Int J Pharm Bio Sci 2013 July; 4(3): (P) 416 - 422

Research Article **Pharmaceutics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ANTIBIOGRAM PROFILE OF PATHOGENIC FUNGI ON SEIDENFIA RHEEDII LEAF EXTRACTS

*** T.FRANCIS XAVIER AND S.R.SENTHIL KUMAR**

Department of Botany, St.Joseph's College College (Autonomous), Tiruchirappalli – 620 002, Tamil Nadu, South India

ABSTRACT

Antibiogram profile of various pathogenic fungi on different solvent extracts of *Seidenfia rheedii* leaf were evaluated using the paper disc agar diffusion method by measuring the diameter of the growth inhibition zones. Among various solvent extracts tested, ethanol leaf extracts exhibited greater inhibition followed by ethyl acetate and chloroform solvent extracts. The petroleum ether, ethanol (1: 1) showed a low degree of inhibition on test fungi whereas no activity was associated with aqueous extract of *Seidenfia rheedii*. The zones of inhibition for each extract was compared with standard antibiotics. The inhibition zones of each solvent extracts found to be either less than or nearly equal to the inhibition zones of standard antibiotics. The leaf extracts showed greater inhibition than the other parts used).From the results achieved there appears to be a rationale for the use of this plant to combat infectious diseases, *Seidenfia rheedii* had an inhibitory effect on the pathogenic fungi causing infections in human beings. Hence, *Seidenfia rheedii* would be the effective plant as it demonstrated good activity against fungi. This would add merit to its traditional use in the treatment of infectious diseases.

KEY WORDS: ***Seidenfia rheedii,*** **greater inhibition, antifungal activity, treatment**

T.FRANCIS XAVIER
Department of Botany, St.Joseph's College College (Autonomous), Tiruchirappalli – 620 002, Tamil Nadu, South India
E.mail: mycofrancis@yahoo.co.in

Corresponding author

INTRODUCTION

Infections induced by fungi are an emerging threat to public Health. Among them *Aspergillus* species are known to cause many important animal and human diseases such as mycotic abortion of sheep and cattle, pulmanory infections in birds and otomycosis, mycotic keratitis, allergy and mycetoma commonly known as Aspergillosis in humans (Rippon, 1988). Among *Aspergilli, A. flavus, A. fumigatus* and *A. parasiticus* produce many toxic compounds such as *ochratoxin* and *aflatoxins. Candida albicans* causes serious systemic infections, including opportunistic infections in patients infected with HIV virus (Srinivasan *et al.,* 2001). Furthermore these organisms which are capable of causing a variety of opportunistic superficial and deep seated mycoses *viz.* Candidiasis, Cryptococcosis and Aspergillosis have increased dramatically in recent years (Caceres et al., 1991). Although there are several antibiotics for treating fungal infections, they are not consistently effective against pathogenic organisms (Gearhart, 1994). Its use is limited by a number of factors such as low potency, poor solubility and drug toxicity (Fromtling and Rahway, 1987; Portillo *et al.*, 2001). Further, the development of resistance in pathogenic fungi against most of the drugs has been reported (Cuenca-Estrella *et al.,* 2000).

Microbial resistance to antibiotics represents a serious problem for human beings since most of the rampant killer diseases are of microbial origin and account for high proportion of mortality in underdeveloped as well as developed countries (Gundidza and Gaza, 1993; Jones, 1998; Guilletmot, 1999). Given the alarming increasing of life threatening pathogens, there is an urgent need for the discovery of alternative, safer and more effective antimicrobial agents in order to control the pathogenic microorganisms (Davis, 1994).One of the methods to reduce the antibiotic resistance and adverse effect on host is by using antibiotic resistance inhibitors and effective antimicrobials of plant origin (Linuma *et al.,* 1994; Kim *et al.,* 1995). Plants are the oldest source of pharmacologically active compounds, and have provided humankind with many medically useful compounds for centuries. Plant based antimicrobials represent a vast untapped source for medicines and have 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. They are effective, yet gentle (Cordell, 1981).

Seidenfia rheedii is one of the most important medicinal orchids with several therapeutic and horticultural values and many species are spread all over the world (Arditti, 1979). Besides their horticultural value, they are used in traditional system of medicine as a remedy for a number of ailments. The medicinal value of the plant has also been discussed in *'Charaka Samhita*' a classic ancient Indian medicinal treatise written by Charaka in Sanskrit a few thousand years ago. This forms the first record of Indian orchids and their uses in Ayurvedic medicine (Manilal and Sathishkumar, 1986). However, the plant *Seidenfia rheedii* has not been distinguished scientifically. Hence, the present investigation was undertaken to determine the antifungal activities of the plant *Seidenfia rheedii* leaf extracts

METHODOLOGY

Plant collection

The plants used in this study were collected from their natural habitats namely Ariyur shola forest range nearly Solakkadu region of Kolli hills, a part of Eastern Ghats ,Namakkal district in Tamil Nadu. South India. A voucher specimen was prepared and it was confirmed in Rapinat Herbarium (RHT), St. Joseph's College, Tiruchirappalli, South India. Healthy leaf samples were taken and dried in the laboratory at room temperature for 5-8 days and they were ground to a fine powder using an electronic blender.

Plant extraction

Aqueous extract is prepared by fifty grams of dried powdered plant materials were extracted with 300 ml of sterile distilled water (1:6 w/v).The aqueous extract was maintained in a soxhlet apparatus over 48 h, filtered and concentrated. Similarly for solvent extract preparation fifty grams of the powdered leaves were soaked separately with 300 ml of each of the solvents viz. ethanol, ethylacetate, chloroform and petroleum ether, ethanol (1: 1) in a soxhlet apparatus for 48 hrs at 78° C until complete extraction of the materials. At the end of 48 hr 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 scapped bottles and the yield of extracts has been weighed. These screw scapped bottles were kept in refrigerator at 40° C. Each of the extract was individually reconstituted using minimal amounts of the extracting solvent prior to use.

Collection of test fungi

The fungal strains consist of *Aspergillus niger* van Tieghem (MTCC 1344), *Aspergillus flavus* Link ex Gray (MTCC 1783), *Aspergillus fumigates* Fresenius (MTCC 2483), *Aspergillus parasiticus* (Vuillemin) Tiraboschi (MTCC 2796) (Molds), *Trichophyton mentagrophytes* Malmsten (MTCC 2657), *Trichophyton rubrum* Malmsten (MTCC 296), *Microsporum gypseum* (Bodin) Guiart and Grigorakis (MTCC 2829) (Dermatophytes), *Candida albicans* Bennett (MTCC 1637) (Yeast like fungus), *Fusarium solani* Saccardo (MTCC 1756) and *Fusarium oxysporum* (Schlech.) Snyder & Hansen were collected from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, Punjab, South India

Antifungal assay

The antifungal activities of *Seidenfia rheedii* lead extracts determined by Paper disc agar diffusion method (Barry and Thornsberry, 1991).Potato Dextrose Agar (PDA) was used as the culture medium for this assay. The spore suspension of 10^6 CFU/ml were added to 15-20 ml of Molten potato dextrose agar medium and mixed well, poured into a sterile petriplates and allowed to set. The sterile filter paper discs (6 mm in diameter) were impregnated with 10 µl of the 3 mg/ml extracts (30 µg/disc) placed on the inoculated agar plates. Negative controls were tested using the same solvents employed in plant extraction. Nystatin (30 µg/disc) was used as positive reference control to evaluate the antifungal activity of plant extract against the test bacteria. The plates were incubated at 30° C for 72 hrs. Antifungal sensitivity was tested by measuring the zones of inhibition against the test organisms. The experiments were carried out in triplicate.

RESULTS AND DISCUSSION

The *in vitro* antifungal activity of various solvent extracts of *Seidenfia rheedii* leaf was evaluated using the paper disc agar diffusion method by measuring the diameter of the growth inhibition zones. (Tables 1).In each extract certain level of activity was observed for some fungi. Based on the results from Table 1, it is clear that the extent of antifungal sensitivity of each extract against each test fungi follows a rank of order. As can be noted from Table1, among various solvent extracts tested, ethanolic leaf extracts exhibited greater growth inhibition followed by ethyl acetate and chloroform extracts. The petroleum ether, ethanol (1: 1) showed a low degree of inhibition on test fungi whereas no activity was associated with aqueous extract of *Seidenfia rheedii*. However, evaluation of the extracts for antifungal property clearly indicated that the fungicidal activity was maximum in ethanol leaf extract. In addition, the inhibition zones formed by standard antibiotic discs (Nystatin 30 mcg/disc-positive control) and those paper discs impregnated with respective extraction solvents (negative control) also given in Table 1.The zones of inhibition for each of the extracts were compared with standard antibiotics. It was noted that the inhibition zones of the each solvent extracts found to be either less than or nearly equal to the inhibition zones of standard antibiotics. The leaf extracts

showed greater inhibition than the other parts used. The zone of inhibition was maximum in *Aspergillus flavus* (4.6 mm), *Aspergillus niger* (4 mm).Their inhibition zones are more or less equal to standard antibiotics. On the other hand the strains of *Aspergillus fumigatus* (3.2 mm), *Aspergillus parasiticus* (3 mm), *Trichophyton rubrum* (3 mm), *and Candida albicans* (3 mm) showed moderately large zones of growth inhibition. However, low degree of inhibition was associated with *Trichophyton mentagrophytes* (2 mm), *Microsporum gypseum* (2 mm), *Fusarium solani* (2.6 mm) and *Fusarium oxysporum* (2.2 mm).The ethanolic leaf extract greatly inhibited the growth of both human and plant pathogenic fungi. Some members of *Aspergillus* and *Fusarium* genera are well known plant pathogens produce aflatoxins. These secondary metabolites are potent carcinogens, hepatotoxins, teratogens and immunosuppressive compounds (Ciegler, 1975). *Fusarium oxysporum* produces phytotoxic fusaric acid and lycomarasmin (Ueno *et al*., 1977). *Aspergillus niger* produces potent mycotoxins on food stuffs and is the most prevalent fungus affecting corn (Marassas, 1991). It was noted from literature, these plant pathogens are more resistant to plant extracts (Leven *et al*., 1979; Naqvi *et al*., 1991; Heisey and Gorham, 1992). In the present study it was surprising to note that these plant pathogens are sensitive to the some of the plant extract especially in ethanol leaf extract more antifungal activity was observed. These observations supported by the works made by other investigators (Heisey and Gorham, 1992; Buwa and Vanstaden, 2006). These results were also in accordance with the report of Irobi and Daramalo (1993) who noted that ethanol leaf extract of *Mitracarpus villosus* having significant antifungal activity against *Aspergillus niger*, *Aspergillus flavus, Fusarium solani, Candida albicans, Microsporum gypseum* and *Trichophyton rubrum*. It is worth noting that similar type of results was reported by Portillo *et al*. (2001). From the results achieved there appears to be a rationale for the use of this plant to combat infectious diseases, as all parts of *Seidenfia rheedii* had an inhibitory effect on the pathogenic fungi causing infections in human beings. Hence *Seidenfia rheedii* would be the effective plant as it demonstrated good activity against fungi. This would add merit to its traditional use in the treatment of infectious diseases.

Table 1
Antifungal activity of leaf extracts of Seidenfia rheedii on pathogenic fungi (Paper disc agar diffusion method)

Inhibition zone diameter in mm (mean ± SD)

Test bacteria	Ethanol extract		Ethylacetate extract		Chloroform extract		Petroleum ether, Ethanol extract (1:1)		Positive control Nystatin
	Experimental	Negative control	Experimental	Negative control	Experimental	Negative control	Experimental	Negative control	
Molds									
A. niger	4.0 ± 0.00	–	3.6 ± 1.88	–	3.0 ± 0.00	1.0 ± 0.00	2.0 ± 0.00	–	5.0 ± 0.00
A. flavus	4.6 ± 0.47	–	3.3 ± 0.47	–	3.0 ± 0.77	1.0 ± 0.00	2.3 ± 0.47	–	6.0 ± 0.00
A. fumigatus	3.2 ± 0.00	1.6 ± 0.47	2.0 ± 0.00	–	2.0 ± 0.00	–	1.4 ± 0.41	1.0 ± 0.09	9.0 ± 0.00
A. parasiticus	3.0 ± 0.00	1.0 ± 0.00	2.0 ± 0.00	–	2.0 ± 0.81	0.6 ± 0.41	1.0 ± 0.00	1.0 ± 0.00	7.0 ± 0.00
Dermatophytes									
T.mentagrophytes	2.0 ± 0.00	–	1.3 ± 0.47	–	1.0 ± 0.00	–	–	–	6.0 ± 0.00
T. rubrum	3.0 ± 0.41	–	1.4 ± 0.31	–	1.3 ± 0.47	–	–	–	6.0 ± 0.00
M. gypseum	2.0 ± 0.47	–	1.0 ± 0.00	–	1.0 ± 0.00	–	–	–	4.0 ± 0.00
Yeast fungi									
C. albicans	3.0 ± 0.00	–	2.6 ± 0.09	–	2.2 ± 0.11	–	1.0 ± 0.09	–	10.0 ± 0.00
Plant pathogens									
F. solani	2.6 ± 0.09	1.0 ± 0.00	2.0 ± 0.00	–	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00	–	8.0 ± 0.00
F. oxysporum	2.2 ± 0.11	1.0 ± 0.00	2.0 ± 0.00	–	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00	–	7.0 ± 0.00

Note: Aqueous extract showed no inhibition
– : No inhibition

ACKNOWLEDGEMENT

Dr.TFX wish to thank University Grants Commission , New Delhi for financial support (F.NO.40-426/2012(SR)) and also thank Management of St.Joseph's College, trichy for providing infrastructure and lab facilities

REFERENCES

1. Rippon, J W. Aspergillosis. *In:* Ed. .T. W. Rippon, *Medical Mycology.* The Pathogenic Fungi and the Pathogenic Actinomycetes. pp 618-650, Saunderes Co., Philadelphia, USA, (1988).
2. Srinivasan D, Nathan S, Suresh T and Lakshmana Perumalsamy P, Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *Journal of Ethnopharmacology,* 74: 217-220, (2001).
3. Caceres A, Lopez B R, Giron M A and Longemann H, Plants used in Guatemala for the treatment *of* dermatophytic infections. Screening for antimycotic activity of 44 plant extracts. *Journal of Ethnopharmacology.* 31: 263-276, (1991).
4. Gearhart M O,Worsening of lung function with fluconazole and review of azole antifungal hepatotoxicity. *Annals of Pharmacotherap,.* 28: 1177-1181, (1994).
5. Fromtling R A. and Rahway N J, Recent trends in the discovery, development and evaluation of antifungal agents. *In:* Proceedings of an International Tele-symposium, J. R. Prous, Barcelona, (1987).
6. Portillo A, Vila R, Freixa B, Adzet T and Canigueral, S. 2001. Antifungal activity of paraguayan plants used in traditional medicine. *Journal of Ethnopharmacology,* 76: 93-98.
7. Cuenca-Estrella M, Mellado E, Diaz-Guerra T M, Monzon A and Rodriguez Tudela J L, Susceptibility of fluconazole-resistant clinical isolates of *Candida* spp., to Echinocandin L Y 303366, itraconazole and amphotericin B. *Journal of Antimicrobial Chemotherapy,* 46: 475-477, (2000).
8. Gundidza and Gaza, Antimicrobial activity of *Dalbergia melanoxylon* extracts. *Journal of Ethnopharmacology,* 40: 127-130, (1993).
9. Jones R N, Important and emerging beta-lactamase mediated resistance in hospital based pathogens, the AMPC enzymes. *Diagnostic Microbiology and Infectious Diseases.* 31: 461-466, (1998).
10. Guilletmot D, Antibiotic use in humans and bacterial resistance. *Current Opinion in Microbiology,* 2: 494-498, (1999).
11. Davis J, Inactivation of antibiotic and the dissemination of resistance genes. *Science.* 264: 375-382, (1994).
12. Linuma M, Tsuchiya H, Sato M, Yokoyama J, Ohyama M and Ohkama Y, Flavanones with potent antibacterial activity against methicillin resistant *Staphylococcus aureus. Journal of Pharmaceutical Pharmacology,* 46: 892-895,(1994).
13. Kim H, Park S W, Park J H, Moon K H and Lee CV, Screening and isolation of antibiotic resistant inhibitors from herb materials.1.Resistant inhibition of 21 Korean plants, *Natural Product Science,* 1: 50-54, (1995).
14. Cordell G A, Introduction to the alkaloids. Biogenetic Approach. John Wiley and Sons, New York. p. 52, (1981).
15. Arditti J, Aspects of the physiology of orchids. In: Advances in Botanical Research. (Ed. Woolhouse, H.W.), Academic Press, London. 7: 421-655, (1979).
16. Manilal K S and Sathishkumar C, Researches on Indian Orchids. In: Biology, Conservation and Culture of Orchids (Ed. Vij, S. P.), Affiliated East West Press Pvt. Ltd., New Delhi, pp. 1-2, (1986).

17. Barry A and Thornsberry C, Susceptibility test and diffusion test procedures. In: Manual of Clinical Microbiology, Washington DC, pp. 1526-1542, (1991).
18. Ciegler A, Mycotoxins, occurrence, chemistry, biological activity. *Lloydia.* 38: 481-483, (1975).
19. Ueno Y, Ishhii K, Sawano M, Ohtsubo K, Mutsuda Y, Tanaka T, Kurata H and Ichinoe, H.Toxicological approaches to the metabolites of *Fusaria*. *Japanese Journal of Experimental Medicine.* 47: 177-184, (1977).
20. Marassas W F O, Toxigenic Fusaria. In: Smith, J. E. and Henderson, R. E. (Eds). Mycotoxin and Animal Foods. CRC Press, Boca Ratan, FL, pp.119-139, (1991).
21. Leven M, Vanden Berghe D , Mertens F, Vlietinck A and Lammens E, Screening of higher plants for biological activities. 1. Antimicrobial activity. *Planta Medica.* 36: 311-321, (1979).
22. Naqvi S A H, Khan M S Y and Vohora S B, Antibacterial, antifungal and anthelmintic investigations on Indian Medicinal Plants, *Fitoterapia.* 62: 221-228, (1991).
23. Heisey R M and Gorham B K, Antimicrobial effect of plant extracts on *Streptococcus mutans, Candida albicans, Trichophyton rubrum* and other microorganisms. *Letters in Applied Microbiology,*14: 136-139, (1992).
24. Buwa L V and Vanstaden J, Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. *Journal of Ethnopharmacology.* 103: 139-142,(2006).
25. Irobi O N and Daramalo S O, Antifungal activities of crude extract of *Mitracarpus villosus. Journal of Ethnopharmacology,* 40: 137-140, (1993).
26. Portillo A, Vila R, Freixa B, Adzet T and Canigueral S, Antifungal activity of paraguayan plants used in traditional medicine. *Journal of Ethnopharmacology.* 76: 93-98, (2001).

Int J Pharm Bio Sci 2013 July; 4(3): (P) 416 - 422