Ethnobotanical Leaflets 12: 1029-34. 2008.

Phytochemical Screening and Antibacterial Activity of Leaf Extracts of *Pterocarpus marsupium* Roxb. (Fabaceae)

S. Ramya[1], M. Kalayansundaram[1], T. Kalaivani[2] and R. Jayakumararaj[3]

[1] Department of Zoology, Raja Duraisingam Government Arts College, Sivagangai, 630561 India
[2] School of Biotechnology, CBME, Vellore Institute of Tech. University, Vellore, 632014 India
[3]Department of Botany, RD Government Arts College, Sivagangai, 630561 India

Issued 17 November 2008

Abstract

The present study was carried out to screen and evaluate the antimicrobial activity of bark and leaf extracts from *Pterocarpus marsupium* Roxb. Hexane, ethyl acetate and methanol extracts were tested against four selected Gram positive and Gram negative bacteria. Result indicated that phytochemical extracts of *P. marsupium* exhibited significant anti-bacterial activity at all dosage tested (1mg/ disc and 5mg/ disc). However, the inhibitory activity was found to be dose dependent. Ethyl acetate and methanol extracts were found to be more active towards the organisms tested than hexane extract. This study depicts that ethyl acetate and methanol extracts of bark and leaves of *P. marsupium* can be used as a potential source of novel antimicrobial agents.

Keywords: *Pterocarpus marsupium;* medicinal plants; antibacterial agents; phytochemicals.

Introduction

Medicinal plants, that form the backbone of traditional system of medicine in India, have in the last few decades been the subject of interest for pharmacological studies. This has been brought about by the acknowledgement of the value of medicinal plants as potential source of novel bioactive compounds of therapeutic prominence (Prusti et al., 2008). Also phytochemicals from the medicinal plants serve as a source of lead compounds in drug discovery and design (Chakravarthy and Gode, 1985; Ebi and Ofoefule, 2000). Medicinal plants are rich source of novel drugs that forms the ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs (Ncube, 2008).

It has been pointed out that more than 80% of world's population depends on plants to meet their primary health care needs (WHO, 2005). In recent years, multiple drug/ chemical resistance in both human and plant pathogenic microorganisms has been developed due to indiscriminate use of synthetic drugs in the treatment of infectious diseases. This drives the need to screen medicinal plants for novel bioactive compounds as a basis for pharmacological studies since the bioactive principles obtained from the plants are biodegradable, safe and have fewer side effects. Therefore, endemic medicinal plants should be investigated to better understand their pharmacological properties and efficacy (Prusti et al., 2008).

According to a survey conducted by WHO (1998), it has been shown that most of the modern prescriptions contain plant derived lead molecules as a base in their drug formulations. Nevertheless, more-than about 42% of 25 top selling drugs marketed world wide are either directly obtained from natural sources or entities derived from plant products (WHO, 2007). The value of plant based prescribed drugs in 2000 was estimated at $35.5 billon which has

been on the raise since then.

Pterocarpus marsupium Roxb. (Fabaceae) popularly known as Indian Kino Tree, Malabar Kino Tree. Kino is locally called as "Vengai" and has restricted distribution to southern part of India. It is commonly found in Western Ghats – Tamilnadu, Karnataka and Kerala. The Indian Kino is a medium to large; deciduous tree and can grow up to 30 m (Gamble, 1935; Mathew, 1983). The count of this tree species is declining in the wild and therefore it has been placed in the red data book. It is exploited for its timber and its medicinal bark and latex.

Traditionally, the plant material has been used as a cooling external application for inflammations and headache, as antipyretic, anti-helminthic, aphrodisiac, alexeteic and in biliousness, mental aberrations and ulcers (Sambathkumar et al., 2006). Parts of the Indian Kino (heart wood, leaves and flowers) have long been used for their medicinal properties in Ayurveda. The heart wood is used as an astringent and in the treatment of inflammation. The wood and bark of *Pterocarpus* are known for their anti-diabetic activity (Ivorra et al., 1989; Kameswara et al., 2001). Phytochemical studies on *P. marsupium* have shown that the plant contains iso-flavonoids, terpenoids and related phenolic compounds, ß-sitosterol, lupenol, epicatechin, and aurone glycosides (Kumar and Seshadri, 1976; Mitra and Joshi, 1983). In the present study we have evaluated the antimicrobial potential of *P. marsupium in vitro*.

Materials and Methods

Collection of Plant Material

Bark and mature leaves of *P. marsupium* were collected from Sivagangai, Tamilnadu, India during July 2008. The Flora of Presidency of Madras (Gamble, 1935) and The Flora of Tamil Nadu Carnatic (Matthew, 1983) were used for identification and authentication of the plants. Collected material was washed thoroughly in running tap water, rinsed in distilled water and shade dried in open air and grounded into powder.

Preparation of Phytochemical Extracts

The powder was extracted by maceration in hexane for 72h. Residuals were further extracted with ethyl acetate and methanol, following the same procedure. The plant extracts were concentrated using rotary flash evaporator (Buchi, Switzerland) and stored at 4^{o} until assay.

Test organisms

Four strains of Gram-positive bacteria - *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Streptococcus pyrogens* and four strains of Gram negative bacteria - *Escherichia coli, Salmonella typii, Serratia marcescens* and *Pseudomonas aeruginosa* were used for antibacterial activity (Table 1). All strains were gift from Prof. Rajsekaran, (School of Biotechnology, Chemical and Biomedical Engineering VIT University, Vellore, India). All bacterial cultures were maintained in NA slants/ plates; stored at 4^{o} and periodically sub-cultured.

Phytochemical screening

Phytochemcial analysis of the extract was carried out as described by Harborne (1998). By this analysis, the presence of several phytochemicals like Sugar, Protein, Alkaloids, Flavonoids, Saponins, Tannins, Cardiac glycoside, Terpenoids and Lipids were tested (Table 2).

Antimicrobial Activity Test

Antimicrobial activity was tested using a modified disc diffusion method originally described by Ncube et al (2008). Plant extracts were dissolved in 20% DMSO treated water. The inoculums for each microorganism were prepared from broth cultures (10^5 CFU/ml). A loop of culture from the NA slant stock was cultured in LB medium overnight and spread with a sterile swab into Petri-plates. Sterile disc (6 mm dia, Hi-media, Mumbai, India) impregnated with the plant extracts (1 mg/ml, and 5 mg/ml) were placed on the cultured plates and incubated for 24

h at 37^{o}. The solvent without extracts served as control. The results were recorded by measuring the zones of growth inhibition. Clear inhibition zones around discs indicated the presence of antimicrobial activity. All data on antimicrobial activity were average of triplicate.

Results and Discussion

Plants are known to have beneficial therapeutic effects documented in Traditional Indian System of Medicine. Much work has been done on ethnomedicinal plants in India. Interest in a large number of traditional natural products has increased. It has been suggested that phytochemical extracts from plants holds promise to be used in allopathic medicine as they are potential sources of antiviral, antitumoral and antimicrobial agents (Nair et al., 2005). The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world.

In the present study most of the bacteria strains tested were inhibited by the bark and leaf extracts of *P. marsupium* and results are given in Table 3-5, the solvent without extracts served as control were no inhibition zone was observed. Ethyl and methanol extracts were more sensitive to the bacteria than extracts made out of hexane. Both the extracts exhibited concentration dependent variation in their anti bacterial activity. Hexane moderately inhibited growth of the bacterial strains tested. All fractions showed a promising activity towards Gram negative bacteria however no inhibition was observed in hexane fractions of *P. marsupium* towards *Bacillus* species. At a concentration of 1mg/ml all fractions showed less activity. Similarly methanol extract of *P. marsupium* (bark) showed maximum activity against *Pseudomonas aeruginosa, Streptococcus pyrogens* and *Staphylococcus aureus*. Similar observations have been reported by (Nair et al., 2005; Sambathkumar et al., 2006) where it has been shown that ethanol extracts of *P. marsupium* exhibited significant anti-ulcer and antioxidant properties in rats.

Conclusion

In the present study antibacterial activity of *P. marsupium* extracts towards drug resistant/ clinically significant microbes are reported and it was observed that the active constituents in the plant material seep-out in organic solvents. Further phytochemical studies for identification and elucidation of active constituents in the plant materials tested is expected to serve as lead in the development of novel bioactive antimicrobial compounds.

References

1. Chakravarthy BK and Gode KD (1985) Isolation of epicatechin from *Pterocarpus marsupium* and its pharmacological action. *Planta Medica* 1:56-9.
2. Ebi GC and Ofoefule SI (2000) Antimicrobial activity of *Pterocarpus osun* stems. *Fitoterapia* 71:433-435.
3. Gamble JS (1935) Flora of the Presidency of Madras. Adlard and Son's Ltd, London, UK.
4. Harborne JB (1998) Phytochemical methods: A guide to modern techniques of plant analysis. 3rd Edition Chapman & Hall Pub., London, UK.
5. Ivorra MD, Paya M and Villar A (1989) A review of natural products and plants as potential antidiabetic drugs *J. Ethnopharmacology* 27:243-275.
6. Kameswara RB, Giri R, Kesavulu MM and Apparao CH (2001) Effect of oral administration of bark extracts of *Pterocarpus santalinus* L. on blood glucose level in experimental animals. *Journal of Ethnopharmacology* 74:69-74.
7. Kumar N and Seshadri TR (1976) A new triterpene from *Pterocarpus santalinus* bark. *Phytochemistry* 9:1877-1878.

8. Manjunatha BK (2006) Antibacterial activity of *Pterocarpus santalinus Indian Journal of Pharmacology Science* 68(1):115-116.
9. Matthew KM (1983) The Flora of Tamil Nadu Carnatic. In The Rapinat Herbarium. St Joseph's College, Tiruchirapalli, India
10. Mitra J and Joshi T (1983) Isoflavonoids from the heart wood of *Pterocarpus marsupiam*. *Phytochemistry* 22:2326-2327.
11. Sambathkumar R, Sivakumar T, Shanmugasundaram R, Sivakumar P, Nethaji R, Gupta M and Kanti U (2006) Antimicrobial and antioxidant activities of *Caerya arborea* Roxb. Stem and bark. *Iranian Journal of Pharmacology and Therapeutics* 5:35-41.
12. Subramanian SV and Madhavan VR (1983) Heritage of the Siddha Medicine. International Institute of Tamil Studies, Chennai, India.
13. Nair R, Kalariya T and Chanda S (2005) Antibacterial Activity of Some Selected Indian Medicinal Flora. *Turk J Biol* 29:41-47.
14. Ncube NS, Afolayan AJ and Okoh A (2008) Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African Journal of Biotechnology* 7(12):1797-1806.
15. Prusti A, Mishra SR, Sahoo S and Mishra SK (2008) Antibacterial Activity of Some Indian Medicinal Plants. *Ethnobotanical Leaflets* 12: 227-230.

Table 1. Phytochemical profile of *P. marsupium* extracts.

S No.	Phytochemicals	Presence/Absence	
		Bark	leaf
1	Sugar	+	+
2	Protein	+	+
3	Alkaloids	+	+
4	Flavonoids	+	+
5	Saponins	+	+
6	Tannins	+	+
7	Glycoside	-	-
8	Terpenoids	+	+
9	Lipids	-	-

Table 2. Bacterial strains used in the present study.

S. No	Bacterial strain	Gram (+/-)
1	*Escherichia coli*	-
2	*Pseudomonas aeruginosa*	-
3	*Serratia marcescens*	-
4	*Salmonella typii*	-
5	*Staphylococcus aureus*	+
6	*Streptococcus pyrogens*	+
7	*Bacillus cereus*	+
8	*Bacillus subtilis*	+

(+ = Gram positive; - = Gram negative)

Table 3. Anti-microbial activity of *P. marsupium* hexane extracts.

Organism	Plant Part	Bark		Leaf	
	Dosage	1 (mg/ disc)	5 (mg/ disc)	1 (mg/ disc)	5 (mg/ disc)
E. coli		+	++	+	++
P. aeruginosa		+	++	+	++
S. marcescens		+	++	+	++
S. typii		+	++	+	++
S. aureus		+	++	+	++
S. pyogens		+	++	+	++
B. cereus		-	+	-	+
B. subtilis		-	+	-	+

(Growth analysis: +++ = abundant; ++ = normal; + = less; - = no)

Table 4. Anti-microbial activity of *P. marsupium* ethyl acetate extracts.

Organism	Plant Part	Bark		Leaf	
	Dosage	1 (mg/ disc)	5 (mg/ disc)	1 (mg/ disc)	5 (mg/ disc)
E. coli		++	+++	++	+++
P. aeruginosa		++	+++	++	+++
S. marcescens		++	+++	++	+++
S. typii		++	+++	++	+++
S. aureus		++	+++	++	+++
S. pyogens		++	+++	++	+++
B. cereus		-	++	-	++
B. subtilis		+	++	+	++

(Growth analysis: +++ = abundant; ++ = normal; + = less; - = no)

Table 5. Anti-microbial activity of *P. marsupium* methanol extracts.

Organism	Plant Part	Bark		Leaf	
	Dosage	1 (mg/ disc)	5 (mg/ disc)	1 (mg/ disc)	5 (mg/ disc)
E. coli		+	+++	+	+++
P. aeruginosa		++	++++	++	++++
S. marcescens		+	+++	+	+++
S. typii		+	+++	+	+++
S. aureus		++	++++	++	++++
S. pyogens		++	++++	++	++++
B. cereus		-	++	-	++
B. subtilis		+	+++	+	++

(Growth analysis: +++ = abundant; ++ = normal; + = less; - = no)