

## Antimicrobial, antidiarrhoeal and analysis of phytochemical constituents of *Sphaeranthus amaranthoides*

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**Abstract:** The ethnomedicinal value of *Sphaeranthus amaranthoides* was investigated through phytochemical study, *in vitro* antibacterial- and antidiarrhoeal- activity of ethanolic extracts. The analysis of the extracts revealed the presence of flavonoids, carbohydrate, tannins, saponins, steroids, glycosides, terpenoids and alkaloids. The extract evinced antibacterial activity on all the tested organisms *viz.* *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Enterococcus faecalis* and *Escherichia coli* at the concentration ranged from 50-400µg/ml as assayed by disc diffusion. *S. amaranthoides* also caused a significant ( $p < 0.001$ ) castor oil induced antidiarrhoeal effect in rats. The results suggest that these activities might be due to the presence of the potent phytochemicals in the extract of *Sphaeranthus amaranthoides* that can be used as a therapeutic agent.

**Keywords:** *Sphaeranthus amaranthoides*, phytochemicals, castor oil induced diarrhea.

### Introduction

Consumption of medicinal herbs is tremendously increasing over a past decade as an alternative approaches to improve the quality of life and maintain a good health. Medicinal plants have been used for centuries as remedies for human diseases (Nostro *et al.*, 2000; Arokiyaraj *et al.*, 2008; Gangadevi *et al.*, 2008). Extensive studies of the adverse effects of these herbal medicines and establishment of a good correlation between biomarkers and plants are essential for ensuring the efficiency and quality of herbal medicines. Recently, there has been growing interest in exploiting the biological activities of flora and fauna owing to their natural origin, cost effectiveness and lesser side effects (Rehan Ahmad *et al.*, 2008; Chellaram & Edward, 2009). Plant-based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc (Gordon, 2001). The medicinal actions of plants unique to particular plant species or groups are consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct (Wink *et al.*, 1999).

The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial herbal extracts (Bisignano *et al.*, 2000; Hammer *et al.*, 1999). Plants containing flavonoides, terpenoids, steroids, phenolic compounds and alkaloids have been reported to have antimicrobial activity (Hostettmann *et al.*, 1977).

The WHO has continued a diarrhoeal disease control programme which includes studies of traditional medicinal practices together with the evaluation of health education and preventive approaches. This wipes out the increasing

mortality rate in developing countries due to diarrhoea. Therefore, it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs.

*Sphaeranthus amaranthoides* Linn. (SPA) is a small procumbent herb, with stems rooting and pubescent with appressed hairs, leaves palmately 3-foliolate. This plant is well known for its medicinal value for the treatment of eczema, blood disorders, stomach worms, filaria, fever and as a remover of kapha, vata and piles. It is also known to cure skin diseases (Kirtikar & Basu, 1971). Many plants being used as an antibacterial and antidiarrhoeal agent, the effect of *Sphaeranthus amaranthoides* Linn against diarrhoea and bacteria has not been investigated. This study was aimed the anti bacterial activity of different solvent extracts of SPA against human pathogenic bacteria and antidiarrhoeal activity of SPA on castor-oil induced diarrhoea in rats. The bacterial organisms used in this study are known for wound infection, diarrhoea, pneumonia and chest pain.

### Materials and methods

#### Animals

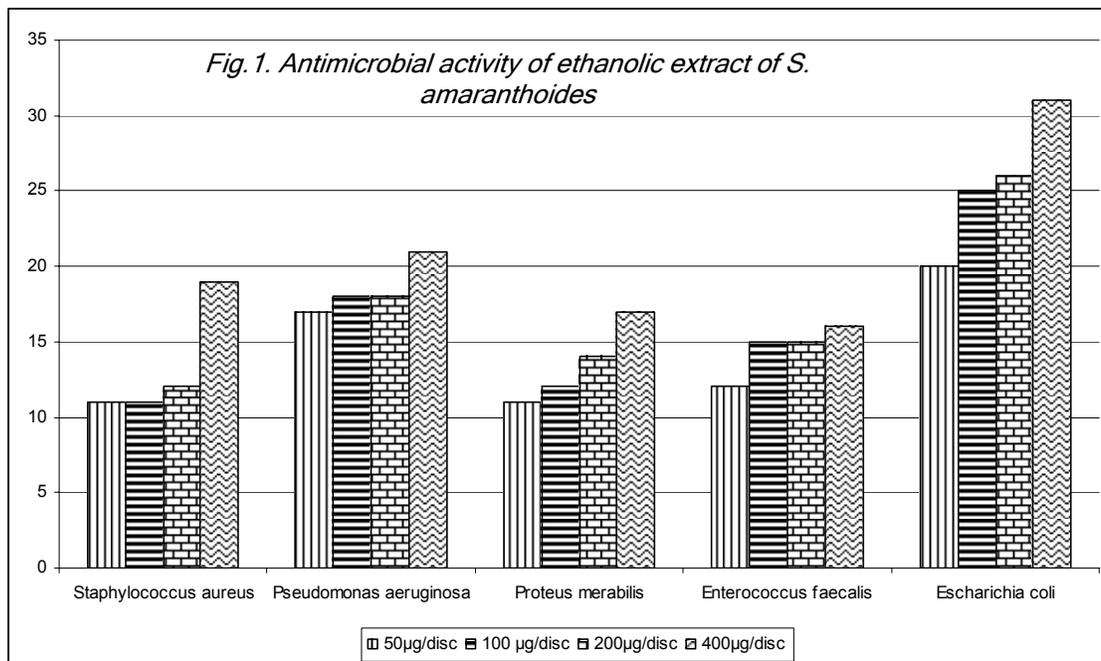
Male Wistar rats (150-200 g) obtained from Experimental Animal Department, King's Institute, Guindy, Chennai were used in the study. The rats were fed standard laboratory diet, given water *ad libitum* and maintained under laboratory condition of temperature 22±1°C, relative humidity 14±1 and 12h light: 12h dark cycle.

#### Phytochemical screening

Conventional standard protocols (Odebiyi & Sofowora, 1978; Trease & Evans, 1983) for detecting the presence of different chemical constituents in the plant extract were employed. The tests for the secondary metabolites *viz.* alkaloids, tannins, saponins, glycosides, flavonoids and phenols were carried out.

**Preparation of the crude extracts:** Hundred grams of *S. amaranthoides* air-dried and coarsely powdered plant material was extracted with ethanol (98.8%) for 2 h. The ethanol extract was filtered and evaporated under reduced pressure using Rota-vapor. The extract was dissolved in dimethyl-sulphoxide to make the final concentrations which kept in refrigerator till used.

**Preparation of the tested organisms:** *In vitro* screening for antimicrobial study was carried out using selected disease causing pathogens which includes two gram +ve bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and three gram -ve bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis*). Five strains each of bacterial species were used in this study. These organisms were identified by following the standard microbiological methods (Collins & Lyne, 1995).



toxicity, mortality and weighed for 14 days (Tijani *et al.*, 1986)

#### *Anti-diarrhoeal activity*

Test was performed according to (Mandal, 1997) with minor modifications. Rats of either sex were tested for 18h and randomly assigned to six groups of six animals each. The doses of the extract (SPA) selected on a trial basis, were administered orally (200, and 400 mg/kg suspended in saline) to three groups of animals. The fourth

The antibacterial screening of the extracts were carried out by determining the zone of inhibition using disc diffusion method (Rath *et al.*, 1999; Sahoo *et al.*, 2006). The strains of microorganisms obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37<sup>o</sup> for 24 h and were referred to as seeded broth. The density of the bacterial suspension was standardized by standard McFarland method (Mc Farland *et al.*, 1987). The extracts were dissolved in dimethyl formamide which was previously tested for antibacterial activity against all test bacteria and found to have no antibacterial activity. The extracts were made solution at a concentration of 50 mg/ml and finally sterilized by filtration using 0.45 µm Millipore filters. The sterile discs (6 mm in diameter) were impregnated with 20 and 2.5 µl of above extract solution to achieve desired concentration of 500 and 250 µg/disc and placed in inoculated agar. Gentamicin (G) (10 µg/disc) and Ciprofloxacin (CF) (25 µg/disc) were used as standards. The controls were prepared using the same solvents employed to dissolve the extracts. The inoculated plates with the test and standard discs on them were incubated at 37<sup>o</sup> C for 24 h.

#### *Acute toxicity (LD50) study*

Acute toxicity study was carried out using the method of (Lorke, 1983). In the first phase, nine rats randomly divided into three groups of three rats per group were given 10, 100 and 1000 mg extract/kg body weight orally (via a cannula), respectively. The rats were observed for signs of adverse effects and death for 24 h and then weighed daily for 14 days. In the second phase of the study, the procedure was repeated using three rats randomly divided into three groups of one rat each, given 1600, 2900 and 5000 mg extract/kg body weight, respectively. The rats were also observed for signs of

group of animals received 5 mg/kg body weight of diphenoxylate orally as a standard drug. The fifth group, which served as a control received 2% v/v saline only. One hour after the treatment each animal received 1 ml of castor oil orally and then observed for detection up to 4 h after. The presence of characteristic diarrhoeal droppings was noted in transparent plastic dishes placed beneath individual cages.

#### *Statistical analysis*

Results were expressed as the mean ± standard error of mean (S.E.M). Statistical analysis of data was carried out using one-way analysis of variance (ANOVA) followed by Student's *t*-test. Differences in mean were considered to be significant when *p* < 0.001.

#### **Results and discussion**

##### *Phytochemical analysis*

Phytochemical analysis of the ethanol extract revealed the presence of steroids, triterpenoids, phenols flavonoids, tannins and glycoside compounds. Further phytochemical analysis of ethanol extract has made SPA medicinally important (Morebise *et al.*, 2002).

##### *Antibacterial activity of ethanolic extract of SPA*

According to World Health Report on infectious diseases 2000, overcoming antibiotic resistance is the major issue for the next millennium. As antibiotics provide the main basis for the therapy of bacterial infections the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Due to lack of information on the screening / evaluation of diverse plants for their antibacterial potential there has been a continuing search for new and more potent antibiotics (Heisig, 2001).

The antimicrobial activity (zone of inhibition) was shown in Fig.1. The ethanol extracts of SPA was

subjected to a preliminary screening for antimicrobial activity against *Staphylococcus aureus*, *P. aeruginosa*, *P. mirabilis*, *E. faecalis* and *E. coli*. Thereby SPA exhibited highest activity against all the tested strains. It showed highest activity against *E. coli* (31mm).

The observed activity may be due to the presence of potent

phytoconstituents in the extracts (Ikram & Innamual 1980a, 1980b) and present investigation on the phytochemical analysis of SPA refers to the presence alkaloids, glycosides, saponins, tannins, triterpenoids, flavonoides, fixed oils and simple phenol compounds which might be responsible for its antimicrobial properties. Though the body system is made in such a way that it tackles invading foreign substances in most cases, the body system is incapable to do so and needs to be protected, enhanced and activated (Murray *et al.*, 1990). This ability to activate the body defense mechanism or to protect the body system has been found to be present in some nature vegetation/herbal sources (Morebise *et al.*, 2002). Table 1 shows the antibacterial activity of SPA in terms of zone of inhibition in mm.

**Antidiarrhoeal activity of ethanolic extract of SPA**

Table 2 shows the effect of ethanolic extract of castor-oil induced diarrhoea in wistar rats. The ethanolic extract of SPA at doses of 200 and 400mg/kg, reduced significantly in a dose dependent way. The frequency of defecation and the wetness of fecal droppings when compared with untreated Tween80, thereby exhibited an anti-diarrhoeal activity. The dose of 400 mg/kg of SPA treated seems to show an equivalent effect of that of 5 mg/kg of diphenoxylate.

Ricinoleic acid the active principle in castor oil caused changes in mucosal cell layer permeability, electrolyte transport and intestinal peristalsis, leading to prostaglandin secretion, which results in an increase in the secretion of water and electrolytes into the small intestine (Zavala *et al.*, 1988). The gut wall contains prostaglandins E and F with prostaglandin synthetase activity mainly in the mucosa that cause intestinal cramps and diarrhoea which might be due to effect on intestinal smooth muscle and secretion (Galvez *et al.*, 1993).

Moreover prostaglandin contributes to the pathophysiological functions in gastrointestinal tract (Sanders, 1984). Further, the studies of Ferreria *et al.* (1972) also revealed an

evidence for our study, that the prostaglandin biosynthesis inhibitors delayed castor oil induced diarrhoea.

Table 1. Antibacterial activity of ethanolic extract of SPA

Name of the Organism	Zone of inhibition in mm Concentration of SPA in µg/ml			
	50µg/disc	100 µg/disc	200µg/disc	400µg/disc
<i>S. aureus</i>	11mm	11mm	12mm	19mm
<i>P. aeruginosa</i>	17mm	18mm	18mm	21mm
<i>P. merabilis</i>	11mm	12mm	14mm	17mm
<i>E. faecalis</i>	12mm	15mm	15mm	16mm
<i>E. coli</i>	20mm	25mm	26mm	31mm

The use of native plants for the treatment of diarrhoea has become a common practice in many folk medicines. As mentioned, various species of *Speranthus* is used

for the treatment of diarrhea. In the present investigation the rats fed with different doses of ethanolic extract of SPA effectively inhibited diarrhoea. The anti-diarrhoeal activity may thus be attributed to the presence of Tannins, the phytochemical which is known to reduce the effect through denaturing the proteins by the formation of protein tannate, thereby causing the intestinal mucosa more resistant and reduces secretion (Tripathi, 1994). Hence tannins present in the extract may be responsible for their medicinal anti-diarrhoeal activity (Yu *et al.*, 2000; Devi *et al.*, 2002) and also, be partly responsible for other pharmacological properties.

To conclude, the present investigation clearly reveals the antibacterial nature of this plant and suggests that this plant could be exploited in the management of diseases caused by the tested bacteria in human. Further ethanolic extract of SPA in graded doses reduced diarrhoea and thereby lending support to use SPA in folklore medicine against chronic diarrhea. The phytochemical analysis revealed that the active principle, a phenolic compound present in the ethanolic extract of SPA, is thus responsible for the antibacterial and antidiarrhoeal activity. It has become expedient to examine scientifically the protective effects of these herbal plants and thus there has been a continuing search for new and more potent antibiotics (Heisig, 2001). Therefore there is an increased need for research in the investigations on plants as a source of human disease management (Aiyelagabe *et al.*, 2000; Mouniswamy *et al.*, 2002; Woldemichael *et al.*, 2003).

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Table 2. Effect of *S. amaranthoides* ethanol extract on castor oil induced diarrhoea in rats

Treatment groups	Mean defecation	Mean number of wet faeces
Control vehicle (p.o)	14.42±0.841	8.8±1.46
Diphenoxylate.HCL5mg/kg(p.o)	0.574±0.095**	0.531±0.08**
SPA extract 200mg/kg (p.o)	5.54±0.89**	3.578±0.59**
SPA extract 400mg/kg (p.o)	4.688±0.78**	0.803±0.13**

Values are expressed as mean ± S.E.M. \*\*p<0.01 compared to control: n=6 animals in each group

"antidiarrhoeal herb"

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