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Bioprospecting of selected medicinal plants for antibacterial activity against some pathogenic bacteria

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Aqueous extracts of 54 medicinal plants were evaluated for antibacterial activity against important human pathogenic and plant pathogenic bacteria by agar well diffusion method. Aqueous extracts of Combretum latifolium BI., Persea macrantha (Nees) Kosterm., Vateria indica L. and Humboldtia brunonis Wall. recorded significant inhibitory activity against both human and plant pathogenic bacteria. These plants were further subjected to solvent extraction successively with petroleum spirit, chloroform, ethyl acetate and methanol based on increasing polarity to identify the suitable solvent for isolation of the antibacterial active principle. Ethyl acetate and methanol extracts of C. latifolium Bl. and V. indica L. significantly inhibited the important human pathogenic bacteria, including Escherichia coli, Salmonella typhi, Staphylococcus aureus and Bacillus cereus. Ethyl acetate extract of P. macrantha (Nees) Kosterm. and Humboldtia brunonis Wall. significantly inhibited Pseudomonas syringae, Xanthomonas oryzae pv. oryzae and Xanthomonas axonopoidis pv. vesicatoria which are important plant pathogenic bacteria. Results of the present investigation suggests that C. latifoliumi BI., Ρ. macrantha (Nees) Kosterm., V. indica L. and H. brunonis Wall. are important candidate plants for further work on isolation and characterization of antibacterial active principle.

Key words: Bioprospecting, medicinal plants, antibacterial activity, *Combretum latifolium* Bl., *Persea macrantha* (Nees) Kosterm., *Vateria indica* L., *Humboldtia brunonis* Wall.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Clinical microbiologists and plant pathologist have great interest in screening of medicinal plants for antimicrobial activities and phytochemicals as potential new therapeutics. The use of plant extracts for medical treatments is enjoying great popularity since 1990s when people realized that the effective life span of antibiotic is limited and over prescription and misuse of traditional antibiotics are causing microbial resistance (Alam et al., 2009). Antibiotic resistance is currently one of the major threats encountered. The increasing prevalence of resistance to most antimicrobial agents complicates their use and the control of infectious diseases. No antimicrobial agent is at present fully effective against dormant facultative bacteria (Jouenne et al., 1998).

Usually bacterial diseases of plants are very difficult to control. Frequently, a combination of control measures is required to combat a given bacterial disease. Only few chemicals are available for control of bacterial diseases of plants (Agrios, 2005; Spiroudi et al., 2001). Medicinal plants are the richest bio-resource of drugs for traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Many of the drugs currently used to treat bacterial and other infections were isolated from natural resources including ethno medicinal plant. Such plants may provide new resources of therapeutic agents against multidrug resistant bacterial infections. It has been estimated that 14 to 28% of higher plant species are used medicinally

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and that 74% of pharmacologically active plant derived components were discovered after following up on ethno medicinal use of the plants. A number of interesting outcomes have been found with the use of a mixture of natural products to treat diseases, most notably the synergistic effects and polyphmacological application of plant extracts. Ethno pharmacologists, botanists, microbiologists, and natural- product chemists are searching the earth for phytochemicals which could be developed for the treatment of infectious diseases especially in light of the emergence of drug-resistant microorganisms and the need to produce more effective antimicrobial agents (Ncube et al., 2008; Anam et al., 2010). Hence, this study was conducted to address the inhibitory activities of selected plants against some human pathogenic bacteria that cause acute diarrhea, food poisoning and other diseases, and plant pathogenic bacteria that causing major blight diseases; when extracted, the different parts with distilled water and different solvents.

MATERIALS AND METHODS

Test plant materials

Fresh, apparently healthy parts of test plants were collected from Mysore, South Canara and Shimoga districts, Karnataka, India. List of the selected test plants and their ethno botanical information are mentioned in Table 1 (Pullaiah, 2006; Trivedi, 2006 Warrier, 2010; and) Herbarium specimen of the test plants are deposited in the Herbarium of Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore.

Extraction of plant material

Aqueous extract

Aqueous extraction was carried out with a modified method of Mahmoudabadi and Nasery (2009). 10 g of the fresh plant material was macerated with 10 ml of distilled water. The macerate was filtered through double layered muslin cloth and then centrifuged at 4000 g for 20 min, the supernatant liquid filtered through Whatmann filter paper No.1 and heat sterilized. The extracts were maintained at 6°C till further use.

Solvent extract

Further, the plants showing significant activity were subjected to solvent extraction. The plants showing significant activity were further extracted with different solvents using Soxhlet apparatus. Air dried and pulverized plant material was initially defatted with petroleum spirit. The defatted pulverized plant material was then successively and exhaustively extracted with solvents based on increasing polarity such as chloroform, ethyl acetate and methanol. The obtained extracts were concentrated and yield recorded (Ndukwe et al., 2008).

Test organisms

From the Microbial Type Culture Collection (MTCC), Chandigarh, the human pathogenic bacteria *E. coli* (MTCC 7410),

Staphylococcus aureus (MTCC 7443), Bacillus cereus (MTCC 121), and Salmonella typhi (MTCC 733), the plant pathogenic bacteria Xanthomonas oryzae (MTCC), Xanthomonas vesicatoria (MTCC 2286) and Pseudomonas syringae (MTCC 1604) were obtained. Ralstonia solanacearum was isolated from infected potato plant and maintained at Kelman's TZC media (French et al., 1995).

Antibacterial activity assay

Antibacterial assay of aqueous extracts was determined using the agar well diffusion method. Approximately 20 ml of molten, sterilized nutrient agar media were poured in sterilized Petri dishes and allowed to solidify.

For aqueous extracts, the bacterial test organisms were grown in nutrient broth for 24 h. A 100 μ l nutrient broth culture of each bacterial organism was used to prepare bacterial lawn. Agar well of 10 mm diameter were prepared with the help of stainless steel cork borer. 100 μ l of the test plant extracts was aseptically added to the well, plates allowed to be incubated at 37 °C for 24 h and then observed for the zone of inhibition, if any. Diameters of the zone was measured in mm (Kurhekar, 2006).

For solvent extracts, antibacterial screening was carried out by standard disc diffusion test. The bacterial test organisms were grown in nutrient broth for 24 h. 100 µl nutrient broth culture of each bacterial organism was used to prepare bacterial lawn. Known concentration of extract were incorporated in 9mm diameter sterile disc and dried. Inoculated plates were incubated at 37 °C for 24 h and then observed for zone of inhibition, if any. Diameter of the zone was measured in mm (Kannan et al,. 2009).

RESULTS

Of the 54 plant screened aqueous extracts of plants, twelve showed inhibition of the different pathogenic bacteria. The plants *V. indica* L. and *Pteris* species show significant activity against the human pathogenic bacteria while *Parkia biglandulosa* Wight and Arn showed good inhibitory activity against plant pathogenic bacteria. *C. latifolium* Bl., *P. macrantha* (Nees) Kosterm. shows good activity against both human and plant pathogenic bacteria. *B. acutangula* (L.) Gaertn shows inhibitory activity against *S. typhi* and *P. syringae*. *H. brunonis* Wall. shows less inhibition of *P. syringae*. *D. viscosa* (L.) Jacq., *A. scholaris* (L.) R Br., *H. brunonis* Wall. shows inhibition of *E. coli* and *S. typhi*, respectively (Table 2).

Solvent extraction shows moderate to high level of inhibitory activity. The spectrum of activity is wide and significantly against Gram negative plant pathogenic bacteria.

Among the solvents extracts, petroleum spirit and chloroform extracts did no major activity. Ethyl acetate and methanol reported to have good spectrum of activity. Ethyl acetate extract of *C. latifolium* Bl. shows inhibition of growth of *P. syringae, Staph. aureus, S. typhi, X. orayzae* and *X. vesicatoria. P. macrantha* (Nees.) Kosterm shows major inhibitory activity against plant pathogens, including *P. syringae, X. orayzae* and *X. vesicatoria. H. brunonis* Wall. recorded inhibition against *S. typhi, P. syringae, X. orayzae* and *X. vesicatoria. Vateria indica* L. shows significant inhibition against

 Table 1. Plants selected for antibacterial activity assay based on ethno botanical information and their uses.

S/N	Name of the plant	Family	Uses
01	Acacia mangifera	Mimosae	As ornamental, shade tree.
02	Achras zapota Linn.	Sapotaceae	Antifungal.
03	Ampellocissus araeosa (Dalzell) Gamble	Vitaceae	Roots as cooling agent, as astringent.
04	Apama siliquosa Lam.	Aristolochiaceae	Bark in colic pain, to treat leprosy, skin diseases, and anthelmintic.
05	Alstonia scholaris (L.) R.Br	Apocynaceae	Bark used to treat colic pain, leprosy, skin diseases, anthelmintic.
06	Averhoa bilimbi L.	Oxalidaceae	Leaves as antipyuritic, fruits used to treat diarrhea, vomiting, and food poisoning.
07	<i>Barringtonia acutangula</i> (L.) Gaertn.	Lecythidaceae	Leaves to treat diarrhea, dysentery, roots used to treat malaria.
08	Canscora diffusa (Vahl) R. Br. ex Roem. and chult.	Gentinaceae	To treat abdominal disorders, intestinal worms, skin diseases, tuberculosi.
09	Canthium parviflorum Lam.	Rubiaceae	Roots and leaves as diuretic, astringent, to treat fever.
10	Callicarpa lanata Linn.	Verbenaceae	Aqueous leaf extract as aseptic to dress wounds, boils, to treat asthma, cough, ulcers.
11	<i>Carallia lucida</i> Roxb. ex Kurz.	Rhizophoraceae	To treat sapraemia, oral ulcer, stomachic.
12	Carmona retusa (Vahl) Masamune	Boraginaceae	Root as antidote, leaf decoction for cough, stomachic.
13	Chloroxylon swietenia <u>DC.</u>	Rutaceae	Bark as astringent, leaves used to heal to wounds, mosquitocidal.
14	Citrullus lanatus (Thunb.) Matsum. and Nakai	Cucurbitaceae	Seed as demulcent, diuretic, pectoral and tonic.
15	Combretum latifolium BI.	Combretaceae	Leaves having insecticidal property.
16	Corchorus trilocularis L.	Tiliaceae	Common weed.
17	Cucurbita pepo L.	Curcubitaceae	Anthelminitc, reduces symptoms of an enlarged prostate, nourishes skin.
18	Dichapetalum genonoides	Dichapetalaceae	As coolant, to treat mouth ulcers.
19	Decalipis hamiltonii Wight and Arn.	Asclepidaceae	Root used to prepare drink which acts as blood purifier, substitute to vanillin, to treat gastric and intestinal disorders.
20	Derris scandens Benth.	Fabaceae	Expectorant, antitussive, diuretic, antidysentery and for treatment of cachexi
21	<i>Dodonaea viscosa</i> (L). Jacq.	Sapindaceae	To treat high blood pressure, burns and boil wounds, stomach problems, also for snake bites.
22	Eleusine coracana <u>Gaertn.</u>	Poaceae	Grain as astringent, to treat renal and vesicle calculi, leaves to control termites.
23	Erythroxylon monogynum Roxb.	Erythroxylaceae	Leaf paste with coconut oil used to treat skin disease and nourishes the skin.
24	<i>Ficus glomerata</i> Roxb.	Moraceae	Bark to treat ulcer, skin disease, urinary infection.
25	<i>Hamelia patens</i> Jacq.	Rubiaceae	Insect bites, menstrual disorders, uterine and ovarian afflictions.
26	Holigarna arnottiana Wt. and Arn.	Anacardiaceae	To treat arthritis, hemorrhoids, skin diseases, also to treat cancer
27	Holarrhena antidysentrica (Linn) Wall.	Apocynaceae	Roots and flowers to treat cough, cold, diarrhea and dysentery.

Table 1. Contd.

28	Humboldtia brunonis Wall.	Caesalpinaceae	In menstrual problems.
29	Hydrocotyle javanica Thunb.	Apiaceae	Leaf stalk to treat toothache, dysentery, blood purifier, indigestion.
30	<i>Ipomoea hederaceae</i> Jacq.	Convolvulaceae	Seeds as purgative, vermifuge, anti inflammatory,
31	Jatropha gosyipifolia Linn.	Euphorbiaceae	Leaves as intermittent fevers, carbuncles, eczema, itches, sores on the tongues of babies, swollen mammae, stomachache, and venereal disease
32	Kigelia pinnata (Jacq.) DC.	Bignonaceae	Stem bark, seeds for syphilis, rheumatism.
33	Lagenaria vulgaris	Cucurbitaceae	Roots as emetic, purgative, anti inflammatory, seeds used to treat fever, cough.
34	Ludwigia perennis Linn.	Onagraceae	Common weed. Sometimes used as aquarium plants.
35	<i>Malphigia</i> sp.,	Malphigiaceae	As antidiabetic, as ornamental.
36	Mesua nagassarium (<u>Burm.f.</u>) <u>Kosterm.</u>	Clusiaceae	Flowers as astringent, anthelminitc, to treat asthama, cough, dysentery, ulcer.
37	Michelia champaca L.	Magnoliaceae	Bark as stimulant, diuretic, febrifuge, dried root as purgative, for liver, heart, and stomach ache.
38	<i>Mirabilis jalapa</i> Linn.	Nyctaginaceae	Ornamental flowering plant.
39	Momardica dioica Roxb. Ex. Wild.	Cucurbitaceae	Fruits used as vegetable, whole plant for skin disorders.
40	Parkia biglandulosa Wight and Arn.	Mimosaceae	Leaves, inflorescence and pods were used to treat dysentery and cold.
41	Persea macrantha (Nees) Kosterm.	Lauraceae	Leaves and bark as arthritis, traumatic injury, edema, and wounds.
42	Piper longum L.	Piperaceae	Roots as expectorant, stomachic, epilepsy, fever, as digestive agent, carminative,
43	Poeciloneuron indicum Bedd.	Clusiaceae	Bark used to treat dysentery, cholera and diarrhea.
44	<i>Pteris</i> sp.		Ornamental.
45	Santalum album L.	Santalaceae	To treat leprosy, jaundice, cough, bronchitis, dysentery.
46	Scheffllera stellata (Gaertn.) Harms.	Araliaceae	Ornamental plant.
47	<i>Selaginella</i> sp.	Selaginellaceae	Coughs, fever.
48	Synedrella vialis (Less.) Gray	Asteraceae	A common weed, as garden floret plant.
49	Taebubia argentia (Bureau and K. Schum.) Britton.	Bignonaceae	As avenue tree for its flowering
50	Urena sianata L.	Malvaceae	Leaves to treat skin disorders.
51	Vateria indica L.	Dipterocarpaceae	To treat skin eruption, wounds, ulcers, as carminative, detergent, expectorant,
52	Vetiveria zizanioides (Linn.) Nash	Poaceae	To treat bilious fever, skin diseases, ulcers, anemia, cough.
53	Viscum articulatum Burm. f.	Viscaceae	Leafless Mistletoe is used as a cure for fever. Paste is applied to cuts.anticacerous.
54	Zizypus xylopyrus (Retz.) Willd.	Rhamnaceae	Antidepressant, anti-ulcer, memory and learning enhancers

Source: Pullaiah (2006), Trivedi (2006) and Warrier et al. (2010).

Table 2. Plants showing inhibitory activity against pathogenic bacteria (Zone of inhibition measured in mm).

	Plant		Test bacteria									
S/N		Part used		G	Gram positive bacteria							
			E.c	S.t	X.o.o	X.a.v	Ra.s	Ps.s	St.a	B.c		
01	Distilled water as control	100 μl/well	00	00	00	00	00	00	00	00		
02	A. scholaris (L.) R.Br.	Leaves	11.33±0.57	11.33±0.57	00	00	00	00	00	00		
03	<i>B. accutangula</i> (L.) Gaertn.	Leaves	00	18.66±1.15	00	00	00	11.66±0.57	00	00		
04	C. latifolium Bl.	Leaves	16.66±0.57	19.33±1.15	22.33±2.30	00	19.33±0.57	19.33±1.15	15.66±0.57	00		
05	<i>H. brunnonis</i> Wall.	Leaves	11.66±0.57	14.33±1.15	00	00	00	00	00	00		
06	P. indicum Bedd.	Leaves	10.66±0.57	00	00	00	11.33±0.57	00	00	00		
07	P. macrantha (Nees) Kosterm.	Leaves	15.33±0.57	00	12.66±0.57	00	12.33±0.57	11.33±0.57	11.00±0.00	00		
08	V. indica L.	Leaves	12.33±0.57	15.66±0.57	15.66±0.57	00	00	00	00	00		

Results of zone of inhibition area in±standard deviation of three replicates. 00, No inhibitory activity; E.c, E. coli; S.t, S. typhi; St.a, Staph. aureus; B.c, B. cereus; X.o.o, X. oryzae pv. Oryzae; X.a.v, X. axonopoidis pv. Vesicatoria; Ra.s, R. solanacearum, Ps.s, Pseudomonas syringae.

Table 3. Antibacterial activity (zone of inhibition) of solvent extracts of selected test plants.

	Plant		Test bacteria									
S/N		Solvent		Gram positive bacteria								
			E.c	S.t	Х.о.о	X.a.v	Ra.s	Ps.s	St.a	B.c		
01	Control	00	00	00	00	00	00	00	00	00		
		PE	00	00	00	00	00	00	00	00		
02	<i>Alstonia scholaris</i> (L.) R.Br. (Family: Apocynaceae)	CF	00	00	00	00	00	00	00	00		
02		EA	00	00	00	00	00	00	00	00		
		М	00	00	00	00	00	11.33±0.57	00	00		
	<i>Barringtonia accutangula</i> (L.)Gaertn. (Family: Lecythidaceae)	PE	00	00	00	00	00	00	00	00		
00		CF	00	00	00	00	00	00	11.33±0.57	00		
03		EA	00	12.00±0.00	00	00	00	00	00	00		
		М	00	11.33±0.57	00	00	00	13.66±0.57	00	00		
	<i>Combretum latifolium</i> Bl. (Family: Combretaceae)	PE	00	00	00	00	00	00	00	00		
04		CF	00	00	00	00	00	00	00	00		
		EA	14.33±0.57	18.33±0.57	16.66±1.15	16.00±1.00	00	24.00±1.00	18.66±0.57	13.66±0.57		
		М	12.66±0.57	18.33±0.57	22.00±2.65	17.33±1.52	00	25.33±2.30	25.33±0.57	15.66±0.57		

Table 3. Contd.

		PE	00	00	00	00	00	00	00	00
05	Humboldtia brunnonis Wall.	CF	00	00	00	00	00	00	11.00±0.00	00
	(Family: Caesalpinaceae)	EA	13.33±0.57	20.33±0.57	21.66±2.08	20.00±1.00	00	19.66±0.57	11.33±0.57	00
		М	00	00	00	00	00	00	00	00
		PE	00	00	00	00	00	00	00	00
06	Poeciloneuron indicum Bedd.	CF	00	11.00±0.00	00	00	00	00	12.33±0.57	00
06	(Family: Clusiaceae)	EA	11.33±0.57	11.00±0.00	00	11.33±0.57	00	00	12.33±0.57	00
		М	00	00	00	00	00	00	00	00
	Persea macrantha (Nees)	PE	00	00	00	00	00	00	00	00
07		CF	00	00	00	00	00	00	00	00
07	Kosterm. (Family: Lauraceae)	EA	11.66±1.15	18.66±3.21	25.66±1.15	18.66±1.16	00	29.33±0.57	17.66±1.15	13.66±0.57
		М	00	00	00	00	00	00	00	00
		PE	00	00	00	00	00	00	00	00
08	Vateria indica L.	CF	00	00	00	00	00	00	00	00
	(Family: Dipterocarpaceae)	EA	00	13.66±0.57	11.66±1.15	16.66±0.57	00	16.33±1.15	00	00
		М	11.33±0.57	20.33±0.57	14.33±0.57	17.66±1.15	14.66±0.57	26.00±2.00	24.33±0.57	00

Results of zone of inhibition are in ± standard deviation of three replicates. 00, No inhibitory activity; *E.c. E. coli; S.t. S. typhi;St.a, Staph. Aureus; B.c. B. cereus, X.o.o, X. oryzae* pv. *Oryzae; X.a.v , X. axonopoidis* pv. *Vesicatoria;, Ra.s, R.solanacearum; Ps., Ps. Syringae*. PE, Petroleum ether; CF, chloroform; EA, ethyl acetate; M, methanol.

P. syringae and *X. vesicatoria.* Methanol extracts of *C. latifolium* Bl. and *V. indica* L. has broad range of activity including both Gram positive and Gram negative test bacteria. Almost all test bacteria were sensitive to these two plant extracts except *E. coli* and *Ralstonia solanaceraum* (Table 3).

DISCUSSION

Compared to aqueous extracts, the solvent extracts showed more antibacterial activity. The initial screening of plants for antibacterial activity typically begin by crude aqueous extracts. The use of aqueous extracts of some plants has been reported from many workers. The agar well diffusion method was suggested as common method for aqueous extracts to determine the antibacterial susceptibility tests and for solvent extracts disc diffusion method is suitable (Cowan, 1999).

Less work has been reported on antibacterial activity of *C. latifolium;* but, the *Combretum* species are known to have more antifungal activity when compared to antibacterial activity. Acetone leaf extracts of *Combretum* species is reported to have antibacterial activity ranging from 0.1 to 6mg/ml. From *Combretum woodi,* Combrestastain-5 isolated from leaves which is having activity against *Staph. Aureus* and *Ps.*

aeruginosa. The Combretum species is known to have flavonoids, alpinetin and chalcone which are having significant antibacterial activity (Masoko and Eloff, 2007). Earlier survey on plants for alkaloids, saponins and tannins shows the presence of alkaloids and tannin in Combretum ovalifolium (Hungund and Pathak, 1971). The leaves of Combretaceae family are known for their pharmacological activity. Forty eight extracts of four plants of the family Combretaceae were screened for antibacterial activity against standard organisms as well as clinical isolates. The extracts in different solvent systems showed high activity against both standard organisms and clinical isolates. Phytochemical screening revealed that the plants were very rich in tannins to which antibacterial activity may be attributed (Elegami et al., 2002). P. macrantha is also a known medicinal plant in Western Ghats. The stem bark of this plant have been reported to have antibacterial activity against Staph. aureus, Ps. B.subtilis aeruginosa, and (Karuppusamy and Rajasekaran, 2009). Apart from this, the petroleum ether and aqueous extracts of P. macratnha is reported to anti-inflammatory anti-arthritic possess and properties(Kulkarni, 2009). Persea species having sesquiterpenes are known to have cytotoxic activity, antibacterial activity (Taha and Raveesha, 2008; Palazzo et al., 2009) and sterols having anti-diabetic effect (Koffi et al., 2009). In this study, more inhibitory activity was recorded against Gram negative bacteria when compared to Gram positive bacteria which represent the role of phytoconstituents towards the action on permeability barrier of Gram negative bacteria, that is, on peptidogylcan laver.

The advantages of plant extracts over synthetic chemicals are possession of low mammalian toxicity, minimal health hazards and environmental pollution. There is practically no risk of developing pest resistance of these products when used in their natural form. There are fewer hazards to non-target organisms but resurgence has not been reported in the use of botanicals. No adverse effect on plant growth, seed viability or food quality has been reported. Botanicals are less expensive and easily available because of their natural occurrence in abundance (Opara and Wokocha, 2008). Although natural products are a vast source of compounds with seemingly unlimited chemical and functional diversity and have been rich source for lead molecules in drug discovery programs, naturally occurring combinations of the phyto compounds can be synergistic and often results in crude extracts having greater antimicrobial activity than the purified individual constituents (Alam et al., 2009; Tu et al., 2010). In conclusion, present bioprospecting study justifies the ethno-medicinal uses of the selected medicinal plants and also reveals the potentialities of medicinal plants such as C. latifolium Bl. and P. macrantha (Nees.) Kosterm. to isolate a promising natural compound for the management of the bacterial diseases.

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