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Evaluation of Antimicrobial Activities of Some Traditional Medicinal Plants of Bangladesh

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ABSTRACT

Plants have been an actual source of medicine and for many centuries people have been trying to alleviate and treat diseases using different plant extracts. The interest in plants antimicrobial properties has been increased because of antimicrobials of plant origin is effective in the treatment of infectious diseases and reduces many side-effects that are often associated with the use of antibiotics. For the development of new effective antimicrobial agents; medicinal plants are prolific sources. The aim of this study was to evaluate the antimicrobial activity of some traditional medicinal plants of Bangladesh for treatment of manifestations caused by microorganisms. Therefore, 80% acetone extracts of the 37 plant extracts were evaluated for their antimicrobial potentials. Among the tested plant extracts, 12 extracts showed the antifungal activity against *Cladosporium herbarum* and 19 extracts exhibited growth inhibition against *Staphylococcus aureus*, while 15 extracts were inactive. The evaluation of the antimicrobial activities of the plant extracts was utilized to justify their traditional uses as traditional medicinal plants. It could be interesting to isolate the active antimicrobial substances from plant species that could be used for medicinal purposes.

INTRODUCTION

Medicinal plants are used as folk medicine in traditional remedies in many developing countries like Bangladesh and India, due to culturally linked traditions, the lack of medical facilities and doctors, the trust the communities have in the medicinal values of traditional medicine and especially microbial resistance in relation to the modern medicine [(1)]. Nevertheless, if the gist of traditional knowledge can be confirmed by scientific experiments, reasonable and reliable cures can be found against the drug. However, scientific studies are insignificant in Bangladesh; therefore, preliminary screening of the antimicrobial activities of the extracts of some plants growing in Bangladesh which have potential of treating infectious diseases and used as folk medicine is of interest. Plants have been used as therapeutic treatments because of their antimicrobial characters, which are due to compounds synthesized in the secondary metabolism of the plant. Secondary metabolites, such as, polyphenols (tannins, flavonoids), terpenoids and alkaloids have a good antimicrobial activity against a large number of bacteria and fungus [(2)]. The demand for finding new antimicrobial agents from plant is intensively increasing, it is significant to recognize them and start the necessary research. With this viewpoint in mind, the objective of this research was to evaluate the antimicrobial activity from various parts of the 37 traditional Bangladeshi medicinal plant extracts. These plants have many folk medicinal uses [(3)] (Table 1) in Bangladesh and India. For antimicrobial activity, 80% acetone extracts were used because 80% acetone extracts are the best extraction solvent of polyphenol compounds.

MATERIALS AND METHODS

Plants:

The 37 traditional medicinal plant samples selected for the study (Table 1) were collected from rural areas of Mymensingh, Bangladesh, in October and identified by Mr. Habib Mohammad Naser, Bangladesh Agriculture Research Institute (BARI), Joydeppur, Gazipur, Bangladesh. The plant samples were air dried in the shade, and coarsely powdered by an electric nailer. Their uses in traditional medicines and previously isolated constituents are listed in Table 1.

Table 1. Uses and previously isolated classes of constituents from traditional medicinal plants [a]

Plant name (family)	Uses in traditional medicine	PICC
Lannea coromandelica (Houtt.) Merr., syn. *L. grandis* (Dennst.) Engl. (Anacardiaceae)	Leprous and obstinate ulcers, impotence, elephantiasis, vaginal troubles, halitosis, heart disease, dysentery, rheumatism (3)	Sterols, flavonoids, physcion anthranol B, phlobatannin, fatty acids (3,4)
Mangifera indica Linn. (Anacardiaceae)	Astringent, ophthalmia, eruptions, haemorrhages, menorrhagia, dysentery, antiscorbutic, laxative (3)	Volatile oil, polyphenols, flavonoids, terpene, sterols, tannins, vitamins (3,5)
Centella asiatica (Linn.) Urban., syn. *Hydrocotyle asiatica* Linn. (Apiaceae)	Dysentery, convulsive disorders, ulcers, eczema, leprosy, urinary, ovarian irritation, eye troubles, sedative, analgesic, antidepressive (3)	Triterpenes, triterpenes saponosides, tannin, saponins, sterols, essential oil, fatty oil, alkaloid (3,6)
Catharanthus roseus (Linn.) G. Don., syn. *Vinca rosea* Linn., *Lochnera rosea* (Linn.) Reic. (Apocynaceae)	Diabetes, stomachic, anti-cancer, leukaemia in children (3)	Indole alkaloids, terpenoids glycosides, fatty acid esters, alcohol, alkanes, vinblastine, vincristine (3,7)
Rauvolfia serpentine	Diarrhoea, dysentery, cholera,	Indole alkaloids, sterol,

(Linn.) Benth. (Apocynaceae)	blood pressure, schizophrenia, epilepsy, hypochondria [3]	ajmalicine, unsaturated alcohols [3,8]
Basella alba Linn., syn. *B. rubra* Linn. (Basellaceae)	Constipation, catarrh, gonorrhoea and balanites [3]	Polysaccharide, sterols, vitamin, flavonoids, saponins, carotenoids [3]
Saraca asoca (Roxb.) De Wilde, syn. *S. indica* Linn. (Caesalpiniaceae)	Menorrhagia, biliousness, dyspepsia, dysentery, ulcers, syphilis [3]	Tannins, sterol, saponin, haematoxylin, galactoside, flavonoids, fatty acid [3,9]
Terminalia arjuna (Roxb.) W. & A. (Combretaceae)	Hypertension, asthma, dysentery, menstrual problems, leucorrhoea, cardiac tonic, astringent, febrifuge [3]	Tannins, alkaloids, flavones, terpene, steroid, lactone, phenolics [3,10]
Terminalia bellirica Roxb. (Combretaceae)	Hepatitis, eye diseases, diarrhoea, dropsy, piles, leprosy, rheumatism, purgative [3]	Tannins, sterol, fatty acid, mannitol, sugars, tripenoids [3,11]
Terminalia chebula (Gaertn.) Retz. (Combretaceae)	Constipation, dysentery, jaundice, painful menstruation, asthma, hiccup, rheumatism, cardio tonic, purgative, astringent [3]	Tannins, steroid, phenolic acids, polyphenols, fatty acids, triterpenoid, flavonoids [3,12]
Lagenaria siceraria (Mol.) Stan., Syn. *L.*	Cholera, jaundice, headache,	Fatty oil, protein, saponins, triterpenoids,

vulgaris Ser., *L. leucantha* (Duch.) Rusby. (Cucurbitaceae)	earache, diuretic [3]	fatty acid, saponin, flavonoids [3,13]
Phyllanthus emblica Linn., syn. *Emblica officinalis* Geartn. (Euphorbiaceae)	Diarrhoea, dysentery, anemia, jaundice, dyspepsia, insomnia, leucorrhoea, tympanitis, carminative, laxative, antacid [3]	Tannins, essential oil, phyllembin, mucic acid, terpenoids, alkaloids, flavonoids, fatty acids [3,14]
Ricinus communis Linn. (Euphorbiaceae)	Constipation, rheumatism, inflammation, nervous disorders, purgative [3]	Fatty acid, alkaloids, proteins, enzymes, sterols [3,15]
Leucas lavandulaefolia Rees., syn. *L. ljinifolia* Spreng. (Labiatae)	Headaches, coughs, scabies, febrifuge [3]	Flavonoids, phenolic acid, terpenoid, steroids alkaloid, linifolioside [3,16]
Cinnamomum verum Presl., syn. *C. zeylanicum* Bl. (Lauraceae)	Vomiting, nausea, stimulant, carminative [3]	Tannin, terpene, eugenol, phellandrrene, caryophyllene, essential oil [3]
Allium cepa Linn. (Liliaceae)	Headache, rheumatism, flatulence, dysentery, aphrodisiac, emmenagogue, emetic, diuretic and antihypertensive [3]	Essential oil, proteins, polyphenols, flavonoids, saponin, steroids [3]
Lawsonia inermis Linn.,	Jaundice, leprosy, soporific, skin	Flavonoids, triterpenes,

syn. *L. alba* Lamk. (Lythraceae)	diseases, emollient poultice, astringent gargle in sore throat [3]	alkaloid, saponins, sterol, essential oil, lawsone, quinones, phenolics, fatty acids [3,17]
Hibiscus rosa-sinensis Linn. (Malvaceae)	Menorrhagia, vaginal and urinary discharges, coughs, chronic dysentery [3]	Flavonoids, vitamins, sterol, taraxerol, cyclopropane compounds [3,18]
Azadirachta indica A. Juss., syn. *Melia azadirchta* Linn. (Meliaceae)	Tumours, smallpox, diarrhoea, cholera, malaria, inflammation, ulcers, eczema, rheumatism, atomic dyspepsia, antiseptic [3]	Saponins, alkaloids, triterpenoids, flavonoids, steroids, tannin, fatty acids, volatile oil [3,19]
Acacia catechu Wild. (Mimosaceae)	Leucoderma, leprosy, psoriasis, erysipelas, ulcer, bronchitis, piles, astringent and aphrodisiac [3]	Tannins, flavonoids, polyphenolic flavonol, alkaloids, sterol [3,20]
Acacia nilotica (Linn.) Bel., syn. *A. Arabica* (Lam.) Wild. (Mimosaceae)	Gonorrhoea, leucorrhoea, piles, diarrhoea, dysentery, diabetes, sexual debility, styptic [3]	Polyphenols, steroids, protein, tannins, flavonoids, alkaloids, terpene [3,21]
Moringa oleifera Lamk., syn. *M. pterygosperma* Gaertn. (Moringaceae)	Tetanus, paralysis, rheumatism, epilepsy, carminative, diuretic, cardiac, circulatory tonic, chronic rheumatism, giddiness, hysteria [3]	Protein, fatty acids, alkaloids, sugars, sterol, pterygospermin, vitamins, polyphenols

		[3,22]
Eucalyptus sp. (Myrtaceae)	Bronchial catarrh, diphtheria, ulcers, febrifuge, carminative, expectorant, diaphoretic, antiseptic [3]	Essential oil, tannin, aldehydes, ketones, phenols, terpenes [3]
Eugenia caryophyllus Thunb (Myrtaceae)	Stomachic, diarrhea, kidney-Yang deficiency, vomiting [23]	Eugenol, eugenol acetate, β-caryophyllene, ylangene, steroid [23]
Papaver somniferum Linn (Papaveraceae)	Analgesics, chronic pain [3]	Alkaloids, terpenoids, flavonoids, polysaccharides, sugars, waxes [3,24]
Trigonella foenum-graecum Linn. (Papilionaceae)	Menstrual disorders, hypertension, diabetes, sexual problems, rheumatism, carminative, astringent, emollient, aphrodisiac [3]	Alkaloids, essential oil, saponins, fatty acids, flavonol glycosides, mucilage, sugars, alkaloid [3,25]
Piper nigrum Linn. (Piperaceae)	Asthma, cholera, fevers, dyspepsia, constipation, gastric troubles, ascites, anemia, pungent, carminative, antiperiodic [3]	Alkaloids, essential oil, terpenes, phenolic, lignans, chalcones, flavonoid, steroid [3,26]
Ampelygonum chinense (L.) Lindley	Healing wounds, tonic and	Flaconoids, essential oil, polyphenols,

(Polygonaceae)	antiscorbutic [3]	steroids, triterpene, plasticizer [3,27]
Nigella sativa Linn. (Ranunculaceae)	Fever, skin eruptions, scorpion-sting, carminative, diuretic, emmenagogue [3]	Fatty acids, essential oil, terpenes, saponins, phenolics, quinones [3,28]
Aegle marmelos (Linn.) Corr. (Rutaceae)	Constipation, dysentery, diarrhoea, melancholia, antiseptic, digestive, stomachic, laxative, astringent and febrifuge [3]	Essential oil, triterpenes, proteins, alkaloids, steroids, coumarine and cinamate derivatives [3,29]
Capsicum frutescens Linn., syn. *C. minimum* Roxb. (Solanaceae)	Headache, dysuria, bronchitis, inflammation, stomachic, carminative, neuralgia, rheumatism, diuretic [3]	Carotenoids, sterols, esters, phenol, terpenoids, saponin, alkaloids, capsaicin, tocopherol [3,30]
Coriandrum sativum Linn. (Umbelliferae)	Syphilis, carminative, stomachic, diuretic, aphrodisiac, anti-rheumatic, antiscorbutic [3]	Essential oil, flavonoids, steroids, coumarins, terpenoid, phenol [3]
Artocarpus heterophyllus Lamk., (Urticaceae)	Skin diseases, asthma, diarrhea, carminative, laxative and diuretic [3]	Tannins, flavonoids, steroids, starch, vitamins, lignin [3,31]

Vitex negundo Linn. (Verbenaceae)	Rheumatism, ulcers, scrofulous sores, febrifuge, expectorant, diuretic, vermifuge, anodyne [3]	Essential oils, alkaloid, sterol, flaconoid glycosides, amino acids, carotene [3,32]
Curcuma longa Linn., syn. *C. domestica* Val. (Zingiberaceae)	Scabies, eye diseases, asthma, gonorrhoea, urinary diseases, parasitic skin diseases, anthelmintic, antacid, carminative, jaundice [3]	Terpenoids, phenolic compounds, sterols, alkaloids, essential oil, curcumin, turmerone, oleoresin [3,33]
Elettaria cardamomum (Zingiberaceae)	Digestive enhancer, gastralgia, enuresis, spermatorrhea, phlegm [3]	Essential oil, terpinene [3]
Zingiber officinale Rosc. (Zingiberaceae)	Dyspepsia, sore throat, constipation, dysentery, earache, vomiting, diarrhoea, carminative, stomachic, digestive, rubefacient [3]	Essential oil, starch, protein, sugars, terpenes, phenolic compounds, gingerol, zingerone [3, 34]

[a] PICC, previously isolated classes of constituents.

Plant extracts:

The powdered plant materials were soaked with 80% acetone. The 80% acetone extracts were used for the antimicrobial activity. The selected plant parts and yields are given in Table 2.

Antibacterial assay:

The antibacterial activity was evaluated by TLC bioautography method [35] using *Staphylococcus aureas* (AHU1142) as the test bacterium. Briefly, the bacterium was grown in nutrient both (20 ml of media in 100 ml flask) at 25^{o}C for 24 hours on a shake at a speed of 100 rpm. A known amount of test extracts (in acetone) was spotted by a micro syringe to give a circular zone (*ca* 14 mm i.d.) on pre-coated TLC plate (Silica gel 60 F_{254} plates, 20x20 cm, 0.25 mm thick, Merck). After the solvent had evaporated, the bacterial culture in nutrient both was sprayed onto the previously prepared TLC plates and incubated for 8 hours at 37^{o}C. The plates were then sprayed with 5 ml of an aqueous solution of INT (*p*-iodonitrotetrazolium violet) (5 mg/ml), and again incubated overnight at 37^{o}C. Antibacterial compounds appeared as clear spots against a pinkish to light purple background. Chloamphenicol was used as a positive control.

Antifungal activity:

Antifungal activity was carried out by modifying TLC bioautography method [36]. A known amount of the test extract and the reference compound (luteone, dissolved in acetone) were loaded onto the TLC plates and developed in $CHCl_3$/ EtOAc/ Acetone/MeOH (40:5:5:1). After the solvent had evaporated, a spore suspension of *Cladosporium herbarum* (AHU9262) in a medium was sprayed over the developed TLC plates, which were incubated at 25^{o}C under humid conditions for 3 days. Antifungal activities appeared as clear white zones against the black colored conidial growth on TLC plate. The diameters of the inhibition zones were measured in mm.

RESULTS AND DISCUSSION

Medicinal plants have a long and rich ethnopharmacological history of traditional knowledge and a large population relies on them for their therapeutic effects. Natural products (secondary metabolites) from medicinal plants may potentially control microbial growth and are a source of many potent and powerful drugs. Antimicrobial screening of plants has been the source of innumerable therapeutic agents. 80% acetone extracts of the different parts of the plant have been screened for their possible antimicrobial activities against *C. herbarum* and *S. aureas*. The different parts used include aerial parts (A), bulb (B), fruit (F), leaves (L), leaves and rhizome

(LR), ripe fruit (RF), rhizome (R), seed (S), stem bark (SB), stem (SM) and whole plant (WP). The data pertaining to the antimicrobial potential of the plant extracts are presented in table (Table 2). The result showed the good antibacterial and antifungal activity against the tested microorganisms.

Table 2. Bioassay results of medicinal plant extracts[a,b]

Plant name (PPI)	*S. aureus* ZID at µg/spot			*C. herbarum* µg/spot of extract loaded		PPI (Yield)
	25	12.5	5	50	10	
Lannea coromandelica	12	10	2	-	-	SB (2.22)
Lannea coromandelica	-	-	-	1	1	L (2.58)
Magnifera indica	8	7	6	-	-	F (3.71)
Centella asiatica	10	9	4	-	-	WP (1.62)
Catharanthus roseus	10	9	6	-	-	WP (2.8)
Rauvolfia serpentine	12	10	2	-	-	A (1.5)
Basella alba	-	-	-	-	-	WP (1.42)
Saraca asoca	-	-	-	-	-	A (2.51)
Terminalia arjuna	8	7	4	1	-	A (7.02)
Terminalia bellirica	-	-	-	1	-	A (2.79)
Terminalia chebula	-	-	-	-	-	A (1.2)
Lagenaria siceraria	10	9	4	-	-	L (1.86)
Phyllanthus emblica	-	-	-	-	-	A (2.5)
Ricinus communis	12	10	3	-	-	A (5.54)
Leucas lavandulaefolia	-	-	-	-	-	SM (6.67)
Cinnamomum verum	-	-	-	2	-	SB (1.61)
Allium cepa	-	-	-	-	-	B (10.3)
Lawsonia inermis	14	12	4	-	-	A (4.79)
Hibiscus rosa-sinensis	-	-	-	-	-	A (4.73)

Azadirachta indica	8	7	4	-	-	A (4.92)
Acacia catechu	-	-	-	1	-	A (2.89)
Acacia nilotica	-	-	-	-	-	A (1.88)
Moringa oleifera	-	-	-	-	-	A (1.16)
Eucalyptus sp.	11	9	4	1	1	A (5.67)
Eugenia caryophyllus	10	9	2	1	-	F (11.46)
Papaver somniferum	-	-	-	-	-	WP (1.33)
Trigonella foenum-graecum	-	-	-	1	1	S (1.99)
Piper nigrum	16	12	2	2	1	S (10.46)
Ampelygonum chinense	-	-	-	-	-	WP (1.29)
Nigella sativa	18	16	10	-	-	S (1.46)
Aegle marmelos	16	14	3	-	-	A (3.18)
Capsicum frutescens	14	13	2	1	-	RF (12.84)
Capsicum frutescens	-	-	-	-	-	WP (1.33)
Coriandrum sativum	12	10	2	-	-	S (1.85)
Artocarpus heterophyllus	-	-	-	-	-	S (1.67)
Vitex negundo	-	-	-	-	-	A (1.99)
Curcuma longa	-	-	-	1	1-	LR (2.64)
Elettaria cardamomum	12	11	-	2	-	F (3.31)
Elettaria cardamomum	14	12	6	-	-	S (3.6)
Zingiber officinale	-	-	-	-	-	RM (2.71)

[a]PPI, plant part investigated; A, aerial parts; B, bulb; F, fruit; L, leaves; LR, leaves and rhizome; RF, ripe fruit; RM, rhizome; S, seed; SB, stem bark; SM, stem; WP, whole plant. Experiments were done in duplicate and results are mean values. ZID, zone of inhibition diameter (mm) -, no inhibition; b, results expressed in number of inhibitory spot (s) on TLC plate; positive control, chloramphenicol at 25 µg/spot (14 mm) and 12.5µg/spot (13 mm) against *S. aureus* and luteone at 10 µg/spot (5 mm) against *C. herbarum*.

TLC bioautugraphy were used for the antimicrobial activity. Clear zones on chromatogram indicated the zone inhibition of growth (ZID=zone inhibition diameter) of organisms after

incubation. The zone inhibition diameter was compared with the positive control. Among 40 extracts of 37 plants, 19 extracts (*L. coromandelica* (SB), *M. indica* (F), *C. asiatica* (WP), *C. roseus* (WP), *R. serpentine* (A), *T. arjuna* (A), *L. siceraria* (L), *R. communis* (A), *L. inermis* (A), *A. indica* (A), *Eucalyptus* sp. (A), *E. caryophyllus* (F), *P. nigrum* (S), *N. sativa* (S), *A. marmelos* (A), *C. frutescens* (RF), *C. sativum* (S), *E. cardamomum* (F) and *E. cardamomum* (S)) exhibited the antibacterial activity against *S. aureas*. As shown in Table 2, six (*L. inermis* (A) (ZID at 25 µg/spot=14 mm), *P. nigrum* (S) (ZID at 25 µg/spot=16 mm), *N. sativa* (S) (ZID at 25 µg/spot=18 mm), *A. marmelos* (A) (ZID at 25 µg/spot=16 mm), *C. frutescens* (RF) (ZID at 25 µg/spot=14 mm) and *E. cardamomum* (S) (ZID at 25 µg/spot=14 mm)) of these nineteen extracts showed greater/equal potency against *S. aureas* than positive control (chloramphenicol at 25 µg/spot (ZID=14 mm). Ten extracts showed promising antibacterial activity (ZID at 25 µg/spot=10~12 mm) against *S. aureas*. Konate *et al*., demonstrated a connection between the concentration of phenolic compounds in the extracts and their antibacterial activity [(37)].

Twelve extracts (*L. coromandelica* (L), *T. arjuna* (A), *T. bellirica* (A), *C. verum* (SB), *A. catechu* (A), *Eucalyptus* sp (A), *E. caryophyllus* (F), *T. foenum-graecum* (S), *P. nigrum* (S), *C. frutescens* (RF), *C. longa* (LR) and *E. cardamomum* (F)) out of 40 extracts were active against *C. herbarum*.

Six extracts (*T. arjuna* (A), *Eucalyptus* sp. (A), *E. caryophyllus* (F), *P. nigrum* (S), *C. frutescens* (RF) and *E. Cardamomum* (F)) out of 40 extracts were shown both antibacterial activity against *S. aureas* and antifungal activity against *C. herbarum.* On the other hand, 15 extracts did not show any antimicrobial activity against the tested microorganisms. The obtained results may provide a support to some of the various traditional uses of these plants. More detailed experiments are essential to identify the active constituents of the active fractions and to verify the therapeutic merits of the active constituents.

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