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In Vitro Antimicrobial Activity of Methanolic Extract of *Sphaeranthus Amaranthoides* Burm

Somnath De*[1], Akalanka Dey[2], A.M.S.Sudhakar Babu[3], Siddabathuni Aneela[1]

1 Dr. Samuel George Institute of Pharmaceutical Sciences, Markapur- 523316, Andhra Pradesh
2. Annamalai University, Department of Pharmacy, Annamalai Nagar, Tamil Nadu, India
3 A.M.Reddy Memorial College of Pharmacy, Narasaraopet, Guntur-522601, AndhraPradesh

ABSTRACT

This study was performed to evaluate the In vitro antimicrobial activity of entire parts of aqueous and methanolic extracts of *Sphaeranthus amaranthoides Burm.* The antimicrobial activities of medicinal plant extracts used for the treatment of parasitic diseases were examined with four bacteria – two from Gram positive group (*Bacillus subtilis and Staphylococcus aureus*) and rest two from Gram-negative group (*Escherichia coli and Salmonella typhi*) following disc diffusion method. The methanolic extract revealed antibacterial activity more than the aqueous extract. Both the extracts showed less activity against *Bacillus subtilis* and where as *Salmonella typhi* no inhibition (NI). It showed more activity on *Escherichia coli* compared to standard. Both the extracts showed minimum inhibitory concentration (MIC) of 25mg/ml on *Bacillus subtilis* and 12.5mg/ml on *Staphylococcus aureus.* Totally the methanolic and aqueous extracts of *Sphaeranthus amaranthoids* showed good anti bacterial activity. Phytochemical screening revealed the presence of steroids, alkaloids, sugars, phenolics, flavonoids, saponins, tannins and amino acids with mottled degree

Keywords: Anti bacterial, Phytochemical screening, Methanolic, *Sphaeranthus amaranthoides Burm*, Disc diffusion

*Corresponding Author Email: somnath.bankura@gmail.com
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INTRODUCTION

The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine[1]. Medicinal plants represent a rich source of anti-microbial agents. A number of medicinal plants have been used to treat microbial infections in man and animals[2]. Plants are used medicinally in different countries and are a source of many potent and powerful drugs[3]. A wide range of medicinal plant parts are used for extract as raw drugs and they possess varied medicinal properties[4]. Within the recent years, infections have increased to a great extent and antibiotics resistance effects become an ever-increasing therapeutic problem[5]. Natural products of higher plants may possess a new source of anti-microbial agents with possibly novel mechanisms of action[6-7]. Anti-microbial agents are undeniably one of the most important therapeutic discoveries of the 20th century[8]. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic microbials[9]. *Sphaeranthus amaranthoides Burm.f.* is a small procumbent herb, with steam rooting and pubescent with appressed hair leaves palmately 3 – foliolate. Features of the herb: low annuals with spreading branches, stem-erect, glabrous, sometimes as thick as the little finger, but short, branches- not winged and 8-12 inches, leaves- 2-4 inches, linear, oblong narrowed at the base. This plant is well known for its medicinal value for the treatment of eczema, blood disorder, stomach worms, filarial, fever and as a remover of kapha, vata and piles. It is also known to cure skin diseases. *S. amaranthoides* belongs to plant kingdom, Dicotyledon class, Gamopetalae sub class, Inferae series, Asterales order and Asteraceae (compositae) family. It is weed of paddy field of southern India particularly in thoothukudi district, Tamil Nadu, India (Dec. 2011). A number of medicinal plants have been used to treat parasitic infections in man and animals[10]. The literature search reveals that still no work have been done on this plant and nobody evaluated anti-microbial activity. Preliminary Phytochemical screening was also conducted in order to identify the chemical profile of active substances.

MATERIALS AND METHODS

Collection of plant material

The entire plant of *S. amaranthoides* collected from the thoothukudi district, Tamil Nadu, India, Voucher No: SGIP, Ref No: 007 in the month of December 2011 and authenticated by Dr. V. Chelladurai, retired research officer-botany, Central council for research in Ayurveda and Siddha[CCRAS], govt. of India; Tirunelveli. Herbarium of the plant was prepared and preserved

in the department of pharmacognosy, Dr. Samuel George Institute of Pharmaceutical Sciences, Markapuram, Prakasam dist. Andhra Pradesh.

Preparation of Extract

Solvent extraction

The collected entire plant materials was washed thoroughly in running tap water and shade dried, then crushed in mortar pestle and exhaustively extracted by soxhlet extractor. 100gms of *S. amaranthoides* air dried and coarsely entire plant materials was extracted with 500ml Methanolic solvent. After extraction, the sample was kept in dark for 72hrs with intermittent shaking. Then the solvent was evaporated by under the reduced pressure using Rota-vapour and to obtain viscous semi-solid masses.

Aqueous extraction

On the other hand 10 gm of dried powder was extracted in distilled water for 6 hrs at slow heat. Every 2 hrs, it was filtered through Whatman filter paper under section and centrifuge at 500 g for 15 min. the supernatant was collected and the process was repeated twice and after 6 hrs the supernatant was concentrated to make the final volume one forth of original volume[11]. It was then autoclave at 121˚c and 15 psi pressure for 15 min. Then stored at 4˚c [12]. Dimethyl sulfoxide (DMSO) was used as an emulsifier at the concentration of 0.05 % in the final test solution.

Microorganisms

Four bacteria, two from Gram-positive group (*Bacillus subtilis and Staphylococcus aureus*) and rest two from Gram-negative group (*Escherichia coli and Salmonella typhi*) were obtained from the stock culture of Microbiology Research Laboratory, NRI Medical College, Mangalagiri, Guntur dist.

Phytochemical screening

The methanolic extract was tested for steroids, alkaloids, sugar, phenolic compounds, flavonoids, saponins, tannins, anthraquinone and aminoacids. Phytochemical screening of the extract was carried out according to the standard method [13].

Screening of antimicrobial activity

The cups are made by sterile cork borer (6 mm) after solidification of the agar medium. The standard and test dilutions are made with DMSO as solvent. 100 mg/ml solution of each extract was introduced into cups at sterile aseptic conditions. Ciprofloxacin (10µg/ml) solution was used as standard and DMSO was used as control. The plates are incubated in refrigerator for diffusion and then transferred to incubator and incubated at 37ºC for 18hrs. The zone of inhibition was measured and recorded. The MIC of the each extract was determined both by broth culture

method and agar diffusion methods. Two fold dilutions of the each extract were prepared and 1ml was introduced into 9 ml of nutrient broth cultures and the growth was observed after 18 hrs incubation. In agar diffusion method different concentrations of each extracts were introduced into cups and the zone of inhibition was calculated.

RESULTS AND DISCUSSION

In the present study the methanolic and aqueous extracts were tested for antibacterial activity against four bacteria – two from Gram positive group (*Bacillus subtilis and Staphylococcus aureus*) and rest two from Gram-negative group (*Escherichia coli and Salmonella typhi*). The methanolic extract revealed antibacterial activity more than the aqueous extract. Both the extracts showed less activity against *Bacillus subtilis* and where as *Salmonella typhi* no inhibition (NI). It showed more activity on *Escherichia coli* compared to standard as shown in Table 1.

Table 1: Anti bacterial activity of methanolic and aqueous extracts of *Sphaeranthus amaranthoids*

S.no.	Type of extract	Diameter of zone of inhibition (mm).			
		Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Salmonella typhi***
1.	Aqueous extract	12	15	17	NI
2.	Methanolic extract	9.4	16	18	NI
3.	Ciprofloxacin (10 µg/ml)	21	18	15	19
4.	DMSO	--	--	--	--

Both the extracts showed minimum inhibitory concentration (MIC) of 25mg/ml on *Bacillus subtilis* and 12.5mg/ml on *Staphylococcus aureus.* The minimum inhibitory concentrations of methanolic and aqueous extracts were 6.25mg/ml and 12.5mg/ml respectively on *Escherichia coli* where *Salmonella typhi* are in 12.5 mg/ml as shown in Table 2.

Table 2: MIC of aqueous and methanolic extracts of *Sphaeranthus amaranthoids.*

S.no.	Bacteria	Aqueous extract (mg/ml)				Methanolic extract (mg/ml)			
		50	25	12.5	6.25	50	25	12.5	6.25
1.	*B. subtilis*	+	+	-	-	+	+	-	-
2.	*S. aureus*	+	+	+	-	+	+	+	-
3.	*E. coli*	+	+	+	-	+	+	+	+
4.	*S.typhi*	ND	ND	+	-	ND	ND	+	+

(+): inhibition present ND – Not Determined (-): inhibition absent

Totally the methanolic and aqueous extracts of *Sphaeranthus amaranthoids* showed good anti bacterial activity. The observed activity may be due to the presence of potent phyto-constituents in the methanolic extract and present investigation on the phytochemical analysis of *Sphaeranthus amaranthoids* refers to the presence steroids, alkaloids, sugar, phenolic

compounds, flavonoids, saponins, tannins, anthraquinone and aminoacids. Simple phenol compounds might be responsible for its antimicrobial properties.

CONCLUSION

It is concluded based on the findings of the present study that the entire plants *Sphaeranthus amaranthoids* shows higher antibacterial activity against bacterial pathogens such as *Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Salmonella typhi.* Phytochemical analysis showed that the antimicrobial activity of *Sphaeranthus amaranthoids* was due to presence of phytocemical compounds like alkaloids, flavanoids, phenols and tannin when compared with other two extracts viz., methanol & aqueous extract. The extract of *Sphaeranthus amaranthoids* showed maximum zone of inhibitionat. The minimum inhibitory concentrations are measured. Both the extracts are having antibacterial activity.

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REFERENCES

1. Dubey R, Dubey K, Sridhar C, Jayaveera KN Human Vaginal Pathogen Inhibition Studies On Aqueous, Methanolic And Saponins Extracts Of Stem Barks Of Ziziphus Mauritiana. Int. J.Pharm. Sci. Res. 2011; 2(3): 659-663.
2. Somnath De, Siddabathuni Aneela, Akalanka Dey, and A.M.S. Sudhakar Babu, 2013. “Phytochemical and GC-MS analysis of bioactive compounds of Sphaeranthus amaranthoides. Burm, Pharmacognosy J 2013;5: 265-268.
3. Srivastava, J., J. Lambert and N. Vietmeyer, 1996. Medicinal plants: An expanding role in development. World Bank Technical Paper. No. 320.
4. Uniyal, S.K., K.N. Singh, P. Jamwal and B. Lal, 2006.Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalayan. J. Ethnobiol. Ethnomed., 2: 1-14.
5. Mahesh B, Satish S. Antimicrobial activity of some important medicinal plants against plant and human pathogens. World J Agric Sci 2008; 4: 839-843.
6. Ahmad I, Aqil F. In vitro efficacy of bioactive extracts of 15 medicinal plants against ESbL-producing multidrug-resistant enteric bacteria. Microbiol Res 2007; 162: 264-275.
7. Barbour EK, Al Sharif M, Sagherian VK, Habre AN, Talhouk RS, Talhouk SN.

Screening of selected indigenous plants of Lebanon for antimicrobial activity. J Ethnopharmacol 2004, 93:1-7.

8. Peterson LR, Dalhoff A. Towards targeted prescribing: will the cure for antimicrobial resistance be specific, directed therapy through improved diagnostic testing.J Anti Chemo 2004; 53: 902-905.
9. Iwu MW, Duncan AR, Okunji CO. New antimicrobials of plant origin In: Perspectives on new Crops and new Uses, eds. J. Janick, ASHS Press, Alexandria, VA, 1999; 457-/462.
10. Nandan dey, Manas kumar Pal, Evaluation of anthelmintic activity of leaves of *paederia foetida.* Int J Pharma and Bio Sci 2011;2(1) :227-233.
11. Nang HLL, May CY, Nagan MA and Hock CC, Extraction and identification of water soluble compounds in palm pressed fiber by sc- co2 and GC –MS, Am J Environ Sci 2007, 3(2):54-59.
12. Nair R and Chanda S., In vitro antimicrobial activity of Psidium guajava L. leaf extracts against clinically important pathogenic microbial strains, Braz J Microbiol 2007; 38: 452-458.
13. Brindha P, Sasikala B, Purushothaman KK. Pharmacological studies on Merugan kizhangu. Bulletin of medico-Ethno-Botanical-Research 1981; 3: 84-96.