African Journal of Biotechnology Vol. 8 (23), pp. 6608-6611, 1 December, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

An evaluation of antibacterial activities of *Seidenfia rheedii* (Sw.) Szlach.

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Accepted 17 October, 2008

Global antibiotic resistance by bacteria is becoming an increasing public health concern and the presence of drug-resistance bacteria does not only hamper the effective treatment of infectious diseases, but also increases the cost of treatment. Therefore there is a need to develop alternate antibacterial drugs for the treatment of infectious diseases. One approach involves the search for new therapeutic agents with novel modes of action from medicinal plants. In this study antibacterial activity of aqueous and various organic solvent extracts of *Seidenfia rheedii* leaf were checked against both gram-positive and gram-negative bacteria. Among them, ethanolic leaf extract was found to possessed significant antibacterial activity against all the test bacteria while aqueous extract did not exhibit any activity against the same organisms. The activity was more pronounced against *Bacillus cereus* (12 mm), *Shigella dysenteriae* (9 mm) and *Sterptococcus faecalis* (8 mm). The results obtained from the present study suggest a new potential application of *S. rheedii* as an alternate source of antibiotics.

Key words: *Seidenfia rheedii*, drug resistance, antibacterial activity.

INTRODUCTION

The search for selective antibacterial agents has gained momentum in recent years due to the growing cases of bacterial resistance to the time honored antibiotics (Davis, 1994; Samie et al, 2005; Francis Xavier and Arun, 2007). With the increasing resistance of bacteria to antibacterial drugs due to misuse and over prescription, there is a serious clinical problem in the treatment of infectious diseases (Leggadrio, 1995). These conditions have been commonly reported from all over the world. The situation is alarming in developing as well as developed countries. Due to the incidence of antibiotic resistance in bacteria of medical importance, there is a distinct and constant need for safe and more effective therapeutic agents from other sources (Martinii and Eloff, 1998). One of the methods to reduce the resistance to antibiotics and adverse effect on host is by using antibiotics of plant origin (Kim et al., 1995). Plant based antimicrobials represent a vast untapped source for medicines and has enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Murray, 1995; Eloff et al., 2005).

One of the unscreened medicinal plants is *Seidenfia rheedii* (Orchidaceae) widely available in the high altitudes of Kolli hills, Namakkal district of Tamil Nadu and it has been used as a traditional medicine in India. Different part of the plants have been claimed to possess medicinal properties in the traditional medicinal system. The medicinal value of the present study plant was also discussed in 'Charaka Samhita - a classic ancient Indian medicinal treatise in Sanskrit few thousands years ago. The people of ancient India were also well aware of the medicinal values of orchids (Manilal and Sathiskumar, 1986; Francis Xavier, 2006). However, the plant *S. rheedii* has not been distinguished scientifically. Thus the present study was undertaken to determine the antibacterial sensitivity of the plant *S. rheedii* leaf extracts.

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MATERIAL AND METHODS

Plant material

Plant materials were collected in fresh condition from Kolli hills of Eastern Ghats of Tamil Nadu and identified after critical examination and the plants were deposited in the Herbarium of the Department of Plant Biology and Plant Biotechnology, St. Joseph's College, Tiruchirappalli, South India.

Extraction of plant material

The plant materials (leaves) were shade dried at 31°C for 10 days. 50 g of the dried powdered materials were soaked separately with 300 ml of the solvent (1:6 w/v) *viz*., water, ethanol, ethyl acetate, chloroform and petroleum ether, ethanol, (1:1) in a soxhlet apparatus for the 48 h at 310°C until complete extraction of the plant materials. At the end of 48 h each extract was filtered through Whatman No.1 filter paper and filtrates were concentrated at room temperature in order to reduce the volume. The paste like extracts were stored in pre-weighed screw capped bottles and the yield of extracts have been weighed and the extracts was individually reconstituted using minimal amounts of extracting solvents prior to use.

Microorganisms

The microorganisms used for the antibacterial screening were purchased from Microbial Type Culture Collection (MTCC), Institute of Microbial Biotechnology (IMTECH), Chandigarh, Punjab. The bacteria includes *Bacillus cereus* Bizio (MTCC 1272), *Streptococcus faecalis* (Klein) Chester (MTCC 655), *Streptococcus pyogens* Rosenbach (MTCC 1928), *Streptococcus pneumoniae* Chester (MTCC 655), *Staphylococcus aureus* Rosenbach (MTCC 1430) (Gram-positive bacteria), *Escheriachia coli* Escherich (MTCC 1195), *Proteus vulgaris* Hauser (MTCC 1771), *Enterobacter aerogens* Bizio (MTCC 2823), *Salmonella typhi* Eberth (MTCC 733),*Shigella dysenteriae* Shiga (MTCC 2405), *Klebsiella pneumoniae* Friedlander (MTCC 2405), *Pseudomonas aeruginosa* Bizia (MTCC 2642) and *Serratia marcescens* Bizia (MTCC 2645). *E. coli* mutant strains viz. $Y_{10}90$. HFrc, KL96, 3006-KL96, $C_2H_5$7, Pi/345 *kan* and K_{12} were collected from Microbiology Division, Madurai Kamaraj University, Madurai.

Assay for antibacterial testing

Antibacterial activity of the above mentioned four different solvent and aqueous extracts were assayed separately using disc diffusion method (Bauer et al., 1966). Petri plates containing 10 ml of Muller Hinton Agar medium were inoculated with 10^8 CFC/ml of each test bacteria. Sterile filter paper discs (6 mm in diameter) were impregnated with 10 µl of the 3 mg/ml plant extracts (30 µg/disc) placed on the surface of the medium. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. A standard disc containing chloramphenicol antibiotic drug (30 µg/disc) was used as a positive control and they were incubated for 24 h. The assessment of antibacterial activity was based on the measurement of diameter of inhibition zone formed around the disc. Three independent trials were conducted. The whole work was conducted from August 2006 to December 2007.

RESULTS AND DISCUSSION

The antibacterial activity of aqueous, ethanol, ethyl acetate, chloroform and petroleum ether, ethanol (1:1) extracts showing both positive and negative results are presented in Table 1. The ethanolic leaf extracts exhibited high degree of inhibition against the bacteria. The zones of inhibition were higher in the case of *Bacillus cereus* (12 mm), *Shigella dysenteriae* (9 mm) and *Streptococcus faecalis* (8 mm). Moderate inhibition was noted against E. *coli* (7.6 mm), *Proteus* vulgaris (7.1 mm). *Enterobacter aerogens* (6.2 mm), *Salmonella typhi* (6.2 mm), *Staphylococcus aureus* (5 mm), *Klebsiella pneumoniae* (6 mm), *Serratia marcescens* (5 mm), $Y_{10}90$ (5.6 mm), HFrc (5 mm), KL96 (4.6 mm) and *Pseudomonas aeruginosa* (4.6 mm) whereas low degree of inhibition zones were associated with pi/345 kan (3.4 mm), 3006 KL96 (3.0 mm), *Streptococcus pneumoniae* (2.6 mm) and $C_2H_5$7 (2 mm) and also very low inhibition was associated with K_{12} (1.6 mm) and *Streptococcus pyogens* (1.4 mm).

The results of antibacterial screening of other solvent extracts of *S. rheedii* leaves were also depicted in Table 1. The zones of inhibition against the pathogenic bacteria were less than the standard antibiotic chloramphenicol (30 µg /disc). Some of the extracts showed complete absence of inhibition zones (Chloroform and petroleum ether ethanol (1:1) extracts) against *S. faecalis*, *S. aureus*, *S. pneumoniae*, *S. pyogens*, *P. vulgaris*, *S. dysenteriae* and some *E. coli* mutants .

The ethanol extracts of *S. rheedii* leaf effectively inhibited the growth of both gram-positive and gram-negative bacteria. Similar results were also drawn by several workers (Rabe and Vanstaden, 2000; Ates and Erdogrul, 2003; Bouhadjera et al., 2005), whereby majority of the significant activity was associated with ethanol extracts. The other solvent extracts showed satisfactory results whereas the aqueous extracts of the plant part showed nil activity. This finding is interesting, because in the traditional method of treating bacterial infection, decoction of the plant parts or boiling the plant, water is employed whereas according to present study, preparing an extracts with an organic solvent was shown to provide a better antibacterial activity, in accordance with the results obtained by Bhattacharjee et al. (2006). This is because most of the antibacterial principles are extracted much through the organic solvents (Chakrabarty and Brantner, 1999; Aburjai et al., 2001). Hence, the aqueous extract did not show activity against pathogenic bacteria. The extracts of *S. rheedii* leaf under study exhibited greater antibacterial activity and the diameter of inhibition zone is higher than that of *Argemone maxicana* (Bhattacharjee et al., 2006), *Salvia officinalis* leaf, *Thymus vulgaris* leaf, and *Rosmarinus officinalis* leaf (Shanab et al., 2004). In addition other plant species; *Boswellia* (Adelakun et al., 2001), *Buxus* (Rahman et al. 1997), *Commiphora* (Asres et al., 1998), *Jatropa* (Aiyelaagbe et al., 2000), *Withania* (Budhadjera et al., 2005) *Cissus* (Beltrame et al., 2002) and *Cleome* (Perumalsamy et al., 1999) have been showed similar

Table 1. Antibacterial activity of leaf extracts of *S. rheedii* on pathogenic bacteria (disc diffusion method).

Test bacteria	Ethanol extract		Ethylacetate extract		Chloroform extract		Petroleum ether : Ethanol extract (1:1)		Positive control chloram-phenicol (30 mcg/ disc)
	Exp*	Negative control	Exp	Negative control	Exp	Negative control	Exp	Negative control	
Gram-positive									
B. cereus	12.0± 0.00	2.6 ± 0.47	10.1 ± 0.09	1.0 ± 0.00	9.3 ± 0.47	1.0 ± 0.00	9.0 ± 0.00	1.0 ± 0.00	10.0 ± 0.00
S. faecalis	8.1 ± 0.98	3.0 ± 0.00	8.0 ± 0.00	–	8.2 ± 0.01	1.0 ± 0.00	7.0 ± 0.25	–	7.0 ± 0.00
S. aureus	5.0 ± 0.00	2.0 ± 0.00	4.0 ± 0.09	–	4.6 ± 0.37	1.0 ± 0.00	4.0 ± 0.00	1.0 ± 0.00	6.0 ± 0.00
S. pneumoniae	2.6 ± 0.09	1.0 ± 0.09	2.2 ± 0.11	–	1.3 ± 0.47	–	1.0 ± 0.00	–	7.0 ± 0.00
S. pyogens	1.4 ± 0.41	0.6 ± 0.41	1.0 ± 0.00	-	1.0 ± 0.09	–	1.0 ± 0.00	–	8.0 ± 0.00
Gram-negative									
E. coli	7.6 ± 0.14	2.0 ± 0.00	7.0 ± 0.09	1.0 ± 0.00	6.6 ± 0.72	2.0 ± 0.00	5.2 ± 0.09	1.0 ± 0.00	10.0 ± 0.00
P. vulgaris	7.1 ± 0.09	1.0 ± 0.00	7.0 ± 0.00	–	6.3 ± 0.14	–	5.0 ± 0.00	–	9.0 ± 0.00
E. aerogens	6.2 ± 0.14	2.0 ± 0.00	6.0 ± 0.14	1.0 ± 0.09	5.0 ± 0.00	1.0 ± 0.00	4.8 ± 0.24	1.0 ± 0.00	9.0 ± 0.00
S. typhi	6.2 ± 0.14	2.0 ± 0.00	6.0 ± 0.00	–	5.0 ± 0.00	–	5.0 ± 0.00	–	9.0 ± 0.00
S. dysenteriae	9.0 ± 0.00	2.0 ± 0.00	7.0 ± 0.09	–	6.0 ± 0.10	–	5.2 ± 0.36	–	8.0 ± 0.00
K. pneumoniae	6.0 ± 0.08	2.0 ± 0.04	6.0 ± 0.00	–	5.4 ± 0.10	1.0 ± 0.01	4.0 ± 0.00	1.0 ± 0.00	8.0 ± 0.00
S. marcescens	5.0 ± 0.00	1.0 ± 0.00	5.0 ± 0.00	1.0 ± 0.00	4.6 ± 0.21	–	3.0 ± 0.00	1.0 ± 0.00	11.0 ± 0.00
Y1090	5.6 ± 0.92	–	5.0 ± 0.00	–	4.5 ± 0.34	–	3.4 ± 0.17	–	7.0 ± 0.00
HFrc	5.0 ± 0.00	1.0 ± 0.00	4.6 ± 0.21	–	4.0 ± 0.00	–	3.0 ± 0.00	–	9.0 ± 0.00
KL96	4.6 ± 0.94	–	4.2 ± 0.47	–	4.0 ± 0.00	–	3.2 ± 0.01	–	14.0 ± 0.00
3006 KL96	3.0 ± 0.77	1.3 ± 0.47	4.2 ± 0.14	–	4.2 ± 0.12	1.0 ± 0.00	3.0 ± 0.00	–	7.0 ± 0.00
C2H57	2.0 ± 0.81	1.0 ± 0.00	2.0 ± 0.00	–	1.0 ± 0.00	–	2.0 ± 0.00	–	9.0 ± 0.00
Pi/345 Kan	3.4 ± 0.47	0.6 ± 0.47	2.0 ± 0.00	–	2.0 ± 0.00	–	2.0 ± 0.00	–	9.0 ± 0.00
P. aeruginosa	4.6 ± 0.09	2.0 ± 0.00	4.0 ± 0.00	1.0 ± 0.00	3.4 ± 0.17	1.0 ± 0.01	2.5 ± 0.33	1.0 ± 0.00	9.0 ± 0.00
K 12	1.6 ± 0.47	1.0 ± 0.00	1.0 ± 0.00	–	1.0 ± 0.00	–	1.0 ± 0.03	–	7.0 ± 0.00

Values are inhibition zone diameter in mm (mean ± SD).
For the exp, 30 µg of the extract per disc was used.
*Subtracted value from negative control.
– : No inhibition.

type of results. This study signifies the potential of *S. rheedii* as a source of antibacterial therapy, which may provide leads in the ongoing search for alternate antibiotics.

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