoxeroides* exhibited a moderate activity with *Klebsiella pneumoniae* (1.3mm) and *Eschericia coli*(1.2mm).The result of antifungal activity indicated that the ethanolic extract of *A.sessilis* was more sensitive than *A.philoxeroides* in *Candida albicans*. Similar results have been reported by Ashok Kumar *et al*.[23]

Table 2.Results of antibacterial and antifungal activity

***Bacterial* species**		**Zone of Inhibition (mm)**									
		Alternanthera sessilis					***Alternanthera philoxeroides***				
Gram positive	*B.subtilis*	10 µg	25 µg	50 µg	Gentamycin /Nystatin	Ethanol	10 µg	25 µg	50 µg	Gentamycin/ Nystatin	Ethanol
		0.8	0.9	1	1.2	1.1	0.5	0.7	0.9	1.3	1
	E.faecalis	0.7	0.9	1.3	1.3	1.4	0.6	0.8	0.8	1	0.9
	S.aureus	1.6	1.4	1.8	2.1	2.3	0.4	0.5	0.6	0.7	0.6
	S.heamolyticus	1	1.5	1.8	2	1.9	0.4	0.7	0.8	1	0.8
Gram negative	*E.coli*	1.2	1.2	1.6	2.5	1.8	1.1	1	1.2	1.5	1.2
	K.pneumoniae	1.2	1.6	1.4	1.6	1.3	1	1.1	1.3	1.4	1.2
	P.mirabilis	0.9	1	1.5	1.5	1	0.3	0.4	0.8	1	1.2
	P.vulgaris	0.7	0.9	0.8	1.9	1	0.6	0.7	0.6	0.9	0.5
Fungi *C.albicans*		1.3	1.4	1.5	1.6	2	1.1	1.2	1.3	1.5	1.0

Alternanthera sessilis leaves showed better results and it may be due to the higher diffusion rate or the degree of sensitivity of the tested micro organisms to the extract is higher. *Alternanthera sessilis* leaves may contain compounds that can be used to control diseases caused by. The different rate of inhibition could be due to the molecular size of the phytochemical compounds present in the extract.[24]

The Gram negative bacteria develop multi drug resistance to many of the antibiotics in the market where *E.coli* is prominent.[25-26] Still the extracts of the *A.philoxeroides* leaves showed more pronounced activity for gram negative than gram positive. The reason is attributed to the sensitivity difference between gram positive and gram negative bacteria. This is mainly due to the morphological constitution between these microorganisms, Gram negative bacteria has thicker cell wall made up of phospholipid membrane which makes it impermeable for antimicrobial chemical components. The gram positive bacteria have only outer peptidoglycan layer that is not an effective impermeable barrier. Therefore gram negative organisms are easily susceptible to antimicrobial agents than gram positive. Inspite of this permeability difference *A.philoxeroides* leaves extract still exerted some degree of inhibition against gram negative organism as well. The phytochemical analysis of ethanolic leaf extract of *A.sessilis* and *A.philoxeroides* also justifies the presence of bioactive compounds alkaloids, terpenoids and steroids responsible for antibacterial activity.

It has been found that more highly oxidized phenols are more inhibitory to micro organisms.[27-28] Sowjanya Pulipati *et al*.[29] reported the results from MIC indicated that *S.aureus* and *E.coli* were the most sensitive bacteria to *A.philoxeroides* leaf extract, inhibited at lowest concentration of 12.5µg/ml. Similar results have been reported by Anjali Rawani *et al*.[30]

CONCLUSION

The pharmaceutical industries are mainly focused towards design and development of new innovative/indigenous plant based drug through investigation of leads from traditional system of medicine. *A.sessilis* and *A.philoxeroides* are important traditional leafy vegetables which contains many bioactive compounds. The phytochemical results of the present study provided supportive scientific evidence that the *A.sessilis* and *A.philoxeroides* possessed maximum bioactive compounds which are pharmacologically active in treatment of various ailments. The findings suggest the usefulness of *A.sessilis* and *A.philoxeroides* against pathogenic bacteria and fungal strains. These plants are also potent source of active principles. Hence, it is anticipated that *A.sessilis* and *A.philoxeroides* would be a useful pharmaceutical material to treat diseases. This investigation may focus research field to develop clinical studies which might be of great scientific contribution for the society.

REFERENCE

1. Watson AJM. Diarrhoea. BMJ, 1992; 1304(4): 304.
2. Cohen ME. Epidemiology of drug resistance implications for a postantimicrobial era. Science, 1992; 257: 1050-1055.
3. Maurice MI, Angela R, Chris OO. New antimicrobials of plant origin. J. Janick (ed.) Alexandra, VA, 1999; 457-462.
4. Farnsworth NR, Loub WD, Information gathering and data bases that pertinent to the development of plant-derived drugs in plants; the potentials for extracting Protein Medicines and other useful

chemicals. Workshop proceedings; OTA-BP-F-23, U.S. Congress. Office of Technology Assessment, Washington, DC, 1983; 178-195.
5. Janovska D, Kubikova K, Kokoska L. Screening for antimicrobial activity of some medicinal plant species of traditional Chinese medicine. Czech. J. Food Sci, 2003; l21: 107-111.
6. The wealth of India: Raw Materials, Vol.I A-J, CSIR, New Delhi, 2004: 51.
7. Scher J. Federal Noxious Weed disseminules of the U.S. Center for Plant Health Science and Technology, Plant Protection and Quarantine, Animal and Plant Health Inspection Service, U.S. Department of Agriculture. Online. Available: http://www.lucidcentral. org/keys/v3/FNW/.
8. Sofowora A, Medicinal plants and Traditional Medicines in Africa. Chichester John Wiley & Sons New York, 1993; 2: 24-36
9. Trease GE, Evans WC, Pharmacognosy. 15th Ed. London: Saunders Publishers; 2002; 42–44. 221–229, 246–249, 304–306, 331–332, 391–393.
10. Ahirwal Laxmi, Singh Siddhartha Mehta Archana. Antimicrobial screening of methanol and aqueous extracts of Swertia chirata. Int J Pharm Sci, 2011; 3(4): 142-146.
11. Purkayastha J, Nath SC, Islam M. Ethnobotany of medicinal plants from Dibru-Saikhowa Biosphere Reserve of Northeast India. Fitoterapia, 2005; 76(1): 121-127.
12. Magana-Arachchi DN, Medagedara D, Thevanesam V. Molecular characterization of Mycobacterium tuberculosis isolates from Kandy, Sri Lanka. Asian Pac J Trop Dis, 2011; 1 (3): 181-186.
13. Dogra JVV, Jha OP, Mishra A. Cheamotaxonomy of Amaranthaceae - study of triterpenes. Biochem J, 1977; 4(1): 14-18.
14. Kapundu R, Mpuza Lami, Nzunzu, Delande, clement. A Saponin from *Alternanthera sessilis*. Bul. Soc. R. Sci. Liege, 1986; 55(5-6): 605-66.
15. Shinne Reni, Chen, Arh- Hwang. Kaohsung Medical college Kaohsung (Taiwan) Tai_Wan_yao Hsueh Tsa chils. 1975; 27(1-2): 103-4.
16. Leung WTW, Busson F, Jardin C. Food composition table for use in Africa. FAO, Rome, Italy, 1968; 306.
17. Gupta AK. Indian medicinal plants New Delhi. ICMAR, 2004;151-7
18. Ghani. Medicinal plants of Bangladesh: Chemical constituents and uses. 2nd ed. Asiatic Society of Bangladesh, 1996; 1-17.
19. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants, 5, Publications & Information Directorate, CSIR, New Delhi, India, 1996; 224.
20. Asolkar LV, Kakkar KK, Chakre OJ. Secondary Supplement to Glossary of Indian Medicinal Plants with Active Principles, Publications and Information Directorate (CSIR), Dr. K.S. Krishnan Marg, New Delhi, 1992; 332-335.
21. Fang JB, Duan HQ, Zhang YW, Yoshihisa T. Chemical constituents from herb of *Alternanthera philoxeroides*. China journal of Chinese material medica. 2006; 31: 1072-1075.
22. Nayak P, Nayak S, Kar DM. Pharmacological evaluation of ethanolic extracts of the plant *Alternanthera sessilis* against temperature regulation. JPR, 2014; 3(6): 1381-1383.
23. Ashok Kumar D, Das M, Mohanraj P, Das A. Antimicrobial activity study of ethanolic extract of *Alternanthera sessilis* Linn. aerial parts. JAPS, 2014; 11(3): 1- 4.
24. Sowjanya Pulipati, Srinivasa Babu P, Sri Devi B. Preliminary phytochemical and anti*bacterial* investigations of the methanol extract of *Alternanthera philoxeroides* (Mart.) Griseb. EJPMR, 2015; 2(4): 347-356
25. Omwenga EO, Okemo PO, Mbugua PK, Ogol C. Ethnobotanical Survey and Antimicrobial Evaluation of Medicinal Plants used by the Samburu Community (Kenya) for treatment of Diarrhorea. Phcog Mag, 2009; 5: 165-75.
26. Higgins PG, Fluit AC, Milatovi D, Verhoef Jand Schmitz, FJ. Antimicrobial susceptibility of imipenem-resistant Pseudomonas aeruginosa. J. Antimicrob. Chemother, 2002; 50: 299-301.
27. Alonso R, Femandez - Aranguiz A, Colorn K, Herreras A, Cistema R. Profile of *Bacterial* Isolates and antimicrobial susceptibility: Multi center study using a one-day cut-off. Revista Espnola de quimioterapia, 2000; 13: 384-393.
28. Scalbert A. Antimicrobial properties of tannins. Phytochemistry, 1991; 30: 3875-3883.
29. Urs NVRR, Dunleavy JM. Enhancement of the bactericidal activity of a peroxidase system by phenolic compounds (Xanthomonas phaseoli var sojensis, soybeans). Phytopathology, 1975; 65: 686-690.
30. Anjali Rawani, Sudin Pal, GoutamChandra. Evaluation of antimicrobial properties of four plant extracts against human pathogens. APJTB, 2011; 2: 71-75.