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Antimicrobial Activity of Different Bark and Wood of *Premna serratifolia* Lin.,

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ABSTRACT

Premna serratifolia Lin., is an important medicinal herb (synonym- *Premna integrifolia*) known as "Munney" in Tamil, "Agnimantha" in Ayurveda and traditionally used for it's as cardio tonic, antibiotic and antihyperglycemic properties. Plant has shown anticoagulant activity and the decoction of leaves exhibited anti-inflammatory and antiarthritic activity. However, its antimicrobial activity has not been investigated still now. Hence it was considered to evaluate the antimicrobial activity. The antimicrobial activity of different extracts of bark and wood of *P.serratifolia* Lin., was studied against nine bacterial and four fungal organisms by disc diffusion method. Ethylacetate, ethanol and aqueous extracts exhibited significant antibacterial activity, n-hexane and chloroform extracts exhibited moderate antibacterial activity and these five extracts exhibited significant antifungal activity. Results revealed that all the extracts possess a significant broad spectrum of antimicrobial activity when compared to Ciprofloxacin and Amphotericin-B may be due to the presence of phytoconstituents like alkaloids, irridoid glycosides and flavonoids present in it.

KEYWORDS

Premna serratifolia Lin., antimicrobial activity, disc diffusion method, Ciprofloxacin and Amphotericin-B.

INTRODUCTION

The World Health Organization estimates that some 80% of the people in developing world rely on traditional medicines and that, of these 85% use plants or their products as the remedies. India is endowed with an estimated 47,000 species of plant that include around 8000 plants which are known to have medicinal properties. Ethno botany has emerged as an important branch of study documenting the age old wisdom and knowledge of the ethnic's societies about the utility of diverse plant species and their medicinal properties. *Premna serratifolia* Lin., is one such a plant species (Verbenaceae), popularly known as Munnai in Tamil and Agnimantha in Ayurveda, is a large shrub 9m in height[1], with a comparatively short trunk and numerous branches and traditionally used as cardio tonic, antibiotic, anticoagulant, stomachic, carminative etc. It is widespread throughout Micronesia and much of the tropical Pacific and tropical Asia. It is common along the Indian and Andaman coasts. Infusion of the leaves is administered with pepper in cold and fever. Leaves are used to cure "weakness of limbs" and the leaves

and leaf sap were used to alleviate headache [2]. Root and wood forms an ingredient of "Dasamula" an Ayurvedic formulation which is used in a variety of affections. Root rubbed into a paste with water is recommended to be taken with clarified butter in urticaria and roseola for a week [3]. From the leaves, a Verbascoside iridoid glycoside conjugate was isolated along with Verbascoside [4] and Premnafolioside, a new Phenylethanoid, and other Phenolic compounds were isolated from stem [5].

Anti-inflammatory and anti-arthritic activity [6] against acute, sub-acute and chronic inflammation induced in both immunological and non-immunological experimental models, anticoagulant activity [7]and anti-hyperglycaemic activity [8] have also been reported, but its antimicrobial activity has not been studied still now. In the present study, the antimicrobial activity of bark and wood of different extracts of *Premna serratifolia* Lin., against different test bacteria and fungal cultures have been evaluated.

MATERIALS AND METHODS

Plant Material and Authentication:

Fresh bark and wood of *Premna serratifolia* Lin., were collected from, The Indian Medical Practitioners Co-operative Pharmacy and Stores (IMPCOPS), Chennai, TamilNadu. The plant was authenticated [9] by Dr. P. Jayaraman, Plant Anatomical Research Centre, Chennai and the voucher specimen (Herbarium No. PARC/2007/80) have been kept in the Department of Pharmacognosy, Madras Medical College, Chennai, for future reference.

Preparation of Extracts: [10]

Freshly collected plant materials were cut into small pieces, shade dried until a constant weight was obtained and coarsely powdered. The air-dried powdered material was extracted successively with n-hexane, chloroform, ethylacetate and finally with ethanol in a Soxhlet extractor. Each time before extracting with the next solvent, the material was dried in hot air-oven below 50˚C. Finally, the marc was macerated with double distilled water for 24 hrs to obtain the aqueous extract. Each extract was concentrated, evaporated to dryness, until semi-solid masses were obtained and then calculated and also the consistency of the extracts were noted and showed in Table – 1 and dissolved in 1% dimethyl sulphoxide (DMSO).

Table - 1

Successive Solvent Extraction of Air-Dried Plant Material of Bark & Wood of Premna serratifolia

S. No.	Solvent	Colour and consistency	Extractive value (% w/w)
1.	n-Hexane	Yellowish brown waxy	1.366
2.	Chloroform	Brownish waxy	2.75
3.	Ethylacetate	Dark brown waxy	3.25

4.	Ethanol	Reddish brown waxy	7.90
5.	Aqueous	Reddish brown powder	7.73

Phytochemical Screening

The extracts were then subjected to preliminary phytochemical screening [11] for the detection of various plant constituents. It gave positive tests for various phytoconstituents like alkaloids, steroids, flavonoids, phenolic compounds and glycosides specifically iridoid glycosides (shown in Table – 2). Fluorescence of bark and wood of *Premna serratifolia* Lin., was observed in day-light and visualized under ultra violet radiations using the successive solvent extracts. The powder was treated with acids like 1N HCl, 50% H_2SO_4, 50% HNO_3 and alkaline solutions like 1N NaOH and fluorescence observed were tabulated [12 & 13] and it is shown in Table 3 and 4. Fluorescence analysis shows the presence of fluorescent compounds in all the extracts and in the drug powder, which indicates the presence of chromophore in this plant. TLC profiles for different active constituents were studied with the extracts and the Rf values were shown Table 5.

Table - 2

Preliminary Phytochemical Test for Different Extracts of Bark & Wood of Premna serratifolia Obtained by Successive Solvent Extraction)

S.No.	Test	n-Hexane	Chloroform	Ethylacetate	Ethanol	Aqueous
1.	Alkaloids	-	-	+	+	+
2.	Glycosides	-	-	-	+	+
3.	Terpenoids	-	-	-	-	-
4.	Carbohydrates	-	-	-	-	-
5.	Proteins	-	-	-	-	-
6.	Steroids	+	+	-	+	-
7.	Flavonoids	-	-	-	+	+
8.	Phenols	-	-	-	+	+

9.	Tannins	-	-	-	-	-
10.	Quinones	-	-	-	-	-
11.	Saponins	-	-	-	-	+
12.	Resin	-	-	-	-	-
13	Fixed oils & Fats	-	-	-	-	-
14.	Volatile oils	-	-	-	-	-

Note: + ve indicates positive result, whereas – ve indicates negative result.

Table - 3

Fluorescence Analysis Of Various Extracts Of Bark & Wood Of Premna serratifolia

S.No	Extracts	Day Light	UV Light	
			Short (254 nm)	**Long (365 nm)**
1.	**n-Hexane**	Yellow	Green Fluorescence	Brown
2.	**Chloroform**	Dark Yellow	Reddish Brown	Brown
3.	**Ethyl Acetate**	Brownish Yellow	Brown Fluorescence	Dark Brown
4.	**Ethanol**	Reddish Brown	Yellow Fluorescence	Brown
5.	**Aqueous**	Brown	Dark Brown	Brown Fluorescence

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Table - 4

Fluorescence Analysis Of Drug Powder Of Bark & Wood Of Premna serratifolia

S.No.	Treatment	Day Light	UV Light	
			Short (254 nm)	Long (365 nm)
1.	**Powder**	Pale brown	Dark brown	Pale brown
2.	**Powder + Water**	Brown	Yellow	Yellow
3.	**Powder + 1N HCl**	Yellowish brown	Pale brown	Brown
4.	**Powder + 1N HNO_3**	Pale yellow	Yellow	Pale brown
5.	**Powder + 1N H_2SO_4**	Reddish brown	Brown fluorescence	Yellow fluorescence
6.	**Powder + 1N NaOH**	Yellowish brown	Dark yellow	Pale yellow
7.	**Powder + Alc.NaOH**	Pale yellow	Brown	Yellow
8.	**Powder + 1N KOH**	Yellow	Yellow	Dark brown
9.	**Powder + Alc. KOH**	Brown	Brown	Brown
10.	**Powder + Ammonia**	Pale yellow	Yellow	Brown

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Table - 5

TLC Profile for Different Extracts of Bark & Wood of Premna serratifolia

Active Constituents	Solvent System	Detecting Reagent	Rf value of the Extracts		
			Ethyl acetate	Ethanol	Aqueous
Alkaloids	Methanol: ammonium hydroxide 200:3	Dragendorff's reagent	0.489	0.542	0.380
			0.39	0.416	0.31
			0.28	0.37	0.25
			-	0.33	-
Flavonoids	Chloroform : Ethylacetate 60:40	UV	-	0.48	0.58
			-	0.420	0.470
			-	0.352	0.332
Glycosides	Chloroform : Methanol : Water 35:25:10	UV	-	0.58	0.63
			-	0.48	0.51
Steroids	Hexane : Ethylacetate 1:1	UV	-	0.38	-

Micro-Organisms Used:

Micro-organisms used includes, Gram positive bacteria like *Coagulase Negative Staphylococcus and Staphylococcus aureus*. Gram negative bacteria like *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B and Vibrio cholerae*. Fungal organisms like *Aspergillus flavus, Aspergillus niger, Penicillium notatum and Candida albicans*. Suitable strains of these organisms were procured from the Institute of Microbiology, Madras Medical College, Chennai - 600 003. The purity of the cultures prior to their use was checked by conventional cultural, morphological and biochemical methods. The bacterial and fungal cultures were maintained and stored in nutrient and Sabouraud's agar medium at 4˚C.

Antimicrobial Activity [14]

The in-vitro antibacterial and antifungal activity of the five extracts at different concentrations were studied by disc diffusion method against various bacterial and fungal organisms. The bacterial and fungal cultures were grown in nutrient agar and Sabouraud's dextrose agar respectively by transferring 25 ml of the media into a pre-sterilized petridish and allowed to solidify at room temperature for 1 hr. 100 µl suspension of different bacterial and fungal cultures were spread into nutrient agar and Sabouraud's dextrose agar. The sterile filter paper disc (6 mm) impregnated with 200 µg/ml of different extracts were placed into

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agar surface at equidistance and petridishes with bacterial and fungal cultures were incubated at 37°C and 27°C for 24 hrs and 72 hrs. The paper disc of standard antibiotic Ciprofloxacin (5 µg/disc) and antifungal, Amphotericin-B (100 µg/disc) were also incorporated in the petridishes. The zone of inhibition zone was measured (shown in Table – 6) and the activity of the different extracts were compared with the standard antibacterial Ciprofloxacin and antifungal, Amphotericin-B agents. An average of three independent determinations was recorded.

Table – 6

Antimicrobial activity different extracts of bark &wood of Premna serratifolia

Name of the organisms	Diameter of zone of inhibition of different extracts in mm						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Bacteria *Staphylococcus aureus*	13	15	19	20	21	23	-
CONS	10	09	13	14	15	15	-
Escherichia coli	12	13	20	23	22	30	-
Klebsiella pneumonia	13	15	16	15	16	18	-
Salmonella typhi	14	15	15	16	17	19	-
Salmonella paratyphi A	10	10	15	17	17	18	-
Salmonella paratyphi B	13	11	16	18	18	19	-
Pseudomonas aeruginosa	11	11	10	12	11	13	-
Vibrio cholerae	12	11	15	14	14	15	-
Fungus *Aspergillus flavus*	09	10	09	10	12	-	14
Aspergillus niger	10	11	10	11	12	-	13
Penicillium notatum	12	12	11	12	11	-	13
Candida albicans	09	10	15	14	15	-	15

1. n-Hexane; 2.Chloroform; 3.Ethylacetate; 4.Ethanol; 5.Aqueous; 6.Ciprofloxacin; 7.Amphotericin-B. (Zone of inhibition is the average of triplicate experiments).

RESULTS AND DISCUSSION

The phytochemical studies when brought to little existence, revealed the presence of phytoconstituents like alkaloids, steroids, flavonoids, phenolic compounds and glycosides specifically iridoid glycosides. In addition, fluorescence analysis, TLC data may help to standardize the active constituents present in it.

The anti-microbial activities of extracts of bark and wood of *Premna serratifolia* Lin., against various micro-organisms have been studied. The results of the antibacterial activity revealed that the control (DMSO) showed no inhibition of growth while the ethylacetate, ethanol and aqueous extracts exhibited significant zone of inhibition of bacterial growth and confirmed the significant antibacterial activity and the n-Hexane, Chloroform extracts produced exhibited moderate antibacterial activity against all the tested bacterial microorganisms at the concentration of 200 µg/ml, when compared with the standard drug Ciprofloxacin (5 µg/ml).

The results of the antifungal activity revealed that the n-hexane, ethylacetate, chloroform, ethanol and aqueous extracts exhibited significant inhibition of growth against the human pathogenic fungi at the concentration of 200 µg/ml, when compared to the standard drug Amphotericin-B (100 µg/ml) and confirmed the significant antifungal activity. *Premna serratifolia* Lin., have been used in the treatment of urticaria, gonorrhea and roseola. Presence of active constituents like alkaloids, glycosides, steroids, phenolic compounds and flavonoids in the extracts as reported earlier are likely to be responsible for the observed significant antimicrobial activity of bark and wood of *Premna serratifolia* Lin., From the above studies, it was concluded that all the four extracts of bark and wood of *Premna serratifolia* Lin., were shown to possess a **significant broad spectrum of anti-microbial activity** against the tested bacterial and fungal organisms. Our findings confirm that the traditional therapeutic claims for this plant, in near future surely be able to replace the conventional anti-microbial agents to which there is increased incidence of drug interactions. Future, study of *in-vivo anti-microbial activity* and experiments involving activity guided fractionation are under way and the study is also aimed at extensive investigation, isolation and purification of active phytoconstituents with broad spectrum of anti-microbial activity.

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REFERENCES

1. Anonymous. The Wealth of India, Dictionary of Indian raw materials and industrial products, vol. 8, New Delhi: Council of Scientific and Industrial Research; 1972. p. 240.
2. Kapoor LD. CRC Handbook of Ayurvedic Medicinal Plants, Herbal Reference Library, CRC Press: Boca Raton London, New York Washington; 2001. p. 70.
3. Natkarni KM, Indian Materia Medica, with Ayurvedic, Unani and Home Remedies, Vol. 1 3rd ed. Popular Prakashan, Bombay, 1976, p.1009-10.
4. Otsuka H, Watanabe E, Yusana K, Ogimi C, Takushi A, Takeda Y. A verbascoside iridoid glucoside conjugate form *Premna corymbosa* Rott. Phytochem. 1993, Mar, Vol. 32 (4) :983-986.

5. Yuasa K, Ide T, Otsuka H, Takeda. Chemical examination of stems of *Premna corymbosa* var. obtusifolia. Jr. Nat. Prod. 1993, Oct, Vol. 56 (10): 1695-1699.
6. Rathore RS, Prakash A, Singh PP. *Premna integrefolia* Lin., A preliminary study of anti-inflammatory activity. Rheum. 1977; 12:130-4.
7. Hamsaveni Gopal R, Purushothaman KK. Effect of plant isolates on coagulation of blood: an in-vitro study. Bull. Med. Ethnobot. Res. 1984, Vol. 5 (3-4), 171-7.
8. Dash GK, Patrolm CP, Maiti AK. A study on the anti-hyperglycaemic effect of roots of *Premna corymbosa* Rottl. Jr Nat Rem. 2005; 5:31-34.
9. Henry AN, Kumari GR, Chitra V. Flora of Tamil Nadu, volume 2, Botanical survey of India southern circle: Coimbatore;1987. pp.167.
10. Kokate CK. Practical Pharmacognosy, 4th ed. Vallabh Prakashan, 1993, p.113-107.
11. Williams Charles Evan WB. Pharmacognosy, 14th ed. Saunders Company Ltd.1996, p.229-493.
12. Chase CR, Pratt RS. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. Jr. A. Pharm Asses. 1949; 38: 384-3314.
13. Kokoshi CJ, Kokoshi RJ, Sharma FT. Fluorescence of powdered vegetable drug under ultraviolet radiation. Jr. Pharm Assoc. 1949, 47; 715-717.
14. Satish Gupta. The short textbook of Medical Microbiology**,** 8th ed. Jaypee brother, Medical Publishers Ltd. New Delhi, 2002, p.159, 350-358.