Journal of Phytology 2009, 1(6): 469–474
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ISSN: 2075-6240
Available Online: www.journal-phytology.com

REGULAR ARTICLE

ANTIMICROBIAL SPECTRUM AND PHYTOCHEMICAL STUDY OF *HOPEA PARVIFLORA* BEDDOME SAW DUST EXTRACTS

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SUMMARY

The present study evaluates the antimicrobial and phytochemical activity of *Hopea parviflora* Beddome an endemic tree belonging to Dipterocarpaceace against certain bacteria (*Escherichia coli, Proteus vulgaris, Pseudomonas aueroginosa, Klebsiella puemoniae* and *Staphylococcus areus*). The aqueous, methanol and ethanol extracts were found to be most effective against *Staphylococcus areus*. Ethanol extracts compared to other extracts was highly effective against most of the test organisms except *Escherichia coli*. TLC seperation of ethanolic extracts yielded 4 fractions with retention factor (Rf) of 0.58, 0.60, 0.77 and 0.99. Fraction with Rf value of 0.99 was shown to contain antibacterial activity.

Keywords: *Hopea parviflora*, Dipterocarpaceace, phytochemicals, antimicrobial activity.

S. Bhatet al. Antimicrobial spectrum and Phytochemical study of *Hopea parviflora* Beddome Saw dust extracts. J Phytol 1 (2009) 469-474
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1. Introduction

Plant based medicines have been part of traditional healthcare in most parts of the world for thousands of years (Chariandy *et al*., 1999; Newman *et al*., 2000). Plants contain numerous biologically active compounds, many of these have been shown to exhibit antimicrobial properties and therefore they were in use as antimicrobial drugs in traditional medicines. Plants have the ability to synthesize aromatic substances, mainly secondary metabolites. These substances serve as the molecules of plant defense against predation by microorganisms, insects and herbivores. They may have also involved in plant odour, pigmentation and flavor (Cowan, 1999). The medicinal value of these plants lies in some chemical substances known as phytochemicals (Hill, 1952).Knowledge of the phytochemicals is desirable not only for the discovery of healthcare products, but also in disclosing new sources of economic materials like tanins, oils, gums etc., (Farnsworth, 1966). And also to commercialize dietary phytochemicals like nutraceuticals, phyto-ceuticals, dietary supplements, functional foods need scientific evidence (Stephen, 1998).

Hopea parviflora is an endemic tree belonging to Dipterocarpaceae family, found in the tropical evergreen forests of south western India distributed all along the Western Ghats of Karnataka (Shivaprasad *et al*., 1987). The tree is economically important as timber; the bark is also a good tanning material and astringent with slow speed of diffusion

(Muralikrishna, 1994). Since nothing much about the chemistry of the tree is known a study on antimicrobial action of the plant extract obtained from the saw dust waste was conducted against different bacterial strains such as *Escherichia coli, Proteus vulgaris, Pseudomonas aueroginosa, Klebsiella puemoniae and Staphylococcus areus*. The extracts were also screened for the presence of different phytochemicals.

2. Materials and Methods

Plant extract preparation

The fresh saw dust of the *Hopea parviflora* tree was collected from the cutting sites, shade dried powdered and weighed. 100 g of this saw dust powder was packed in Whatmann No 3 filter paper and soxhletted in 250 ml of methanol and ethanol at 80°C and 70°C respectively for 12 hours. The extracts were then filtered through a layer of whatman No 1 filter paper, evaporated to dryness at 37°C on a water bath and stored at 4°C. Aqueous extract was prepared by soaking 100g of powdered sawdust for 72 hours with stirring every 12 hours. At the end of extraction period it was centrifuged and supernatant was filtered through whatmann No 1 filter paper, evaporated to dryness on a water bath and stored at 4°C

Phytochemical analysis

The condensed extracts were used for preliminary screening of phytochemicals such as alkaloids (Wagner and Dragendorff's tests), flavonoids (Shinda and NaoH tests), Phenols (ellagic acid and phenol tests), tanins (gelatin tests), saponins (foam tests), sterols (Liberman-Burchard and Salkowski tests) (Dey and Harborne, 1987; Evans, 1989).

Bacterial cultures and Media

Clinical isolates of *Escherichia coli, Proteus vulgaris, Pseudomonas aueroginosa, Klebsiella puemoniae and Staphylococcus areus* were obtained from the department of microbiology, M. S. Ramaiah Medical College, Bangalore. All bacteria were grown on Nutrient agar (NA) at 37°C. For antibacterial assays, bacteria were inoculated into Nutrient broth (Himedia, India) and incubated overnight at 37°C.

Assay for anti bacterial activity

The plate-hole diffusion assay of Ivan *et al* (1979) as given by Kudi *et al* (1999),with modifications was used to determine the growth inhibition of bacteria by the *Hopea parviflora* extract. Two hundred microlitre of overnight Nutrient broth culture were added to 15 ml of molten Muller-Hinton Agar (MHA) mixed well, poured into a sterile petridish and allowed to set. A sterile cork-borer (8mm diameter) was used to make wells in the set agar. 100 microlitre of plant extract dissolved in 50% DMSO (a final concentration of 1 mg/ml) were added to each well, the two control wells received 100 microlitre 50% DMSO (negative control) and streptomycin (1mg/ml) dissolved in sterile water (positive control).The plates were incubated overnight at 37°C.

Antibacterial activity was recorded by measuring the zone of growth inhibition around the well. All the antibiotic assays were carried out in triplicates.

Determination of Minimum inhibitory concentration (MIC)

MIC of the *Hopea parviflora* extracts was determined by using different concentrations of extracts in Muller-Hinton broth by macro dilution method (NCCLS, 1993; Akinyemi *et al*, 2005).

Thin layer chromatography (TLC)

TLC analyses were made on 0.25 mm thick silica gel G (Merck), prepared on glass. Plant extract samples (5µl) were applied 2.5 cm from the base of silica plates. After drying the plates were developed with chloroform: methanol (27:0.3) solvent mixture for phenols. After the solvent was allowed to evaporate, the colour and retention factor (Rf) values of these phenols were recorded under visible light after spraying the plates with Folin-Ciocalteue reagent heating at 80°C for 10 min (Harborne,

1998). The TLC analyses were carried out in duplicates using Gallic as reference standard.

3. Results

Percentage yield of the powdered *Hopea parviflora* crude extracts obtained using various solvents is shown in Table 1.

Table 1. Percentage yield of the crude extracts of *Hopea parviflora*

Extraction solvent	Percentage yield
Distilled water (Aqueous)	1.5
Methanol	8
Ethanol	10.33

The highest yield of 10.33 % was obtained in ethanol extracts. The phytochemical screening of the crude extracts of *Hopea parviflora* revealed the presence of some bioactive compounds (Table 2).

Table 2. Qualitative analysis of Phytochemicals in *Hopea parviflora.*

Phytochemicals analyzed	Aqueous extract	Methanol extracts	Ethanol extracts
Alkaloids	+	+	+
Saponins	+	+	+
Tannins	+++	+++	+++
Phenolics	+++	+++	+++
Steroids	-	-	-
Flavonoids	+	+	+

``—``:- Not detected, +:- Low concentration, ++:- High concentration, +++: Very high concentration

All the extracts were positive for alkaloids, saponins, tannins, phenolics and flavonoids where negative for steroids. Phenolics and tannins were observed to be present in high concentrations. Antibacterial activity of crude extracts of *Hopea parviflora* against bacterial species is presented in Table 3. The test organisms showed susceptibility to *Hopea parviflora* extracts in varying degrees. *Staphylococcus areus* showed a highest degree of sensitiveness to all the three extracts i.e. aqueous, methanol and ethanol with an inhibition zone diameter of 23,25 and 28mm respectively, followed by *Proteus vulgaris* which had zone diameter of 16, 18 and 23mm for aqueous, methanolic and ethanolic extracts respectively.

The highest zone of growth inhibition was shown by ethanolic extract against *Staphylococcus areus* (28 mm), *Proteus vulgaris* (23mm), *Pseudomonas aeuroginosa* (16mm) and *Klebsiella pneumoniae* (8mm). Whereas *E. coli* showed highest zone of inhibition (14mm) for aqueous extract.

Table 3. Antibacterial activities of *Hopea parviflora* extracts (Values are mean of three replications)

Test organism	Aqueous extract (1 mg/ml)	Methanol extract (1mg/ml)	Ethanol extract (1mg/ml)	DMSO 50%	Streptomycin (1mg/ml)
E. coli	14	12	5	No zone	25
Klebsiella pneumoniae	5	2	8	No zone	30
Pseudomonas aeuroginosa	12	15	16	No zone	25
Proteus vulgaris	16	18	23	No zone	35
Staphylococcus aureus	23	25	28	No zone	40

The MIC of *Hopea parviflora* was detected in the range of 0.8-2.4 mg ml^{-1} against all the five bacterial cultures (Table 4). A significant MIC of 0.8 mg/ml was observed for ethanol extract against *Staphylococcus areus.* Aqueous and methanol extracts showed MIC of 1mg/ml against *Staphylococcus areus*.

Table 4. MIC of *Hopea parviflora* extracts (mg ml^{-1})

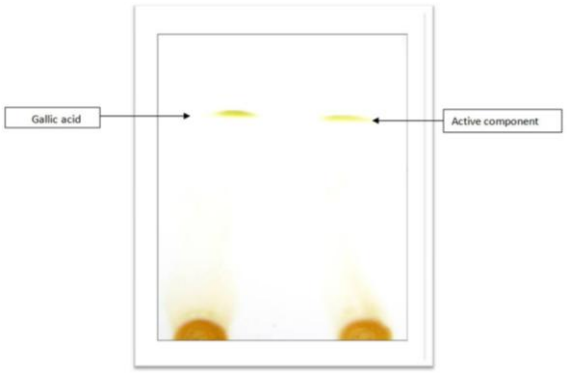
Test organisms	Aqueous extract	Methanol extract	Ethanol extract
E. coli	1.8	1.8	2.0
Klebsiella pnuemoniae	2.4	2.4	1.8
Pseudomonas aeuroginosa	1.8	1.8	1.6
Proteus vulgaris	1.4	1.2	1.2
Staphylococcus aureus	1.0	1.0	0.8

TLC using Chloroform: Methanol (27:03) as the developing solvent was able to separate Phenolics in the ethanolic extract (which yielded good antimicrobial results) into four distinct visible fractions with retention factor (RF) values 0.58, 0.60, 0.77 & 0.99. All the four fractions, plus the origin were purified from the developed duplicate plate by collecting the silica placing it into a polypropylene test tube and redissolving the fraction in 50% DMSO (a volume of 100 µl).

Following centrifugation to remove silica particals the supernatant was collected. All fractions were tested in a plate-hole diffusion assay against *Staphylococcus areus* to determine antibacterial activity of the separated components. In those fractions only fraction (RF=0.99) was active (with diameter of zone of

inhibition =18mm), while some activity within origin was also detected.

Fig. 1. Thin Layer Chromatographic separation of active component in *Hopea parviflora* extracts



4. Discussion

The phytochemical compounds detected alkaloids, tannins, phenolics, saponins and flavonoids have potentially significant application against the bacteria (El-Mahmood *et al*., 2008). Several authors have linked the presence of these bioactive compounds to the antimicrobial properties of plant extracts (Adesokan *et al*., 2001; Nwolobi *et al*., 2007; Oyeleke *et al*., 2008). Aqueous and alcoholic extracts of several Indian have shown antibacterial activity against *Bacillus subtilis*, *E. coli*, *Proteus vulgaris, Pseudomonas aeuroginosa* and *Staphylococcus areus* etc (Ahmed *et al*, 1998). In vitro and In vivo studies conducted by Madani and Jain (2008) have shown the anti-salmonella activity of *Terminalia balerica.* Susceptibility of the test organisms was of varying degrees to the plant extract. The susceptibility varied according to strains and species (Karou *et al*, 2006). The streptomycin which served as positive control at the given concentrations produced larger zone diameter for the test organisms. This difference may be due to the fact that synthetic antibiotics are in pure form where as crude plant extracts contains some impure substances that may be inert and do not have any antibacterial activities (El-Mahmood, 2009).The extract with low activity against a particular organism has given high MIC, while highly reactive extract has given low MIC value. The MIC technique is used to evaluate the efficacies of antimicrobial agents (Junaid *et al*., 2006). In our study MIC values tend to support the results obtained in the antibacterial screening above showing clearly that ethanol extract were potent than aqueous and methanol extracts for test organisms other than *E. coli*. Also the MIC values clearly support the antibacterial screening of *S. areus*, since values are more potent. In our study we suspect that abundant amount of tannins and phenols in the extracts could be responsible for the antibacterial activity as reported earlier by Trease and Evans (1989); Reddy *et al*., (2007).

In TLC separation of ethanol extract the fraction (RF=0.99) which was purified from the developed TLC plate was further separated on pre coated silica gel (Merck) along with Gallic acid as reference standard. It was found that the fraction (RF=0.99) which had potential antimicrobial activity had the same retention factor that of standard Gallic acid.

In summary, the anti bacterial activity of *Hopea parviflora* extract was possibly due to the presence of phytochemicals like phenols and tannins in high concentration, it may be also localized to a single component, possibly Gallic acid if a detailed study is conducted. However the results showed activities agreeing with comparable results of previous researchers using extracts of other plant species like *Azadirachta indica* (Ram *et al*., 2000),*Chukrasia tubularis* (Nagalakshmi *et al*.,2001),*Toona ciliata* and *Amoora rohituka* (Chowdhury *et al*.,2003), *Aglaia spectabilis* (Lavanya *et al*.,2006).Further work on these lines is needed

References

Adesokan, A. A., Akanji, M. A., Yakubu, M. T. 2007. Antibacterial potentials of aqueous extract of *Enantia chlorantha* stem bark. *Afr. J. Biotechnol*, 6 (22): 2502-2505.

Ahmad, I., Zafar, M and Mahammad, F. 1998. Screening of some Indian medicinal plants

for their antimicrobial properties. *J. Ethnopharmocol*. 62: 183-193.

Akinyemi, K. O., Oladapo O., Okwara, C. E., Ibe, C. C and Fasure, K.A. 2005. Screening of crude extracts of six medicinal plants used in south-west Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococus aureus* activity. *BMC Complement Alter Med*, 5: 6

Bai, D. 1990. Traditional Chinese material: A respect and prospect. *Plant Medica*.56:5002.

Chariandy C M., Seaforth, C E., and Phelps, R H. 1999. Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. *J. Ethnopharmacol*, 64: 265-270.

Chowdhury, R. M., Choudhury, H., Mohammad and Rashid, A. 2003. Antimicrobial activity of *Toona ciliata* and *Amoora rohituka*. *Fitoterapia*, 74: 155-158.

Cowan, M M.1999.Plant products as antimicrobial agents. *Clin. Microbiol. Rev*, 12: 564-582.

Dey, P.M and Harborne, J.B. 1987. Methods in plant Biochemistry, Academic Press, London.

El-Mahmood, A.M. 2009. Antibacterial activity of crude extracts of Euphorbia hirta against some bacteria associated with enteric infections.Journal of Medicinal Plants Res. 3(7): 498-505.

El-Mahmood,A M., Doughari, J H., Chanji, F J. 2008. In-vitro antibacterial activities of crude extracts of *Nauclea latifolia* and *Daniella oliveri*. *Sci. Res .Essay*, 3(3): 102-105.

Fransworth, N.R. 1966. Biological and Phytochemical screening of plants. *J Pharm Sci*, 35: 225-276.

Harborne J.B. 1998. Phytochemical analysis: A Guide to Modern Techniques of Plant analysis, 3rd edition. Chapman and Hall. London.

Hill, A.F. 1952. A textbook of useful plants and plant products. McGraw-Hill Book Company Inc, Newyork.

Ivan, M., Vanden Berghe, D.A., Mertens, F. 1979. Screening of higher plants for biological activity1. Antibacterial activity. *Planta Med* , 36: 311-321.

Junaid, S. A., Olabode, A. O., Bonwulin,F.C., Okworiu, A E J., Agina, S. E. 2006.The antimicrobial properties of *Ocimum gratissimum* extracts on some selected bacterial gastrointestinal isolates. *Afr. J. Biotechnol*, 5(22):2

Karou, D., Savadogo, A., Canini, A., Yameogo, S., Montesano, C., Simpore, J., Colizzi, V., Traore, A. S. 2006. Antibacterial activity of alkaloids from *Sida acuta*. *Afr. J. Biotechnol*. 5(2): 195-200

Kudi, A. C., Umoh, J U., Eduvie,L.O and Gefu, J. 1999.Screening of some Nigerian medicinal plants for antibacterial activity. *J Etanopharmacol*, 67: 225-228.

Lavanya, T.M., Rama Murty, K., Srinivasa Reddy, N and Sambasiva Rao, K.R.S. 2006. Phytochemical and Antimicrobial study of *Aglata spectabilis* leaf extracts. *J. Trop. Med. Plants*. 7: 163-168.

Madani, A and Jain, S. K. 2008. Anti-Salmonella activity of *Terminalia belerica*: In vitro and in vivo studies. *Indian Journal of Experimental Biology*. 46: 817-821.

Mallikarjuna, P.B., Rajanna, L.N., Seetharam, Y.N and Sharanabasappa. 2007. Phytochemical studies of *Strychnos potatorum* L.f. - A Medicinal Plant. *E-Journal of Chemistry* 4(4): 510-518.

Nagalakshmi, M.A.H., Thangadhuri, D., Muralidhar Rao and Pullaiah. 2001. Phytochemical and antimicrobial study of *Chukrasia tabularis* leaves. *Fitoterapia*, 72:62-64.

NCCLS. 1993. Methods for dilution in antimicrobial susceptibility tests: Approved standard, Villanova Pa, NCCLS, M2-A5 . 315-2321.

Newman, D. J., Cragg, G. M and Snader, K. M. 2000. The influence of natural products upon drug discovery. *Nat Prod Rep*, 17: 175-285.

Owolabi, O.J., Omogbai, E K I., Obasuyi, O. 2007. Antifungal and antibacterial activities of the ethanolic and aqueous extracts of Kigelia Africana (Bignoniaceae) stem bark. *Afr. J. Biotechnol*, 6(14): 1677-680.

Oyeleke, S. B., Dauda BEN., Boye, O. A. 2008.Antibacterial activity of *Ficus capensis*. *Afr. J. Biotechnol*. 7(10): 1414-1417.

Stephen, A.M. 1998. Functional Foods: Biochemical and Processing Aspects. Technomic Publishing Co. Inc, Lancaster. USA.

Ram, M.S., lavazhagan, S.K., Sharma, S.A., Dhanraj, B., Suresh, M.M., Parida., A.M., Jana., A.M., Devandra, K and Selvamurthy, W. 2000. Antimicrobial activity of a new vaginal contraceptive NIM-76 from neem oil (*Azadirachta indica*). J. Ethnopharmacol, 71:377-382.

Ramamurthy and Nagamani Kandimalla. 2008. Antimicrobial spectrum and phytochemical study of *Walsura trifoliate* (A.Juss.) Harms. (Meliaceae) Bark extracts. *J Pharmacology and Toxicology*, 394:267-271.

Reddy, M.K., Gupta, S.K., Jacob, M.R., Khan, S.I and Ferreere, D. 2007. Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from *Punica granatum* L. Planta Med, 73(5): 461-7

Toma, I., Karumi and Geidam, M. A. 2009. Phytochemical screening and toxicity studies of the aqueous extract of the pods pulp of *Cassia sieberiana* DC. *Afr. J. Pure and Applied Chemistry* 3 (2): 26-30.

Trease, G.E and Evans, W. C. 1989. Trease and Evans Pharmacognosy: A Physicians guide to Herbal Medicine. 13th Edition, Bailliere Tindall. London