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Anti bacterial and antifungal activity of various leaves extracts of *Hardwickia binata roxb.* (Caesalpinaceae)

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Abstract : The antimicrobial activity of petroleum ether, chloroform and ethanolic leaves extracts of *Hardwickia binata* roxb(Caesalpinaceae) were tested against 12 human pathogens such as *Bacillus subtilis, E.coli, P.aeruoginosa, S.typhi, S. aureus, S.pneumonia, P.vulgaris and V. vulnificus* bacterias *and Aspergillus niger, Aspergillus flavus, C. albicans and A.fumigatus* fungus by using agar well diffusion method.Among the three extracts the ethanolic extract of *Hardwickia binata* roxb showed the highest range of activity against all tested human pathogens. *S.pneumonia, A. niger, A. flavus, C. albicans and A.fumigatus* were found to be susceptible forming highest zone of inhibition diameter of 28-31mm at concentration of 100mg/ml, suggesting that *Hardwickia binata roxb* was strongly inhibitory towards these organisms. These results indicate that *Hardwickia binata roxb* (*Caesalpinaceae)* possessed potential Antibacterial and Anti fungal activities

Keywords: Antibacterial, Antifungal, Humanpathogens, Agar well diffusion method.

Introduction :

Hardwickia. binata Roxb., (*Caesalpinaceae*) is commonly known as anjan synonym is *Hardwickia trapeziformis* R.Grah. *Hardwickia binata* is a moderate-sized to large tree, up to 24-30 m tall, girth 1.8-3 m with a clean cylindrical bole up to 12-15 m; graceful, drooping slender branches; crown conical in early life, becoming broader later. Leaves small, 2-6 cm long by 2-3 cm wide, alternate, pinnate, almost kidney shaped and greyish-green. Flowers small, pale yellowish-green in axillary and terminal lax panicled racemes. The pod flat and samaroid, 5-7.6 x 1-1.5 cm, oblong lanceolate, coriaceous, narrowed at both ends, with parallel longitudinal veins, containing 1 seed near the apex. The seed is exalbuminous, flat, averaging 0.8 x 0.3 in, in sub-reniform, pointed at one end and rounded at the other, with a fairly hard testa.The natives of Chhattisgarh Plains used this leaves for headache. The leaves are collected and by crushing it in water, an aqueous paste is prepared. This paste is applied externally on painful parts as

treatment [(1).]The natives of Bagbahera region used the piece of its wood rubbed on stone, like the wood of Chandan (Santalum indicum) and the paste prepared was applied on small boils common in summer. The natives of Kanker region used leaves for purgative and can be used in treatment of constipation. Balsam—used for sexually transmitted diseases. The balsam is similar to Copaiba balsam (*Copaifera langsdorffii* Desf., *Leguminosae*) of Brasil and is used in leucorrhoea, chronic cystitis, gonorrhoea, combined with Cubebs and sandal. The resin (not the oleo-resin) is used as diuretic. The bark has a good absorption capacity for mercury from water. Seeds are used for dysentry · The methanolic extract of the heartwood yields β-sitosterol, (+)-taxifolin, eriodictyol, (+)-catechin, (+)-epicatechin and (+)-mopanol[(2.]. Medicinal plants are an important

therapeutic aid for various ailments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century [3]. In India, from ancient times, different parts of medicinal plants have been used to cure specific ailments. Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects. Natural antimicrobials can be derived from plants, animal tissues, or microorganisms[4]. The shortcomings of the drugs available today, propel the discovery of new pharmacotherapeutic agents in medicinal plants[5].To determine the potential and promote the use of herbal medicine, it is essential to intensify the study of medicinal plants that find place in folklore[6,7,8].Therefore, the aim of the present study is to evaluate the antimicrobial activity of *Hardwickia binata roxb.,* and also the literature survey reveals that no reports were found on the antimicrobial activity of the leaves extracts of *Hardwickia binata roxb.*

Materials and Method:

Collection of Plant Material

The plant materials (leaves) of *Hardwickia binata* Roxb., (Caesalpiniaceae) were collected from foot of the Arunachala hills, Tiruvannamalai, Tamil Nadu and the collected plant materials were botanically identified and confirmed by A. C. Tangavelou, Director, Bio-Science Research Foundation, Pondicherry. The herbarium specimen was prepared and preserved in Department of Pharmacognosy, MTPG&RIHS, Puducherry.

Preparation of the Extracts

The collected materials (leaves) were chopped into small pieces separately, shade-dried, and coarsely powdered using a pulverizor. The coarse powders were subjected to successive extraction with organic solvents such as petroleum ether, chloroform and ethanol by Soxhlet method. The extracts were collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvents was removed *in vacuo*. The resulted extracts were used for antimicrobial studies.

Bacterial and fungal used:

Pure culture of like *B. subtilis, E.coli, P.aeruoginosa, S.typhi, S. aureus, S.pneumonia,P.vulgaris and V. vulnificus* and fungs like *Aspergillus niger, Aspergillus flavus, C. albicans and A.fumigatus* were obtained from the Bio-Science Research Foundation, Pondicherry.

Preparation of Inoculum

Stock cultures were maintained at 4 ^{0}c on slopes nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth for bacteria and fungi that were incubated without agitation for 24 hours at 37^o C and 25^0 C respectively.

Antimicrobial activity

Nutrient agar for bacterias and potato dextrose agar (HI-Media) for fungus were prepared according to the manufacturer recommendations. The agar well diffusion method was used for the inoculation of the bacterias and fungus. Petri dishes containing 0.5ml 0f sterile nutrient agar each were inoculated with standardized (1.5x 108 cells /ml) sterile Pasteur pipette[10]. Wells of 5mm diameter were made at the centre of each plate and 0.15 ml of the various concentrations (25mg/ml, 50mg/ml and 100mg/ml) of the plant extracts dispensed into each well. The extracts were allowed to diffuse into the medium for 1 hour at room temperature. This was then incubated at 37^{0}C for 24 hours for bacterial strains and at 27^{0}C for 72 hours for the fungi after which the zones of growth inhibition were measured and recorded in mm[9]. The control was set up in a same manner except that the extract replaced with sterile distill water and ciprofloxacin ,gentamycin sulphate and nystatin were used as positive standard. The experiments were conducted in triplicate.

Antibiotic assay

The selected antibiotics are obtained from a chemist. These drugs, in their high concentration, were diluted with sterile water reducing them in to a lower concentration. Wells were bored on the prepared agar with a cork borer and with the use of sterile needle and syringe; the antibiotics were poured in to the well .The zone of inhibition was observed after 24 hours and recorded.

Table:1: Anti bacterial and antifungal activity of Petroleum ether leaves extract of *Hardwickia binata roxb.,*

Microorganisms used	Zone Of Inhibition in mm					
	Test concentration (mg/ml)			Standard concentration (mg/ml)		
	100	50	25	Cip	Gen	Nys
B. subtilis	17	16	21	37	25.5	NT
E. coli	22	18	16	36.5	-	NT
P. aeruginosa	18	15	14	36.5	-	NT
S. typhi	21	20	18	38.5	NT	NT
S. aureus	23	22	18	35.5	-	NT
S. pneumonia	17	16	15	38	30.8	NT
V. vulnificus	24	23	22	37.5	31	35.5
P. vulgaris	27	19	14	36.5	NT	NT
A. niger	16	13	12	38	NT	38.5
A.fumigatus	15	13	12	39	NT	35
A. flavaus	16	14	12	38	NT	30.5
C. albicans	15	14	12	39	NT	36

NT :not tested, "-":not active
Standard drug used –Cip: Ciprofloxacin, Gen: gentamycin sulphate, Nys:nystatin

Table:2: Anti bacterial and antifungal activity of chloroform extract leaves extract of *Hardwickia binata roxb.,*

Microorganisms used	Zone Of Inhibition in mm		
	Test concentration (mg/ml)		
	100	50	17
B. subtilis	19	17	12
E. coli	17	14	12
P. aeruginosa	17	15	13
S. typhi	-	-	--
S. aureus	22	21	12
S. pneumonia	16	13	13
V. vulnificus	18	17	14
P. vulgaris	-	-	-
A. niger	18	17	16
A.fumigatus	17	16	13
A. flavaus	14	13	12
C. albicans	14	12	12

"-":not active

Table:3: Anti bacterial and antifungal activity ethanolic extract leaves extract of *Hardwickia binata roxb.,*

Microorganisms used	Zone Of Inhibition in mm		
	Test concentration (mg/ml)		
	100	50	25
B. subtilis	19	16	13
E. coli	24	23	22
P. aeruginosa	26	22	20
S. typhi	23	21	19
S. aureus	23	21	17.5
S. pneumonia	28	22	20
V. vulnificus	20	16	14
P. vulgaris	24	22	17
A. niger	28	22	20
A.fumigatus	30	29	27
A. flavaus	30	27	26
C.albicans	31	24	23

Results and Discussion

The three different extracts from leaves of *Hardwickia binata roxb.,* like petroleum ether, chloroform and ethanol extracts at various concentrations (25mg/ml, 50mg/ml and 100mg/ml) were tested against both gram negative ,gram positive bacterias *like B.subtilis, E.coli, P.aeruoginosa, S.typhi, S. aureus, S.pneumonia, P.vulgaris and V. vulnificus and also tested against fungus like Aspergillus niger, Aspergillus flavus, C. albicans and A.fumigatus*. Gram negative bacteria like *E.coli, P.aeruoginosa, S.typhi* may cause the urinary tract infections, GIT infections, skin infections etc. The gram positive organisms like *S. aureus, B.subtilis* may cause the infections such as nosocomial infections, food poisoning, pyrarthirtis, endocardilis, suppurations, abscess formation, osteomyetilis and toxic shock syndrome etc [(11-14).] *Aspergillus niger* is food borne pathogens if inhaled with large amounts of spores, causes a serious lung disease, aspergillosis. , otomycosis pain. *Aspergillus flavus* are widely distributed in nature, hearing loss and, in severe cases, damage to the ear canal causing considerable mortality and morbidity in the tympanic membrane [(15).] As most of the *Aspergillus* infections are caused by *A. fumigatus,* the majority of studies have focused on this species, and our understanding of other *Aspergillus* species is far from satisfactory[(16,17).] *C. albicans* which resides as commensal in the mucocutaneous cavities of skin, vagina and intestine of humans [18], can cause infections under altered physiological and pathological conditions such as infancy, pregnancy, diabetes, prolonged broad spectrum antibiotic administration, steroidal chemotherapy as well as AIDS [(19-26)].

The petroleum ether extract showed the concentration dependent antimicrobial activity. The activity of the pet. ether extract showed the highest zone of inhibition with concentration of 100mg/ml against tested organisms. The pet. ether extract of *Harwickia binata* (100mg/ml) inhibits the growth of micro organism such as *P.vulgaris, S. aureus, and V. vulnificus* and it is also produced the highest zone of inhibitions diameter of 22 mm to 23mm respectively. Its showed the moderate zone of inhibition diameter of 20mm to 21mm against organisms like *B.subtilis, E.coli,* and, *S.typhi.* It was showed the lowest zone of inhibition of diameter 17 to 18mm against *P.aeruoginosa,* and *S.pneumonia*. It also produced the activity against all tested fungus strains with zone of inhibition of diameter 15mm to 19mm at concentration of 100mg/ml it was indicated in table 1. The chloroform extract leaves of *Hardwickia binata* showed the activity against bacterias like *basilus subtilis, E.coli, P.aeruoginosa, S. aureus, S.pneumonia* and *V. vulnificus*. The chloroform extract not produced the activity against bacteria like *S.typhi and P.vulgaris*. Its showed the highest zone of inhibition against *S. aureus* with diameter of 22mm and it produced the moderate zone of inhibition diameter of 19-17 mm against *B. subtilis and E.coli* . Its produced the lowest zone of inhibition diameter of 16mm against *P.aeruoginosa, S.pneumonia and V. vulnificus*. Its also showed the activity against all tested fungus strains with zone of inhibition of diameter 14mm to 18mm at concentration of 100mg/ml it was indicated in table2.

The ethanol extract leaves of *Hardwickia binata* showed the high range of activity against all tested organisms when compared to pet.ether and chloroform extracts .The diameter of zone inhibition of ethanol extract denoted in table 3. The results of preliminary phytochemical analysis indicated the presence of saponins, steroids, flavonoids, coumarins and tannins. Alkaloids and anthraquinones were not present in the extracts. The sensitivity test results indicated that the organic extract of *Hardwickia binata* was active against all the test isolates. Tannins have been traditionally used for protection of inflammed surfaces of the mouth and treatment of catarrh, wounds, haemorrhoiods, and diarrhoea, and as antidote in heavy metal poisoning. Flavonoids are naturally occurring phenols which possess numerous biological activities including anti-inflammatory, antiallegic, antithrombotic and vasoprotective effects [(27, 28).] Presence of these active constituents may responsible for the antimicrobial activity.

Conclusion

The leaves extracts of *Hardwickia binata* roxb (Caesalpinaceae) in this study showed a broad-spectrum of activity against both gram-positive and gram-negative bacteria and fungi. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial and fungal infections including gonorrhea, pneumonia, eye infections and mycotic infections. Isolation, identification and purification of these phytoconstituents and determination of their respective antimicrobial potencies and toxicological evaluation with the view to formulating novel chemotherapeutic agents should be the future direction for investigation.

References :

1. Pankaj Oudhia, medicinal herbs of chhattisgarh,india having less traditional uses,2003
2. C.P.Khare , Indian medicinal plants,spinger referecence,2007
3. Zaika LL. Spices and herbs: their antimicrobial activity and its determination. J Food Safety 1975;9:97-118.
4. Gordon MC, David JN. Natural product drug discovery in the next millennium. Pharm Biol 2001; 139:8-17.
5. Cordell GA. Pharmacognosy: New roots for an old science. In: Atta - ur - Rahman, Basha FZ, editors. Studies in natural products chemistry. 1993,Vol. 13: Bioactive natural products (Part A). Elsevier;
6. Awadh Ali NA, Juelich WD, Kusnick C, Lindequist U. Screening of yemeni medicinal plants for antibacterial and cytotoxic activities. J Ethnopharmacol 2001;74:173-9.
7. Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected Indian medicinal flora. Turk J Biol 2005;29:1.
8. R Nair, S Chanda, Activity of some medicinal plants against certain pathogenic bacterial strains,2006 ,Volume : 38 , Issue : 2 , Page : 142-144.
9. Odunbaku,O.A and llusanya O.A.,Antibacterial activity of the ethanolic and methanolic leaf extracts of some tropical plants on some human pathogenic microbes ,Research journal of agriculture and biological sciences,4(5):373-376,2008.
10. Olafimihan.CA, Fawole MO., Antibacterial properties of the stem bark of azardica indica (the neem tree).J.Pure Appl.Sci. 18:1407-1412,2003
11. Nurdan sarac, aysel ugur, antimicrobial activities and usage in folkloric medicine of some laminaceae species growing in mugla, turkey., *Eurasian jounal of biosciences* 4, 28- 37(2007).
12. Todd Jk .,Toxic shick syndrome. Clinical microbiology reviwes1, 1998, 432-446.
13. Hajjeh Ra, Reingold A., Weil A Et Al., Toxic shock syndrome in the united states: surveillance update, 1979-1996. Emerging infectious diseases 5, 1999, 807-810.
14. Rubin R J , Harrington Ca, Poon A, Dietrich K., Grene Ja, Moiduddin A .,The Economic impact of staphylococcus infection in new ork city hospitals. Emerging infections diseases 5, 1999) 9-17.
15. R. Hema, S. Kumaravel and N. Elanchezhiyan Antimicrobial Activity of Some of the South-Indian Spices and Herbals Against Food Pathogens Global Journal of Pharmacology 3 (1): 38-40, 2009 ,ISSN 1992-0075.
16. Hedayati MT, Pasqualotto AC, Warn PA, Bowyer P., Denning DW. Aspergillus flavus: human pathogen, allergen and mycotoxin producer. Microbiology 2007; 153: 1677_1692.
17. Alessandro C. Pasqualotto Differences in pathogenicity and clinical syndromes due to Aspergillus fumigatus and Aspergillus flavus, Medical Mycology 2008, S1_S10, iFirst article
18. Kaufman HK: Opportunistic fungal infections: Superficial and systemic candidiasis. Geriatrics 52: 50–54, 1997.
19. Friedman S, Richardson SE, Jacobs SE, O'Brien K: Systemic infection in extremely low birth weight infants: Short term morbidity and neuro developmental outcome. Ped Infect Disease J, 2000 19: 499–504.
20. Young GL, Jewell D: Topical treatment for vaginal candidiasis in pregnancy. 2000,Cochrane Datab System Rev 2: CD000225.
21. Rippon JW: Candidiasis and the pathogenic yeasts. In: J.W. Rippon(ed), Med Mycol, PA, 1988, pp 532–581.
22. Kennedy WA, Laurier C, Gautrin D, Ghezzo H, Pare MJL, Contandriopoulos AP: Occurrence of bad risk factors of oral candidiasis treated with oral antifungals in seniors using inhaled steroids. J Clin Epidem,2000,53: 696–701.
23. Sallah S: Hepatosplenic candidiasis in patients with acute leukemia: Increasingly encountered complication. Anticancer Res,1999 , 19: 757–760.
24. Jagarlamudi R, Kumar L: Systemic fungal infections in cancer patients.Trop Gastroenterol 2000,21: 3–8.
25. Mackenzie DWR, Cauwenberg G, Van Cutsem J, Drouhet E, Dupont B:Mycoses in AIDS patients: An overview. In: H. Vanden Bossche (ed), Mycoses in AIDS Patients. New York, Plenum Press, 1990, p 27–33.
26. Vijaya Manohar,1 Cass Ingram,2 Judy Gray et al.,Antifungal activities of origanum oil against Candida albicans, *Molecular and Cellular Biochemistry* **228:** 111–117, 2001.
27. Ref: D. S. OgunleyeF and S. F. Ibitoye, Studies of antimicrobial activity and chemical constituents of *Ximenia Americana,* Tropical Journal of Pharmaceutical Research, December 2003; 2 (2): 239-241
28. Finnermore H, Cooper JM, Stanley MB, CobcroftJH, Harris LJ. ……indicate title of article here …. J. Soc. Chem. Ind., 1988; 57:162-9.

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