Antimicrobial screening of ethnobotanically important stem bark of medicinal plants

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ABSTRACT

Background: The stem barks are the rich sources of tannins and other phenolic compounds. Tannins inhibited the growth of various fungi, yeast, bacteria and virus. Hence, ten stem barks of ethnomedicinally important plants were screened for antibacterial and antifungal activities against human pathogenic strains. **Methods:** Air-dried and powdered stem bark of each plant was extracted with 50% aqueous ethanol, lyophilized and the dried crude extracts were used for the screening against 11 bacteria and 8 fungi. Antibacterial and antifungal activities were performed according to microdilution methods by NCCLS. **Results:** The plants *Prosopis chilensis*, *Pithecellobium dulce, Mangifera indica* showed significant antibacterial and antifungal activities against *Streptococcus pneumonia, Enterobacter aerogenes, Klebsiella pneumonia* and *Candida albicans* with MIC of 0.08mg/ml. *Pithecellobium dulce* bark also showed significant antibacterial activity against Bacillus cereus. **Conclusion:** The bark of *Pithecellobium dulce* has more or less similar activity against the known antibiotic and may be considered as potent antimicrobial agent for various infectious diseases.

Key words: Antibacterial, antifungal, bark, ethnobotany, MIC.

INTRODUCTION

Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 people every day; especially the burns remain a large public health issue and approximately 50-75% of hospital deaths are reported to be due to secondary infections.[1] Another important factor is that drug resistance to human pathogenical bacteria has been increasing not only in the developing countries but throughout the world due to indiscriminate use of antibiotics. The drug resistance bacterial and fungal pathogens have further complicated the treatment of infectious diseases in immunocompromised AIDS and cancer patients. In the present scenario due to emergence of multiple drug resistance to human pathogenic bacteria and fungi, especially the antibiotic penicillins, cephalosporins and chloromphenical types involve the enzymic inactivation of the antibiotic by hydrolysis or by the formation of an active derivative. This has opened a new vista for the search of new antimicrobial substance.

Search for newer drugs from plant has been increasing day by day due to the emergence of new diseases and alarming side-effects of synthetic drugs. Several plant species are being used by ethnic groups for the treatment of various diseases like dysentery, skin diseases, asthma, malaria, and a horde of other indications.[2-4] Natural products of higher plants may give a new source of antimicrobial agents. Tannins are water-soluble polyphenols and their presence in plants mostly located in dead or dying cells. They exert an inhibitory effect on many enzymes due to protein precipitation and hence they may contribute a protective function in barks and heartwood.[5] The antimicrobial activities of tannins are well documented. The growth of many fungi, yeast, bacteria, and viruses are inhibited by tannins.[6] Generally barks are considered a rich source of tannins. This paper present the antimicrobial screening of stem barks of ten medicinal plants. The selection of the plants for evaluation was based on traditional uses.

MATERIALS AND METHODS

The stem bark of medicinally important plants viz. *P. chilensis*

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(Molina) Stuntz, *Mangifera indica L., Ceiba pentandra* (L.) Gaertn, *Senna siamea, Tectona grandis, Semecarpus anacardium, Holoptelea integrifolia, P. dulce, Cedrella toona,* and *Strychnos nux-vomica* were collected from the field. The herbarium were prepared and deposited in the pharmacognosy and ethnopharmacology division herbarium wide voucher specimen numbers as shown in Table 1.

Air-dried and powdered stem bark of each plant was percolated with 50% aqueous ethanol (v/v) at 25°C±2°C for 24 h (consecutively three times). The extracts were decanted, filtered with Whatman No. 1 filter paper, and concentrated under reduced pressure and low temperature (below 45°C). The concentrated extracts were lyophilized and the dried crude extracts were used for the screening. The test microorganisms used for the antimicrobial screening were 11 bacteria and 8 fungi and procured from Institute of Microbial Technology (IMTECH) Chandigarh, India. *Micrococcus luteus* MTCC (106), *Bacillus subtilis* MTCC (121), *Bacillus cereus* MTCC (430), *Enterobacter aerogenes* MTCC (111), *Escherichia coli* MTCC (443), *Klebsiella pneumonia* MTCC (109), *Proteus mirabilis* MTCC (1429), *Pseudomonas aeruginosa* MTCC (424), *Staphylococcus aureus* MTCC (96), *Salmonella typhimurium* MTCC (98), *Str. pneumonia* MTCC (2672), *Candida albicans* MTCC (183) and *Cryptococcus albidus* MTCC (2661), *Trichophyton rubrum* MTCC (296), *Aspergillus niger* MTCC (16404), *A. flavus* MTCC (1973), *A. spinulose* MTCC (16919), *A. terreus* MTCC (1782), and *A. nidulans* MTCC (11267).

Stock bacterial inocula suspensions were obtained from 6-12 h culture on Mueller Hinton Broth (MHB, Oxoid) at 37°C. These final suspensions served for the inocula preparation. The cell density of each suspension was determined by NCCLS[7] using a counting chamber and then adjusted to 0.5 McFarland turbidity at the concentration of 10^5-10^6 CFU/ml by dilution with MHB. The fungi were grown on Sabouraud Dextrose Agar (SDA, Oxoid) at 28-30ºC for 3±7 days, to induce conidia formation. Then, the culture was washed with 2 ml of peptone water (HiMedia Lab. Pvt. Ltd. India) to prepare a suspension. This suspension was transferred to a sterile tube and kept for 5 min. The upper homogenous suspensions were transferred in a new sterile tube, and added appropriate

Table 1: Details of the stem bark material procured along with their medicinal properties

Species (family) voucher specimen number	Local name	Distribution	Medicinal uses
Prosopis chilensis (Fabaceae) 218665	Vilayati kikar	Cultivated in India, Native of America.	Fruits used as beverage; fodder[11]
Mangifera indica (Anacardiaceae) 218673	Am	Probably indigenous in Sikkim Assam, Khasia hills, higher hills of Satpura range mostly cultivated in tropics.	Bark-abortifacient, used in antifertility, stomachache; leaf - in cancer and cholera; fruit - in diarrhea, digestion, tonic; ripe fruit pulp - laxative[11]
Ceiba pentandra (Malvaceae) 218672	Safed simal	Distributed in the forests in the hotter parts of western and southern India	Root-diuretic, in diabetes and in scorpion sting; gum-tonic, astringent, laxative, in bowl complaints; unripe fruits-astringent, demulcent[12]
Senna siamea (Lam.) H. S. Irwin & Barneby (Fabaceae) 218674	Kassod	Western Peninsula	Antioxidant, analgesic and anti-inflammatory[13]
Tectona grandis L. F. (Verbenaceae) 218674	Sagon	Konkan, Western Ghats, Ghats of Mumbai and Madras States, Circars Deccan Carnatic and Madhya Bharat	Stem bark - diarrhea, stomachache; wood oil - in eczema, ring worm; wood - in eye diseases, headache swelling; fruit - hair tonic[11]
S. anacardium L. F. (Anacardiaceae) 221328	Bhilwa	Sub-Himalayan tract, Assam, Khasia hills, Chittagong, Madhya Bharat, Gujarat, Konkan, Kanara and forests of all districts of Madras states	Bark - gonorrhea; fruit - in psoariasis, skin diseases;[11] nut - in syphilis, skin diseases, nervos debility neuralgia, asthma, dyspepsia, piles and abortifacient[14]
H. integrifolia (Roxb.) Planch (Ulmaceae) 218675	Chilbil	Sub-Himalayas, Ajmer, Bundelkhand, Bihar, Assam and West Peninsula	Stem bark - in bone fracture, rheumatism, ringworm, scabies, ulcers; leaf - inflammation of body[11]
P. dulce (Roxb.) Benth (Fabaceae) 2186710	Jangal-Jalebi	Cultivated throughout India, native of tropical America	Bark - used as febrifuge; root - antirabic in dog bite; leaves - in leprosy and promotes hair growth[11]
Cedrella toona Roxb. (Meliaceae) 2186700	Toon	Sub-Himalayan tract, Chitta-Gong, Assam, Chota Nagpur, Ganjam, Western Ghats of Bombay, hills of West Peninsula	Bark - antiseptic, astringent, tonic, in chronic infantile dysentery, in fever, ulcers; leaf - Bronchitis[11]
Strychnos nux-vomica L. (Loganiaceae) 218159	Kuchla	Forests of Gorakhpur, Bihar, Orissa, Konkan, Deccan, Carnatic West coast of Madras states in deciduous forests and Travancore	Seed - dyspepsia, chronic, dysentery, diarrhea, epilepsy chronic constipation, gout, chronic rheumatism,[14] chickenpox fever, eczema, piles, rheumatism and skin diseases;[11] root bark - in cholera; leaves - as poultice to sloughing wounds and ulcers[12]

quantity of Mycological peptone (MP. HiMedia Lab. Pvt. Ltd. India). This suspension was adjusted microscopically about 10^4 CFU/ml.

Antibacterial activity was performed according to microdilution method by NCCLS[8] and Zgoda and Porter[9] with slight modifications. Briefly, extract was dissolved 2.5% dimethyl sulphoxide (DMSO, Sigma) and filtered through 0.2 micron nonpyrogenic filter and serially diluted (twofold) with 2.5% DMSO to give a range 0.08 – 50 mg/ml. Tests were performed in sterile U bottom 96 - well by dispensing in to each well 95 µl of MH broth and 5 µl of inoculums (0.5 McFarland turbidity). Here 100 µl of test extract was finally added to each appropriate well. The final volume in each well was 200 µl. Standard antibiotic - gentamicin (Sigma) was used as positive control. The plates were covered with sterile sealer and incubated at 37° C for 18-24 h. To indicate bacterial growth, 40 µl of 0.2 mg/ml p-iodonitrotetrazolium violet (INT, Sigma) solution was added to each well and incubated for further 30 min. Inhibition of bacterial growth was visible as a clear well and the presence of growth detected by the presence of pink red color.

The method used for antifungal activity was M27-T described by the NCCLS[10] with some modifications. The culture medium was mycological peptone. Ketoconzole (Sigma) was used as standard. Concentration range was 0.08-50 mg/ml. After inoculation, the plates were incubated at 28-30ºC for 24-96 h. The minimum inhibitory concentration (MIC) values of extract/compound of antifungal agent were estimated by lack of visual turbidity (matching the negative growth control).

RESULTS AND DISCUSSION

The ethnobotanical data of these barks and their distributions have been compiled in Table 1. The inhibitory concentration of aqueous ethanolic (50%) extracts of all the bark has been established.

The results of the present work indicate that bark selected have significant antibacterial activity as compared to antifungal. Very weak activity was observed against *C. albidus* and *A. spinulose* in comparison to known antibiotics [Table 2]. Some of the extracts like *P. chilensis* and *P. dulce* gave very low MIC values and inhibited the growth of *S. pneumonia, K. pneumonia,* and *E. aerogenes* with a concentration of 0.08 mg/ml. The extract of *P. dulce* bark show promising activity against *B. cereus, E. aerogenes, K. pneumonia, Str. pneumonia,* and *C. albicans* [Table 2]. It is interesting to note that this bark has significant inhibition against *K. pneumonia* and *E. aerogenes* as compared to known antibiotic gentamicin, which is considered more potent antibiotic against gram negative bacteria. The extracts of *H. integrifolia* and *S. nux-vomica* showed better antibacterial activity against *B. cereus* and the extracts of *P. chilensis* and *S. anacardium* possesses similar inhibition against *B. subtilis* as compare to the positive control.

The stronger and broader spectrum of antifungal activity was also observed in the extract of *M. indica* and *P. dulce* against *C. albicans* with MIC values of 0.08 mg/ml [Table 2]. The ongoing results indicated that the plant species assayed possess more antibacterial property and explain the use of these plants in folk medicine for the treatment of various diseases, which may be caused by

Table 2: MIC values of aqueous ethanolic extracts (50%) of ten stem bark against microorganisms by microdilution method (conc. mg/ml)

Microorganisms	Pc	Ct	Mi	Cp	Cs	Tg	Sa	Hi	Pd	Sn	Ge
Bacterial strains *Micrococcus luteus* (MTCC-106)	0.31	0.31	0.31	0.31	0.62	0.62	-	1.25	0.16	0.16	0.08
Bacillus subtilis (MTCC-121)	0.16	-	0.62	1.25	1.25	0.62	0.16	-	-	-	0.16
B. cereus (MTCC-430)	-		0.62	1.25	1.25	-	1.25	0.16	0.31	0.16	0.31
Enterobactor aerogenus (MTCC-111)	-	0.62	-	-	-	-	-	-	0.08	-	0.16
Escherichia coli (MTCC-443)	-	0.31	-	-	-	-	-	0.62	-	0.31	0.08
Klebsiella pneumonia (109)	-	0.62	-	-	-	-	-	-	0.08	-	0.16
Proteus mirabilis (MTCC1429)	-	-	-	-	-	-	-	0.62	0.31	-	0.08
Staphylococcus aureus (MTCC-96)	0.62	-	-	-	-	-	-	-	-	-	0.16
Salmonella typhimurium (MTCC-98)	-	0.31	-	-	-	-	-	0.31	0.16	-	0.02
Str. pneumonia (MTCC-2672)	0.08	0.31	0.62	0.61	1.25	-	-	0.16	0.16	0.31	0.04
Pseudomonas aeruginosa (MTCC-424)	-	-	-	-	-	-	-	-	-	-	0.08
Fungal Strain											Kt
C. albicans (MTCC 183)	-	-	0.08	-	-	-	-	-	0.08	0.62	0.08
Cryptococcus albidus (MTCC 2661)	0.62	1.25	0.31	-	-	0.31	-	0.16	-	-	0.04
Aspergillus niger (MTCC 16404)	-	-	-	-	-	-	-	-	-	0.62	0.08
A. spinulose (MTCC 16919)	-	-	-	-	-	0.62	1.25	1.25	1.25	0.62	0.16
Trichophyton rubrum (MTCC 296)	-	0.31	-	-	-	-	-	-	-	-	0.04

MTCC - microbial types of culture collection; Ge - gentamicin (sigma); Kt, ketoconzole (sigma); Pc - *Prosopis chilensis*; Ct - *Cedrella toona*; Mi - *Mangifera indica*; Cp - *Ceiba pentandra*; Cs - *Senna siamea*; Tg - *Tectona grandis*; Sa - *Semecarpus anacardium*; Hi - *Holoptelea integrifolia*; Pd - *Pithecellobium dulce*; Sn - *Strychnos nux-vomica*.

bacterial infection and also suggest the importance of the ethnobotanical claims for the development of bioactive compounds.

From the present screening, it has been concluded that the bark of *P. dulce* is more potent antimicrobial agent and has more or less similar activity against the known antibiotic. Further, the detailed phytochemical research is required to identify the active principal responsible for aforesaid activities.

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