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IN VITRO ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF SOME TRADITIONAL HERBS

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ABSTRACT

Fourteen medicinal plants known as traditional herbs were investigated for *in vitro* antibacterial activity against Gram-positive *Enterococcus faecalis* and *Staphylococcus aureus* and Gram-negative *Salmonella typhimurium*, *Klebsiella pneumoniae* and *Escherichia coli* bacteria using disc diffusion method. The results of screening are encouraging as out of 14 plants, 11 plant extracts (Activity Profile 78.57%) showed antibacterial activity against one or more tested bacterial species. Eight extracts (Activity Profile 57%) inhibited the growth of *S. aureus. E. faecalis* was the most susceptible bacteria inhibited by 11 plant extracts (Activity Profile 78.57%). Among screened plants only two species (Activity Profile 14%) i.e., *Cassia spectabilis* and *Lantana camara* were found active against *E. faecalis, S. aureus* and *K. pneumonia*e and between these two species *C. spectabilis* was found more effective antibacterial agent than *L. camara*. Phytochemical analysis revealed presence of various phytoconstituents and thus validating their uses in various herbal remedies.

KEYWORDS: Antibacterial activity, Phytochemical analysis, Disc-diffusion assay, Herbal remedies

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INTRODUCTION

The emergence of multiple-drug resistant human pathogenic organisms and undesirable side effects of currently used drugs have been pushing researchers to search for new antimicrobial substances from other sources including plants. Plants produce biologically active compounds which possess many biological properties such as antimicrobial, allelopathic, antioxidant etc. These compounds are produced either as part of their normal program of growth and development or in response to pathogen attack or stress. So, for some decades traditionally used plants are gaining more attention in searching of bioactive compounds with described properties[1]. A focused research will not only achieve definitive knowledge about the plant and its biological properties, but also it may facilitate the synthesis of more potent drug with reduced toxicity and better efficacy. So there is always a need to screen a number of plants with medicinal value for promising biological activity. Antimicrobial properties of traditionally used plants are being reported from all over the world in recent years[2-6]. To make best use of traditional knowledge about antimicrobial properties of medicinal plants, a systematic study is required which involves 1) Screening of medicinal plants known for antimicrobial property, 2) Selection of one among them possessing promising antimicrobial activity and 3) Isolation and identification of active principles as well as subsequent determination of the spectra and potency of isolated compounds. For example, in one systematic study researchers screened 140 medicinal plants used in Mediterranean region as anti-infection agents[7,8] and then selected one of them i.e. *Helichrysum stoechas* for isolation and identification of active principles as well as determination of their potential as antimicrobial agents. Ten antimicrobial principles were isolated from this plant and four of them exhibited activity in a range of 3-25 µg/ml against Gram positive bacteria[9, 10]. Keeping these points of view, in an ongoing project some plants were selected which are known to be used to treat various infections, and to check their antimicrobial potency against some human pathogenic bacteria. The selection of medicinal plants for screening was based on their traditional uses as well as reported for antimicrobial properties in literature. Phytochemical analysis of selected plant species was also performed in order to facilitate further research focused on the isolation and identification of active antimicrobials from selected plant species. Moreover, knowledge about the presence of phytoconstituents in plants are desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of many economically important materials such as- tannins, oils, saponins, flavonoids etc.

MATERIALS AND METHODS

(i) Chemicals

All the chemicals were of analytical grade. Mueller Hinton agar (MHA), Mueller Hinton broth (MHB), sterile discs, standard antibiotics were purchased from Hi-Media (Mumbai, India). Whatman no.1 filter paper from Axiva Sichem Biotech, Delhi, (India).

(ii) Plant material

Fourteen medicinal plant species known to be used to treat various infections were selected to check their antibacterial potency. Their scientific names, known phytoconstituents and traditional uses are presented in table 1. These plants were collected locally and taxonomically identified by Dr. M.C. Rajanna, Professor (Curator), Botanical Garden, University of Agricultural Sciences, Gandhi Krishi Vigyan Kendra, Bangalore. Plant material was washed, dried under shade, powdered and stored in dark until use.

Table 1
Ethnobotanical and phytochemical data of Medicinal plants screened for antibacterial activity

Medicinal Plant (Family)	Part used traditionally	Known Phytoconstituents	Traditional uses	Ref.
Abrus precatorius (Papilionaceae; Fabaceae)	Root, Leaves, Seeds	Abrin, toxalbumin, precol, abrol, glycyrrhizin, alkaloids-abrasine, precasine, triterpenoids-abruslactone A, methyl abrusgenate, abrusgenic acid	Purgative, emetic aphrodisiac, uterine stimulant, abortifacient, toxic, used in nervous disorders, antimicrobial	11, 12
Andrographis paniculata (Acanthaceae)	Whole plant	Andrographolide, deoxyandrographolide, diterpenes	Hepatoprotective, cholinergic, antispasmodic, stomachic, anthelmintic, alterative, blood purifier, used in jaundice, flatulence, cold and upper respiratory tract infections	11
Annona squamosa (Annonaceae)	Whole plant	Quinoline, squamone, bullatacinone, leaves contains anonaine, benzyltetrahydro-isoquinoline, borneol, camphene, camphor, car-3-ene, carvone, ß-caryphyllene, eugenol, farnesol, geraniol, hexacontanol, higenamine, isocorydine, limonine, linalool, linalool acetate, menthone, methylanthranilate, methylsalicylate, methylheptenone,n-octacosanol, á-pinene, ß-pinene, rutin, stigmasterol, ß-sitosterol, thymol& n-triacontanol.	Insecticide, abortifacient, purgative, antibilious, antiemetic, expectorant, antiulcerogrnic, astringent, used for diarrhea and dysentery	11, 13
Calotropis procera (Asclepediaceae)	Whole plant	Calotropin, calotropagenin, latex-uscharin, calotoxin, calactin, benzoyallinenolone, benzolisolineolone, evanidin 3-rhamnoglucoside, proceragenin	Used in fever, bronchial asthma, dyspepsia, flatulence, constipation, mucus in stool, leaves, antimicrobial	11, 14
Cassia spectabilis (Fabaceae)	Leaves, Pods	Sennosides, aloe-emodin, 1,3,8-trihydroxy-2-methylanthraquinone, chrysophanol, physcion, stigmasterol, β-sitosterol and piperidine alkaloids	Laxative, purgative, antimicrobial, antipyratic, anti-inflammatory, antiviral, in treatment of cold & flu	15,16, 17,18
Clerodendrum phlomides (Verbenaceae)	Whole plant	Scutellarein, Pectolinarin, β-sitosterol, D-mannitol, Ceryl alcohol, Clerodin, Clerosterol, Clerodendrin A	Used in dyspepsia, stomachache, colic, cholera, dysentery, postnatal fever, during convalescence from measles, in nervous disorders	11
Costus speciosus (Zingiberaceae)	Rhizome, Leaves	Dioscin, gracillin, β-sitosterol-β-d-glucoside, sapogenin, diogenin	Astringent, purgative, depurative, anti-inflammatory (used in gout, rheumatism, bronchitis, asthma, catarrhal fevers, dysuria), anthelmintic, antivermin, maggoticide, antifungal	11
Ficus religiosa (Moraceae)	Leaves, Twigs, Bark	Bergaptol, bergapten, β-sitosteryl-d-glucoside, vitamin k, n-octacosanol, methyl oleanolate, lanosterol, stigmasterol, lupen-3-one	Astringent, antiseptic, alterative, laxative, haemostatc, vaginal disinfectant, used in diabetes, diarrhea, leucorrhoea, menorrhagia, nervous disorders, skin diseases, also applied on ulcers and wounds, laxative	11, 14
Lantana camara (Verbenaceae)	Leaves	Carmine, isocamerene, micramene, lantadenes A &B, lancamarone, lantanine	Antirheumatic, antimalarial, used in tetanus &ataxy of abdominal viscera, pounded leaves are applied to cuts, ulcers and swellings, decoction is used as a lotion for wounds	11, 14
Ocimum sanctum (Labiatae)	Whole plant	Eugenol, carvacrol, methyl eugenol, caryophyllene, nerol, ursolic acid, apigenin, luteolin, apigenin-7-o-glucuronide, luteolin-7-o-glucuronide,	Carminative, stomachic, antispasmodic, antiasthmatic, antirheumatic, expectorent, stimulant, hepatoprotective,	11, 14

		orientin, molludistin	antiperiodic, antipyretic, diaphoretic, antimalarial, antistress, adaptogenic, antibacterial, antifungal	
Plumbago zeylanica (Plumbaginaceae)	Roots	Plumbagin	Used in dyspepsia, leprosy, piles, influenza, black-water fever and skin diseases,	11
Syzygium cumini (Myrtaceaae)	Bark, seeds, fruits & Leaves	Jambosine, ellagic acid, gallic acid, β-sitosterol, friedelan-3-alpha-ol, kaempferol, quercetin, betulinic acid, crategolic acid	Stomachic, carminative, diuretic, antidiarrhoeal, hypoglycaemic, antibacterial, antidysentric	11
Tinospora cordifolia (Menispermaceae)	Whole plant	Berberine, columbin, chasmanthin, palmarin, tinosporon, tinosporic acid and tinosporol	Antipyretic, antiperiodic, anti-inflammatory, antirheumatic, spasmolytic, hypoglycaemic, hepatoprotective, antacid, antidiarrhoeal, antidysentric	11
Vitex negundo (Verbenaceae)	Seeds, Leaves, Flowers	Iridoid glycosides, isomeric flavanones, luteolin-7-glucoside, hentriacontane, β-sitosterol, stigmasterol, vitexicarpin, betulinic acid, ursolic acid, *p*-hydroxybenzoic acid, 5-oxyisophthalic acid, vitextriterpene, 5,7,3' trihydroxy-6,8,4'-trimethoxy flavone	Used in spermatorrhoea, as a rejuvenating tonic, anti-inflammatory, analgesic, astringent, febrifuge, antidiarrhoeic	11

(iii) Preparation of Extracts

Air dried leaves were powdered in a grinder. Ten gm of air dried powder was placed in 100 ml. of 70% methanol in a conical flask, plugged with cotton and then kept on a rotary shaker at 190-200 rpm for 24 h. After 24 h., the mixture was filtered with whatman no. 1 filter paper. Filtrates were evaporated using rotary evaporator and dried extracts were stored at 4^0 C until use.

(iv) Microorganisms

Five clinical isolates of human pathogenic bacteria tested in the present study were *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium, Klebsiella pneumoniae* and *Enterococcus faecalis* kindly provided by Dr. D. C. Mohana, Microbiologist, Department of Microbiology and Biotechnology, Bangalore University, Bangalore, India. The bacterial species were cultured at 37 ^{0}C on MHA medium, stored at 4 ^{0}C and subcultured regularly after every two weeks.

(v) Determination of antibacterial activity

Antibacterial activity of plant extracts was determined by agar disc diffusion method[19]. Bacterial suspension (10^6 CFU/ml, 100 µl) was swabbed evenly onto the surface of Mueller-Hinton agar plate with a sterile cotton-swab. The extracts were dissolved in DMSO (100mg/ml). Sterile paper discs of 6mm diameter were impregnated with 40 µl of each test sample. These discs were placed on the culture inoculated agar plates. Discs with equal amount of DMSO were used as negative control. Antibiotic susceptibility testing of human pathogenic bacteria Antibiotic sensitivity of tested human pathogenic bacteria was determined against a number of antibiotics. The potency of antibiotics per disc is given in table-2.

Table 2
Antibiotics used to test antibiotic sensitivity of human pathogenic bacteria

S.no.	Antibiotic	Potency/disc
1.	Bacitracin	10 U
2.	Chloramphenicol	30 µg
3.	Penicillin G	10 U
4.	Polymixin B	300 µg
5.	Gentamicin	10 µg
6.	Neomycin	30 µg

(vi) Phytochemical analysis

Plant extracts were tested for the presence of bioactive compounds by using standard methods[20, 21, 22].

1) Test for Alkaloids- 1 ml of filtrate with 2 ml of dragendroff's reagent shows turbid orange colour.
2) Test for Phenols and Tannins- 1ml filtrate was mixed with 2 ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.
3) Test for Saponins- Crude extract was mixed with 5 ml. of distilled water in a test tube and it was shaken vigorously. Development of foam on the surface of the mixture, lasting for about 10 minutes indicates the presence of saponins.
4) Test for Cardiac Glycosides (Keller-Kilani test) - To 1 ml. of filtrate add 1 ml. of $FeCl_3$ reagent (Mixture of 1 vol. of 5% $FeCl_3$ solution + 99 vol. of glacial acetic acid) and a few drop of conc. H_2SO_4. Greenish blue colour appears within few minutes.
5) Test for flavonoids (Alkaline reagent test) – Crude extract was mixed with 2 ml. of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

RESULTS AND DISCUSSION

1. Determination of antibacterial activity

The antibacterial activity of all plant extracts is given in Table 3.

Table 3

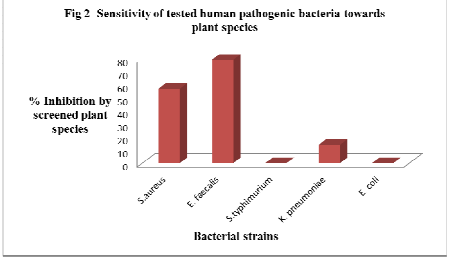
Antibacterial activity of methanol extracts of screened medicinal plant species

S.no.	Plant	Zone of Inhibition (mm)*				
		SA	EF	ST	KP	EC
1.	*Abrus precatorius*	7.3±0.57	7.6±0.57	-	-	-
2.	*Andrographis paniculata*	-	7.3±0.57	-	-	-
3.	*Annona squamosa*	-	7.0±0.00	-	-	-
4.	*Calotropis procera*	-	-	-	-	-
5.	*Cassia spectabilis*	11.3±0.57	12.6±0.57	-	9.3±0.57	-
6.	*Clerodendrum phlomides*	-	8.3±0.57	-	-	-
7.	*Costus speciosus*	-	-	-	-	-
8.	*Ficus religiosa*	7.6±0.57	8.0±0.00	-	-	-
9.	*Lantana camara*	8.6±0.57	10.0±0.00	-	7.6±0.57	-
10.	*Ocimum sanctum*	7.3±0.57	8.6±0.57	-	-	-
11.	*Plumbago zeylanica*	9.6±0.57	8.6±0.57	-	-	-
12.	*Syzygium cumini*	7.0±0.00	7.6±0.57	-	-	-
13.	*Tinospora cordifolia*	7.3±0.57	7.6±0.57	-	-	-
14.	*Vitex negundo*	-	-	-	-	-

** Diameter of inhibition zone including diameter of disc (6mm) expressed as Mean±SD (mm).*
'- '-' denotes no activity. Key of Bacterial strains- SA, Staphylococcus aureus;
EF, Enterococcus faecalis; ST, Salmonella typhimurium; KP, Klebsiella pneumoniae; EC, Escherichia coli

'The results of screening are encouraging as out of 14 plants, 11 plant extracts (Activity Profile (AP) 78.57%) showed antibacterial activity against one or more tested bacterial strains. Among screened plant species many were found active against Gram positive bacteria (*E. faecalis* and *S. aureus*) and only two species i.e., *C. spectabilis* and *L. camara* were found active against one Gram negative bacterial strain *K. pneumonia*e and between these two species *Cassia spectabilis* was found more effective antibacterial agent than *Lantana camara* based on diameter of inhibition zones (Table 3). This is noteworthy that many diseases such as pneumonia, urinary and respiratory tract infections are caused by *Klebsiella* species. The overall sensitivity of tested human pathogenic bacteria towards screened plant species is shown in Figure 1.

Figure 1
Sensitivity of tested human pathogenic bacteria towards plant species



The sensitivity order of tested human pathogenic bacteria was found as: *E. faecalis>S. aureus>K. pneumoniae>S. typhimurium, E. coli*. The reason for different sensitivity between Gram positive and Gram negative bacteria could be due to their different morphology. Gram negative bacteria have an effective permeability barrier, comprised of the outer membrane, which restricts the penetration of many compounds. Six plant species in this study were also screened previously against other pathogenic organisms[2] and showed different results to this study. The difference in activity may be due to collection of plant material from a different environment, extraction procedure and sensitivity of bacterial strains.

2. *Antibiotic susceptibility testing of human pathogenic bacteria*

The sensitivity of the tested human pathogenic bacteria was checked towards various antibiotics as well. The response of tested human pathogenic bacteria towards various antibiotics is shown in Table 4.

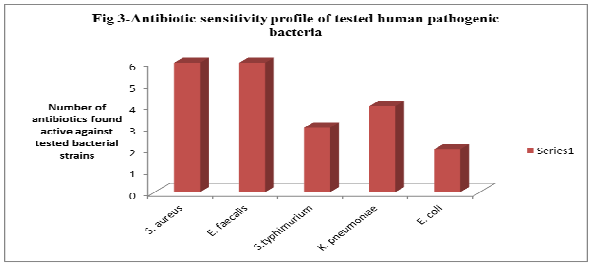
Table 4
Antibiotic susceptibility of tested human pathogenic bacteria

S.no.	Antibiotics (Potency/disc)	Diameter of Zone of Inhibition (mm)*				
		SA	EF	ST	KP	EC
1.	Bacitracin (10U)	8±0.00	11±0.57	-	-	-
2.	Chloramphenicol (30 µg)	11±0.57	17±0.00	13±0.57	10±0.57	14±0.00
3.	Penicillin G (10U)	22±0.57	24±0.57	-	-	-
4.	Polymixin B (300 µg)	7±0.00	11±0.57	10±0.57	10±0.00	-
5.	Gentamicin (10 µg)	14±0.57	14±0.00	10±0.00	5±0.57	12±0.00
6.	Neomycin (30 µg)	10±0.00	10±0.57	-	7±0.00	-

** Diameter of inhibition zone including diameter of disc (6mm) expressed as Mean±SD (mm). '-'denotes no activity.*
Key of Bacterial strains- SA, Staphylococcus aureus; EF, Enterococcus faecalis; ST, Salmonella typhimurium; KP, Klebsiella pneumoniae; EC, Escherichia coli

Chloramphenicol and Gentamicin were found most potent antibiotics inhibiting all the tested human pathogenic bacteria followed by Polymixin B (active against four tested bacterial strains) and then Neomycin (against three). Penicillin G and Bacitracin exhibited activity against Gram positive organisms only.The order of sensitivity of human pathogenic bacteria to antibiotics can be seen as *E. faecalis*>*S. aureus*>*K. pneumoniae*>*S. typhimurium*> *E. coli*. So, among tested human pathogenic bacteria *S. typhimurium* & *E. coli* was found resistant to many antibiotics. The antibiotic sensitivity profile of tested human pathogenic bacteria is shown in Figure 2.

Figure 2
Antibiotic sensitivity profile of tested human pathogenic bacteria



Comparative analysis between screened plant extracts and antibiotics suggests that extract of *C. spectabilis* is more effective than antibiotics Bacitracin, penicillin G and Neomycin (based on zone of inhibition).

3. Phytochemical analysis

Phytochemical analysis of various 11 plant extracts (possessing antibacterial activity) revealed the presence of alkaloids (36.3%), flavonoids (63.6%), glycosides (45.4%), phenols & tannins (81.8%) and saponins (36.3%). These compounds are known to be biologically active compounds possessing medicinal value. Table 5 summarizes the presence of various phytoconstituents in studied plant species.

Table 5
Preliminary phytochemical analysis of alcoholic extract of screened plant species

S.no.	Plant Sp.	Phytocompounds				
		Alkaloids	Phenols/ Tannins	Flavonoids	Glycosides	Saponins
1.	*Abrus precatorius*	+	-	-	-	+
2.	*Andrographis paniculata*	-	+	+	-	+
3.	*Annona squamosa*	+	+	+	-	-
4.	*Calotropis procera*	NT	NT	NT	NT	NT
5.	*Cassia spectabilis*	+	+	+	+	+
6.	*Clerodendrum phlomides*	-	-	-	-	-
7.	*Costus speciosus*	NT	NT	NT	NT	NT
8.	*Ficus religiosa*	-	+	-	+	-
9.	*Lantana camara*	-	+	-	-	+
10.	*Ocimum sanctum*	-	+	+	+	-
11.	*Plumbago zeylanica*	+	-	+	-	-
12.	*Syzygium cumini*	-	+	-	-	-
13.	*Tinospora cordifolia*	-	+	+	+	-
14.	*Vitex negundo*	NT	NT	NT	NT	NT

Tannins exhibit antimicrobial activity by iron deprivation, hydrogen bonding or non-specific interactions with vital proteins such as enzymes[23]. These compounds are potent antioxidants as well and herbal drugs containing tannins are astringent in nature and used for treating intestinal disorders such as diarrhea and dysentery[24]. Alkaloids are reported to have various medicinal properties[25] for example- anticancer[26] and anti-inflammatory[27]. Saponins also exhibit antimicrobial activity[28]and anti-inflammatory effect[29]. Flavonoids are synthesized by plants in response to microbial infection so they are found quite effective antimicrobial agent against many microorganisms. These compounds form complexes with many extracellular and soluble proteins. Some flavonoids exert antimicrobial action by inhibition of nucleic acid synthesis for example-robinetin, myricetin & epigallocatechin, some by inhibition of cytoplasmic membrane function for example- Catechins and some by inhibition of energy metabolism example- Licochalcones[30]. Moreover, these compounds also exhibit other biological activities like anti-inflammatory, anti-angiogenic, analgesic, anti-allergic, cytostatic and antioxidant properties[31]. These observations support the use of studied medicinal plants in various herbal remedies.

CONCLUSION

From the present study, we can conclude that *C. spectabilis* may possess potent antimicrobial compounds that can be used further as antibiotics or in combination with currently used antibiotics. So this plant will be explored to isolate and identify various antibacterials. It may also possible that other plant species possess more potent antimicrobials because the solubility of phytoconstituents depends upon the nature of solvent and extraction procedure. Presence of various phytochemicals in medicinal plants confirms their medicinal value and validates their uses in herbal remedies as they are reported to possess a definite physiological action on the human body.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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