JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2008. Vol. **2**(1), p. 161-164

Antimicrobial Activity of Leaf Extracts of *Pterocarpus santalinus* L. (Fabaceae)

K. Balaraju[1], S. Arokiyaraj[1], P. Agastian[1]*, K. Perinbam[1] and S. Ignacimuthu[2]

[1]Department of Plant Biology and Biotechnology, School of Life Sciences, Loyola College, Chennai - 34, India.
[2]Entomology Research Institute, Loyola College, Chennai - 600 034, India.

(Received: 30 December 2007; accepted: 21 January 2008)

The present study was carried out to evaluate the antimicrobial activity of leaf extract from *P. santalinus*. Hexane, ethyl acetate and methanol extracts were obtained by cold percolation method. Result indicated that *P. santalinus* exhibited significant antimicrobial activity at all the dosage tested (1.25 mg/disc, 2.5 mg/disc and 5 mg/disc). Ethyl acetate and methanol extracts were found to be active towards drug resistant strains. This study showed that leaf extract of *P. santalinus* can be a potential source of new antimicrobial agents for the tested drug resistant bacterial strains and fungi.

Key words: Multidrug resistant, medicinal plants, secondary metabolites.

Medicinal plants, which form the backbone of traditional medicine, have in the last few decades been the subject for very intense pharmacological studies; this has been brought about by the acknowledgment of the value of medicinal plants as potential sources of new compounds of therapeutic value and as sources of lead compounds in the drug development. World Health Organization (WHO) noted that 90% of the world's population depends on traditional medicine for primary health care. There arises a need therefore to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies. In recent years, multiple drug/chemical resistance in both human and plant pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation has forced scientists to search for new antimicrobial substances in various sources like medicinal plants[1]. *Pterocarpus santalinus* L.f., (Fabaceae) commonly called red sanders is restricted to part of Andra Pradesh and neighboring areas of Chennai and Mysore state of India. The paste of the wood has been used as a cooling external application for inflammations and headache, as antipyretic, anthelmintic, aphrodisiac, alexeteric and in biliousness, mental aberrations and ulcers. The wood and bark of the

* To whom all correspondence should be addressed.
Tel.: +91 9840687163, Fax: +91 44 28174644
E-mail: agastian@loyolacollege.edu, k_balaraju1980@yahoo.co.in

other species of the *Pterocarpus* are well known for their antidiabetic activity[8]. Previous phytochemical studies with *P. santalinus* revealed isoflavonoids[2], terpenoids and related phenolic compounds[3], β-sitosterol[4], lupeol[5], epicatechin[6], and aurone glycosides[7]. *P. osun* has been reported to have antimicrobial activity[9]. In the present study we have studied the antimicrobial activity of *P. santalinus in vitro.*

MATERIAL AND METHODS

Collection of plant material

Healthy, disease free mature leaves of *P. santalinus* were collected from Chittor, Andhra Pradesh, India during February 2007 and authenticated at the herbarium, and voucher specimen was deposited at the department of Plant Biology and Biotechnology, Loyola College, Chennai. Collected material was washed thoroughly, shade dried in open air and grounded into powder. The powder was extracted by maceration in hexane for 72 hr. Residuals were further extracted with ethyl acetate and methanol, following the same procedure. The plant extracts were concentrated using rotary flash evaporator (Buchi, Flawil, Switzerland) and preserved at 5°C in air tight bottle until assay.

Preparation of test organisms

Three strains of gram-positive bacteria *Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (MTCC 441), Methiciline Resistant *Staphylococcus aureus* (MRSA) and five strains of gram-negative bacteria *Vibro cholera* (ATCC 3241), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 10536), *Shigella dysenteriae* (ATCC 13313), *Salmonella typhi* (ATCC 43579) were used for antibacterial activity. Fungal organisms such as *Candida albicans* (MTCC 227) and *Aspergillus flavus* (ATCC 9643) were also employed. All strains were obtained from department of Microbiology, Christian Medical College, Vellore, India. All bacterial and fungal stocks were maintained in MHA slants;

Table 1. Antimicrobial activity of the extracts of *P. santalinus* (Leaf)

Fractions	Mg/ disc	Diameter of the inhibition zone (mm)									
		VC	EC	SD	ST	PA	BS	SE	MRSA	CA	AN
I	1.25	-	-	-	-	7	7	-	-	-	-
	2.5	10	10	8	-	11	10	-	-	-	8
	5	12	13	9	10	13	14	8	13	9	10
II	1.25	8	7	-	-	-	8	8	8	-	8
	2.5	11	10	9	10	8	10	10	12	10	9
	5	13	13	13	12	10	12	13	15	12	11
III	1.25	-	-	-	-	7	-	10	8	-	-
	2.5	-	8	9	-	10	8	9	10	8	10
	5	9	10	12	10	13	13	11	12	11	12
O	5 µg/disc	15	15	15	12	17	13	15	-	ND	ND
M	40 µg/disc	ND	ND	ND	ND	ND	ND	ND	ND	15	17

I- Hexane, II- Ethyl acetate, III- Methanol, VC- V.*cholerae*, EC - *E. coli*, SD- *S. dysenteriae*, ST- *Salmonella typhi*, PA- *P. aeruginosa*, BS – *B. subtilis*, SE – *S. epidermidis*, MRSA- Methiciline Resistant *S. aureus*, CA- *Candida albicans*, AN – *A. niger,* ND – Not done, O – Ofloxacin (5 µg/disc), M – Miconzole (40 µg/disc). - no activity.
The results are the mean values of triplicate tests repeated three times after 24 h of incubation at 37^{0}C

stored at 4°C. Fungus was maintained on Sabouraud-dextrose agar.

Antimicrobial activity test

Antimicrobial activity was tested using a minimum modification of the disc diffusion method originally described by Bauer[10]. Plant extracts were dissolved in 20 % DMSO in water. The inoculums for each microorganism were prepared from broth cultures (10^5 CFU/ml). A loop of culture from the MHA slant stock was cultured in Muller Hinton broth overnight and spread with a sterile cotton swab into petri plates containing 15 ml MHA. Sterile Hi-media disc (6 mm in diameter) impregnated with the plant extracts (1.25 mg/ml, 2.5 mg/ml, 5 mg/ml) were placed on the cultured plates and incubated for 24 hours at 37^0C. The solvent without extracts served as negative control. Standard antibiotics of Ofloxacin (5 µg/disc) and Miconazole nitrate (40 µg/disc) were used as positive controls. The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicated the presence of antimicrobial activity. All data on antimicrobial activity are the average of triplicate analyses.

RESULTS AND DISCUSSION

Most of the bacteria and fungi species were inhibited by the leaf extract of *P. santalinus* and the results are shown in table 1. Ethyl acetate and methanol extracts were sensitive to majority of bacteria and fungi tested. Both extracts exhibited concentration dependent activity. Hexane moderately inhibited the growth of bacteria and fungi at 2.5 and 5 mg/disc. All fractions showed a promising activity towards *B. subtilis, P. aeruginosa* and MRSA. At 1.25 mg all fractions showed less activity. Similarly methanol extract of *P. santalinus* (stem bark) showed maximum activity against *Pseudomonas aeruginosa, Proteus vulgaris, Bacillus cereus, Bacillus subtilis* and *Staphylococcus aureus*[11]. Also the ethanol extracts of *P. santalinus* showed antiulcer and antioxidant properties in rats[12]. In the present study antibacterial activity of *P. santalinus* (leaf) towards drug resistant microbes are reported for the first time and their active constituents are leached out in ethyl acetate and methanol solvents. Further phytochemical studies for identification and elucidation of active constituents in this plant may provide useful lead to the development of new and effective antimicrobial compounds.

ACKNOWLEDGMENTS

The authors are grateful to University Grants Commissions, New Delhi, India, for providing financial assistance (Sanction No. F.31-238/2005(SR) dated 05.04.2006).

REFERENCES

1. Sambath Kumar, R., Sivakumar, T., Shanmuga Sundaram, R., SivaKumar, P., Nethaji, R., Gupta, M., Kanti, U. Antimicrobial and antioxidant activities of *Caerya arborea* Roxb. Stem Bark. *Iranian Journal of Pharmacology & Therapeutics.*, 2006; **5**: 35-41.
2. Mitra, J., Joshi, T. Isoflavonoids from the heartwood of *Pterocarpus marsupium*. *Phytochemistry.,* 1983; **22**: 2326-2327.
3. Kumar, N., Seshadri, T.R. A new triterpene from *Pterocarpus santalinus* bark. *Phytochemistry.,* 1976; **9**: 1877-1878.
4. Ivorra, M.D., Paya, M., Villar, A. A review of natural products and plants as potential antidiabetic drugs. *Journal of Ethnopharmacology.,* 1989; **27**: 243-275.
5. Harborne, J.B., Baxter, H. (Eds). Phytochemical dictionary: A Handbook of Bioactive Compounds from Plants. Taylor and Amp Frost, London, 1983; 791
6. Chakravarthy, B.K., Gode, K.D. Isolation of (-) epicatechin form *Pterocarpus marsupium* and its pharmacological action. *Planta Medica.,* 1985; **1**: 56-59.
7. Moham, P., Joshi, T. Two anthochlor pigments

from heartwood of *Pterocarpus marsupium*. *Phytochemistry.*, 1989; **28:** 2529-2530.

8. Kameswara Rao, B., Giri, R., Kesavulu, M.M., Apparao, Ch. Effect of oral administration of bark extracts of *Pterocarpus santalinus* L. on blood glucose level in experimental animals. *Journal of Ethnopharmacology.,* 2001; **74**: 69-74.
9. Ebi, G.C., and Ofoefule, S.I. Antimicrobial activity of *Pterocarpus osun* Stems. *Fitoterapia.,* 2000; **71**:433-435.
10. Bauer, Kirby, Sherris and Turck. *Am. J. Clin. Path.,* 1966; **45**: 493.
11. Manjunatha, B.K. Antibacterial activity of *Pterocarpus santalinus*. *Indian J. Pharm. Sci.,* 2006; **68**(1): 115-116.
12. Taiwo, O., Xu, Hx, Lee, S.F. Antibacterial activities of extracts from Nigerian chewing sticks. *Phytother Res.,* 1999; **13**(8): 675-9.