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Research Article

ANTIMICROBIAL ACTIVITY OF THE LEAF, FLOWER AND STEM EXTRACTS OF *SPHENOCLEA ZEYLANICA*

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Abstract

The present study was carried out with an objective to investigate the antimicrobial potentials of leaves, flowers and stem extracts of *Sphenoclea zeylanica*. The aim of the study was to evaluate the antimicrobial activity and to determine the zone of inhibition of extracts against medically important bacterial and fungal strains. The antimicrobial activity was determined in the extracts using zone of inhibition method. The antibacterial and antifungal activities of extracts (40, 50, 60 and 70) of *Sphenoclea zeylanica* were tested against *B. subtilis, P. vulgaris, S.aureus, E.coli, C. albicans, A. niger, S. typhi* and *B. cereus*. Zone of inhibition of extracts were compared with that of control for antimicrobial activity. The results showed that the leaf extract showed notable inhibition of the microbial growth against the tested organisms. The microbial activity of the *Sphenoclea zeylanic a* might be due to the presence of various secondary metabolites. Hence, this plant can be used to identify the specific bioactive natural products which may serve as leads in the development of new antimicrobial agents.

Keywords: *Sphenoclea zeylanica*; Antimicrobial Activity; Antibiotic Resistance.

Introduction

Medicinal plants are a rich source of antimicrobial agents and are used as a source of many potent drugs (Srivastava *et al*., 1996). A range of medicinal plant parts possess varied medicinal properties and are used as raw drugs. Root, stem, flower, fruit, twigs exudates and modified plant organs are used. While some of these raw drugs are collected by the local communities and folk healers in smaller quantities, but some raw drugs are traded in the market as the raw material for many herbal industries and collected in larger quantities (Uniyal *et al*., 2006).

Despite hundreds of plants being tested for antimicrobial properties but still a vast majority of them have not been effectively assessed (Balandrin *et al*., 1985). Herbal medicines are used by about 75-80% of whole population, and involves the use of plant extract and their active constituents (Akerele, 1993). Though conventional drugs are widely used, herbal medicines are utilized due the presence of secondary metabolites such as alkaloids, flavonoids, tannins, and terpenoids reported to have antibacterial activities (Cowan, 1999; Adenkunle and Adekunle, 2009; Lewis and Ausubel, 2006).

Interest in medicinal plants has prospered due to the higher efficiency of plant derived drugs and the concern about the side effects of modern medicine. The resistance to antibiotic has increased substantially in the recent years. Usage of antibiotic resistance inhibitors from plants is one of the ways to reduce the antibiotic resistance (Kim *et al*., 1995; Alagesaboopathi, 2011). Plants produce a wide range of compounds in order to protect themselves against pathogens. The plant extracts which have target sites other than those used by antibiotics will be active against drug resistant pathogens (Ahmad and Beg, 2001). Antimicrobials derived from plants are a vast untapped source of medicines despite the studies on their wide spread therapeutic potential and effectiveness in the treatment of infectious disease, further research of plant antimicrobials are required (Parekh *et al*., 2007). The screening of plant extracts and their product has shown that higher plants are a potent source of novel antibiotics (Afolayan, 2003). Because of the availability of chemical diversity, natural products provide unlimited opportunities for the development of new drugs (Cos *et al*., 2006).

Though the discovery and development of antibiotics are the most important achievements of modern science and technology for controlling of infectious diseases. However, the rate of resistance of microorganisms tocommonly used anti-microbial agents is increasing with in startling

frequency (Ge *et al*., 2002; Nair and Chanda, 2005; Neogi *et al*., 2008).

Fungal diseases are a critical health problem to health and are one of the main causes of morbidity and mortality worldwide (CSIR, 1998). In tropical and subtropical developing countries human infections, especially those of the skin and mucosal surfaces are a serious problem (Portillo *et al*; 2001). An alternative to the conventionally used fungicides is the use of compounds extracted from plants as they contain flavonoids, phenols, tannins, alkaloids, quinones, saponins and sterols (Burt, 2004).

Though the use of synthetic fungicides is a quick and effective management for most of plantpathogenic fungi their massive use has severe environmental impact (Osman and Al-Rehiayam, 2003). Their inappropriate use lead to adverse effects on ecosystems and also carcinogenic risk than insecticides and herbicides together (Stranger and Scott, 2005). Moreover, fungicides have become ineffective due to resistance by pathogens (Zhonghua and Michailides, 2005; Cohen, 1992; Nascimento*et al*., 2000).

Sphenoclea zeylanica Gaertner belongs to family Campanulaceae, it is pantropical to India. It is found in swampy areas, along the banks of water courses and in rice fields. It is an erect annual herb, and the inflorescence is a dense, terminal spike with small, greenish yellow flowers. The young plants and tips of older plants are streamed and eaten as vegetable with rice in Java (Usher, 1984). In the present study, the methanol extract of *Sphenoclea zeylanica* leaf, stem and flower extracts were evaluated in order to know their antimicrobial activity against clinically important microorganisms.

Materials and Methods

Screening of Antibacterial Activity

Preparation of Inoculums

Stock cultures were maintained at 4^{o}C on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loop full of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hrs at 37^{o}C and 25^{o}C respectively. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to $2.0 \cdot 10^{6}$ colony forming units (CFU/ml) for bacteria.

Antimicrobial Susceptibility Test

The disc diffusion method (Bauer *et al.*, 1966) was used to screen the antimicrobial activity. *In vitro* antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Hi-media (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The concentration of extracts is 40 mg/disc was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37^{o}C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Antifungal Activity

Culture Media

The media used for antifungal test was Sabouraud's dextrose agar/broth of Hi media Pvt. Bombay, India.

Determination of Antifungal Activity

The agar well diffusion method (Perez, 1993) was modified. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabourauds dextrose broth. A total of 8 mm diameter disc were placed in the agar and filled with plant extracts. Standard antibiotic (Fucanazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37^{o}C for 72 hrs. The diameters of zone of inhibition observed were measured.

Results and Discussion

Antibiotics are most important weapons in fighting bacterial infections and have greatly enhanced the health-related quality of human life since their discovery. But over the past few decades, these benefits are under threat as many commonly used antibiotics have become less effective against certain illnesses not, only because many of them produce toxic reactions, but also due to emergence of drug-resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Antimicrobial screening of plant extracts and phytochemicals, then, represents a starting point for antimicrobial drug discovery (Cseke *et al*., 2016).

In this study, the antimicrobial activity of the methanol extracts of the leaf, stem and flower of *Sphenoclea zeylanica* at different concentrations was studied using the organisms *B. subtilis, P. vulgaris, S. aureus, E. coli, C. albicans, A. niger, S. typhi* and *B. cereus* (Table 1-3). It was seen that the inhibition displayed by the extracts of concentrations 50, 60 and 70 was higher than the inhibition showed by control in some cases. No inhibition was seen at the lowest concentration except for the inhibition of *B. cereus* by the leaf extract. The leaf extract showed maximum antimicrobial activity against the organisms at the highest concentration (Table 1). The most susceptible organisms to the leaf extract were found to be *P. vulgaris, C. albicans, A. niger* and *B. cereus*. The least susceptible organisms were *S. aureus and E. coli*. Stem extract of *Sphenoclea zeylanica* had the lowest antimicrobial activity compared to the other two extracts (Table 2). Thus, it can be concluded that the leaves of *Sphenoclea zeylanica* have significant antimicrobial activity.

Table 1: Screening of Antimicrobial activity in leaf extract of *Sphenoclea zeylanica*

S.N.	Organisms	Control	Concentrations			
			40	50	60	70
1.	*B. subtilis*	17±0.70	0	5.66±1.08	10±0.70	12±0.70
2.	*P. vulgaris*	12.66±2.16	0	0	8±1.41	14.33±1.47
3.	*S. aureus*	13.33±1.47	0	4.66±1.78	7±1.41	9.66±1.08
4.	*E. coli*	13±1.87	0	6±1.87	8.33±1.08	11.66±1.08
5.	*C. albicans*	16.33±1.08	0	5.66±2.48	11.33±1.47	15.66±1.47
6.	*A. niger*	16±1.87	0	10±1.41	10.33±1.47	17.66±1.08
7.	*S. typhi*	16.33±0.40	0	5.33±1.08	8±1.87	13.33±1.08
8.	*B. cereus*	14±1.22	0	10±0.70	9.33±2.16	14.33±2.27

Table 2: Screening of Antimicrobial activity in stem extract of *Sphenoclea zeylanica*

S.N.	Organisms	Control	Concentrations			
			40	50	60	70
1.	*B. subtilis*	17±0.70	0	9±0.70	13.33±1.47	13±0.70
2.	*P. vulgaris*	12.66±2.16	0	10.33±1.08	11±0.70	16±1.87
3.	*S. aureus*	13.33±1.47	0	6.66±1.08	10.66±0.81	14.33±1.47
4.	*E. coli*	13±1.87	0	11.33±2.27	9±0.70	11±0.70
5.	*C. albicans*	16.33±1.08	0	5.66±1.47	12±1.87	14.33±1.08
6.	*A. niger*	16±1.87	0	7.66±1.78	7.66±1.08	17.33±1.08
7.	*S. typhi*	16.33±0.40	0	7±0	9±0.70	15±2.12
8.	*B. cereus*	14±1.22	0	10±1.41	14.33±1.08	

Table 3: Screening of Antimicrobial activity in flower extract of *Sphenoclea zeylanica*

S.N.	Organisms	Control	Concentrations			
			40	50	60	70
1.	*B. subtilis*	17±0.70	0	7.33±1.08	11±0.70	15±0.70
2.	*P. vulgaris*	12.66±2.16	0	6.33±1.08	11.33±1.08	11.66±1.08
3.	*S. aureus*	13.33±1.47	0	7.66±0.40	12.66±1.77	13±1.41
4.	*E. coli*	13±1.87	0	7.66±1.08	13±1.87	11±0.70
5.	*C. albicans*	16.33±1.08	0	11.33±1.08	11.33±1.08	18±0.70
6.	*A. niger*	16±1.87	0	6.66±1.08	12.33±1.08	15±0.70
7.	*S .typhi*	16.33±0.40	0	7.33±0.81	13±1.41	13±1.87
8.	*B. cereus*	14±1.22	0	7±0.70	8±0.70	19±0.70

Conclusion

Sphenoclea zeylanica is an herb found commonly in India. In the present study, the methanolic extracts of *Sphenoclea zeylanica* leaves, flower and stem were analyzed to understand the antimicrobial activity they exhibit. Eight organisms viz *B. subtilis, P. vulgaris, S. aureus, E. coli, C. albicans, A. niger, S. typhi* and *B. cereus* were taken as test organisms. The most susceptible species were *P. vulgaris,*

C. albicans, *A. niger* and *B. cereus.* The leaf extract had higher antimicrobial activity when compared to other extracts. This study proves that *Sphenoclea zeylanica* has significant antimicrobial activity. Therefore the compounds can be isolated from this plant and can be developed as antimicrobial agents against microorganisms causing infectious diseases.

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