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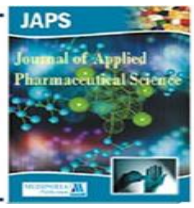

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Phytochemical investigations and antibacterial activity of some medicinal plants against pathogenic bacteria

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ABSTRACT

The antibacterial activity of various solvent extracts of medicinal plants was evaluated against the human pathogenic bacteria *Escherichia coli, Klebsiella pneumonia, Bacillus subtilis Bacillus cereus, Salmonella typhi, Enterobacter aerogenes* and *Staphylococcus aureus* by agar cup diffusion method. Methanol extracts of *Clerodendrum inerme* L., *Terminalia chebula* Retz., *Curcuma amada* Roxb., *Anacardium occidentale* L., *Duranta repens* L., *Eucalyptus camaldulenis* Dehnh *and Euphorbia cotinifolia* L. showed significant activity. The petroleum ether and chloroform extracts of *Terminalia chebula, Curcuma amada and Piper betel* also showed promising results. The antibacterial activity of promising plant extracts when compared with standard drugs streptomycin and gentamycin recorded siginificant inhibition. Phytochemical analysis of the different extracts of the screened plants indicated the presence of flavanoids, terpinoids, tannins steriodas, alkaloids and glysocides. The positive results of screening of medicinal plants for antibacterial activity forms primary platform for further phytochemical and pharmacological studies.

Key words: Medicinal plants, phytochemistry, antibacterial activity, human pathogens.

INTRODUCTION

Diseases are the major causes of death in the developing countries and accounts to 50% of it. The extensive use of the antibiotics to control these diseases has led to the emergence of multidrug resistance (Westh et al., 2004). Bacterial resistance to antibiotics increases mortality likelihood of hospitalization and length of stay in the hospital (Winstanley, 1997). The use of plants as source of remedies for the treatment of many diseases dates back to history and people of many continents have this old tradition. The advent of science into the search for antibiotics largely depends on some of these plants as raw materials.

Plant based antimicrobials represent a vast untapped source. The use of plant extract for medicinal treatment has become popular 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). At present, nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly from plants and their extracts dominate in homeopathic or ayurvedic medicines (Murugesan et al., 2011; Jabeen et al., 2007; Banso, 2009; Ahamunthunisa and Hopper, 2010).

Medicinal plants are finding their way into pharmaceuticals, cosmetics, neutraceuticals. Plants have given Western Pharmacopoeia about 7000 different pharmacologically important compounds and a number of top selling drugs of modern times eg. quinine, taxol, campothecine etc.(Tshibangu et al., 2002). The objective of this research is to evaluate the potentiality of some common medicinal plant extracts against standard microorganisms which would lead to the

Preparation of Fill Solution

Drug fill solution was prepared by accurately weighing required quantities of ibuprofen and diphenhydramine
discovery of some active secondary metabolites.

MATERIALS AND METHODS

Selection of plant material

Ten medicinal plants *viz*., *Clerodendrum inerme* L., *Terminalia chebula* Retz, *Curcuma amada* Roxb., *Foeniculum vulgare* Mill., *Piper longum* L., *Anacardium occidentale* L., *Duranta repens* L., *Piper betle* L., *Eucalyptus camaldulenis* Dehnh. , *and Euphorbia cotinifolia* L., were selected based on ethnomedicinal importance. Healthy leaves, stem, bark, fruits and seeds of the above medicinal plants were collected in and around Mysore and were used for the preparation of solvent extracts. The family name, different part and also the uses of these plants are tabulated in Table 1.

Test Organisms

Authentic cultures of human pathogenic bacteria viz., *Escherichia coli* (MTCC 7410), *Klebsiella pneumonia* (MTCC 7407), *Bacillus subtilis* (MTCC 121), *Bacillus cereus* (MTCC 1272), *Salmonella typhi (*MTCC 733), *Enterobacter aerogenes* (MTCC 7325) and *Staphylococcus aureus* (MTCC 7443) were obtained from Microbial Type Culture Collection, Chandigarh, India and they served as test bacteria.

Preparation of extract

Solvent extract

The selected medicinal plant material such as leaves, bark, stem or seeds were washed and dried. Each sample was powdered with the help of warning blender. Accurately weighed 50 g of the respective plant powder was filled in the thimble and extracted successively with petroleum ether, chloroform, ethyl acetate and methanol using soxhlet extractor for 48 h. All the extracts were concentrated using rotary flash evaporator and preserved at 5 °C in an airtight bottle until further use.

All the extracts were subjected to antibacterial activity assay.

Antibacterial activity assay

Antibacterial activity of different solvent extracts of the studied plants was determined by agar cup diffusion method on nutrient agar medium (Karthikeyan et al., 2009). Cups were made in nutrient agar plate using cork borer (5mm) and inoculums containing 10^6 CFU/ml of bacteria were spread on the solid media with a sterile swab moistened with the bacterial suspension. The dried solvent extracts were reconstituted in methanol to a concentration of 100 mg/ml. 100 µl solvent extract of each plant was placed in the cups made in the inoculated plates. Also 100 µl of methanol was placed in the cups separately as a control. Antibiotics Gentamycine (1 mg/ml) and Streptomycine (streptomycine sulphate IP; 1mg/ml) at their respective dosage were also tested for comparative efficacy. The plates were incubated for 24 h at 37 °C and zone of inhibition if any around the cups was measured in millimeter. For each treatment, three replicates were maintained.

Phytochemical analysis

Phytochemical analysis of petroleum ether, chloroform, ethyl acetate and methanol extract of the screened plants were done for the presence or absence of active secondary metabolites or different constituents such as tannins, alkaloids, flavanoids, terpenoids, steroids, carbohydrates, proteins and saponins. The dried extract was reconstituted in methanol and subjected to standard phytochemical analysis following the procedures of Harborne (1998).

RESULTS AND DISCUSSION

The antibacterial activity of test plants is shown in Table 2. The antibacterial efficacy of various solvent extracts namely petroleum ether, chloroform, ethyl acetate and methanol of the selected plants against the human pathogenic bacteria showed varied level of inhibition. The activity of the different extracts of all the screened plants were compared with standard drugs Streptomycine and Gentamycine.

Table 1 List of studied medicinal plants and their uses.

Sl. No	Plant name	Family	Part used	Uses
1	*Clerodendrum Inerme* L.	Verbenaceae	Leaves	Anti inflammatory, digestive, carminative, anthelmentic, external application for cephalalgia and ophthalmia
2	*Terminelia chebula* Retz.	Combretaceae	Fruits	Astringent, laxative, purgative, cardiotonic, antiseptic, jaundice, epliepsy, lepsory, neuropathy, general debility
3	*Curcuma amada* Roxb.	Zingiberaceae	Rhizome	Aromatic, cooling, appetizer, carminative, demulcent aphrodisiac, dyspepsia, wounds, chronic ulcers, sprains
4	*Piper longum* L.	Piperaceae	Fruits	Thermogenic,diuretic,purgative,expectorant,dyspepsia, anorexia,asthma,,bronchitis,gonorrhoea,&haemorrhoids
5	*Anacardium occidentale* L.	Anacardiaceae	Leaves	Roots purgative,,snakebite,leprosy,obstinate ulcers,aphrodisiac,prevent hair loss and increase growth
6	*Duranta repens* L.	Verbenaceae	Leaves	Febrifuge, as diuretic, treatment of abscesses, malaria and intestinal worms.
7	*Euphorbia cotinifolia* L.	Euphorbiaceae	Leaves	Cytotoxic,cauterize wounds,purgative
8	*Piper betel* L.	Piperaceae	Leaves	Astringiant,carminative,anthelmentic,expectorant,bronchitis.impotency,cough,rheumatism,diarrhoea,&laryngitis
9	*Eucalyptus camaldulenis* Dehnh	Myrtaceae	Bark	Antiseptic,,deodrant,stimulant,astringent,digestive, cardiotonic,asthma,skin diseases,cardic debility,&interment fever
10	*Foeniculum vulgare* Mill.	Apicaceae	Seeds	Emllient,refrigentexpectorant,haematinic,galactagogue, aphrodiosic,nepthropathy,vomiting,cardiac diseases&dysentry

Table 2. Antibacterial activity of different solvent Extracts of screened medicinal plants.

		Zone of inhibition* (mm)						
Plant	**Extract**	***B.c***	***B.s***	***E.cl***	***Ent***	***Kl .p***	***S.t***	***S.a***
Clerodendrum	P.Ether	-	-	-	-	-	-	-
inerme L.	Chloroform	-	10		10		12	
	E. acetate	-	12		15	10	16	12
	Methanol	13	14	11	13	10	15	12
Terminelia	P.Ether							
chebula Retz.	Chloroform	17	28	18	20	17	20	23
	E. acetate	20	20	22	23	18	20	23
	Methanol	20	22	23	22	23	23	25
Curcuma	P.Ether	18	20	19	20	20	23	13
amada Roxb.	Chloroform	19	20	18	20	23	24	13
	E. acetate	20	20	28	19	22	24	26
	Methanol	20	19	20	19	22	26	18
Piper	P.Ether	-	-	-	-	-	-	-
longum L.	Chloroform	-	-	-	12	-	-	15
	E. acetate	-	-	-	15	12	-	18
	Methanol	-	-	-	15	14	-	20
Anacardium	P.Ether	-	-	-	-	-	-	-
occidentale L.	Chloroform	-	-	-	-	-	-	-
	E. acetate	14	10	15	16	20	10	17
	Methanol	19	15	24	17	23	12	27
Duranta	P.Ether.	-	-	-	-	-	-	-
repens L.	Chloroform	20	19	12	18	12	14	16
	E. acetate	10	12	12	10	15	13	13
	Methanol	10	23	20	13	20	23	23
Euphorbia	P.Ether	-	08	-	-	08	-	-
cotinifolia L.	Chloroform	9	08	08	10	09	08	-
	E. acetate	11	13	11	13	13	12	11
	Methanol	13	18	14	17	17	16	16
Piper betel L.	P.Ether	-	-	10	15	12	12	12
	Chloroform	18	18	25	27	19	15	20
	E. acetate	16	15	20	15	17	12	15
	Methanol	12	10	13	11	11	10	12
Eucalyptus	P.Ether	-	-	-	-	-	-	-
camaldulenis	Chloroform	-	-	-	-	-	-	-
Dehnh	E. acetate	-	-	-	-	-	-	-
	Methanol	18	23	23	18	20	23	23
Foeniculum	P.Ether	-	-	-	-	-	-	-
vulgare Mill.	Chloroform	-	15	-	10	-	10	15
	E. acetate	-	16	-	15	-	12	20
	Methanol	-	15	-	18	-	13	20
Streptomycin		22	21	24	22	20	23	19
Gentamycin		25	28	24	26	23	24	23

E.cl-Escherichia coli ,Kl. p- Klebsiella pneumonia ,B.s- Bacillus subtilis, B.c-Bacillus cereus , S.t-Salmonella typhi ,Ent.ar-, Enterobacter aerogenes and S.a-Staphylococcus aureus. *Average of three replicates.

All the solvent extracts of *T. chebula* and *C. amada* showed very good activity against the test bacteria ranging from 17-26 mm. Ethyl acetate and methanol extracts of *Clerodendrum inerme* , *Anacardium occidentale* , *Duranta repens*, *Piper betel*, *Eucalyptus camaldulenis* Dehnh and *Euphorbia cotinifolia* showed significant inhibition zone. The maximum inhibition zone of methanol extract was 20- 22 mm found in *Anacardium occidentale*, *Euphorbia cotinifolia* and *Eucalyptus camaldulenis* Dehnh. The chloroform extract of *P. betel* and *Duranta repens* showed inhibition zone ranging from 15-25 mm and 12-20 which was slightly less than the standard drugs. *P. logum* and *F. vulgare* showed activity against some bacteria and it was less than 10 mm which is not significant.

All the studies indicate that plants have potential antibacterial activity. Because of the differences in plants and the plants parts that are extracted it is natural that there is differences in antibacterial activity. Methanol extract of almost all the screened plants showed activity against all the test bacteria and the zone of inhibition varied from 13 mm in *F. vulgare* to 25 mm in *T. chebula* and *C. amada* extracts. Many of the researchers (Parekh and Chanda, 2007; Alam et al., 2009) have reported that methanol is highly potent solvent for extracting the phytochemicals from the plant material. The significant activity of methanol extract, which is equal or slightly lesser than the standard antibiotics tends to show that the active compounds of the plants are better extracted with methanol.

Followed by methanol extracts, ethyl acetate extracts of *Terminalia chebula, Curcuma amada, Aancardium occidentale, Duranta repens., Piper betle* , and *Euphorbia cotinifolia,* showed significant activity with the inhibition zone ranging 18- 23, 19-26, 10-21,10-15,15-20 and 12-20 mm, respectively. Chloroform extract exhibited very good activity in *T. chebula, C. amada* , *Duranta repens* and *P. betel* which ranged 17-23, 13-24,12-20 and 15-25, respectively. While the chloroform extracts of the other test plants recorded negligible activity. Petroleum ether extracts of most of the plants except *T. chebula* and *C. amada* did not show any activity, which indicated that, the insolubility of the active compounds in this medium.

The results of the present investigation highlight the fact that the organic solvent extracts exhibit greater antibacterial activity because the active principles were either polar or non-polar and were extracted only through successive organic solvents (Mohanasundari, 2007; Britto, 2001).

The results of phytochemical analysis of the test plants are given in Table 3. The secondary metabolites commonly present in the test plants are flavanoids, terpenoids, tannins, steroids, alkaloids and glycosides. The presence of one or more of these secondary metabolites indicated that the antibacterial activity is due to these active compounds present in different parts of the test plants. The gram positive bacteria were slightly more susceptible to the extracts and showed greater inhibition zone than the gram negative bacteria, which in recent years has widely been reported in literature (Jigna and Sumitra, 2006). The antibacterial activity of *T. chebula* and *C. amada* has been reported in literature (Chanda and Baravalia, 2010; Chattopadhyay et al.,2009, Ahamed and Beg, 2001) but the test bacteria and zone of inhibition varied. The results of antibacterial activity of *P. betel*, *D. repens*, *E. cotinifolia* and *A. occidentales* is highly encouraging and gives new lead plants for isolation and characterization of the active compounds. In the present screening, antibacterial activity of *E. cotinifolia* has been reported for the first time. The activity of this plant against the human pathogens has added one more plant to the pharmacological drug discovery. The demonstration of antibacterial activity against the test bacteria is an indication that there is the possibility of sourcing alternative antibiotic compounds from the screened plants leading to the discovery of newer compounds.

Table 3: Phytochemical results of different solvent extracts of screened medicinal plants

Plant	Extract	alkaloids	tannins	flavanoids	terpenoids	steroids	glycosides	saponins	carbohydrate	proteins	anthraquinone
Clerodendrum inerme L.	PE	-	-	-	-	-	+	-	-	-	-
	Chloroform	+	+	+	-	+	-	-	-	-	-
	E. acetate	-	-	-	-	+	+	-	-	-	-
	Methanol	+	+	+	-	+	-	+	+	-	-
Jerminelia chebula Retz.	PE	-	-	-	-	-	+	-	-	-	-
	Chloroform	-	+	-	-	-	-	-	+	+	-
	E. acetate	-	+	+	-	+	+	-	+	-	+
	Methanol	-	+	+	-	+	-	-	+	-	-
Curcuma amada Roxb.	PE	+	-	-	+	++	+	-	-	-	-
	Chloroform	+	-	-	+	++	+	-	+	+	+
	E. acetate	+	-	-	+	+	+	-	-	-	-
	Methanol	+	-	-	+	+	+	-	-	-	-
Piper longum L.	PE	+	-	-	+	-	+	-	-	-	-
	Chloroform	+	-	-	-	+	+	-	-	-	-
	E. acetate	-	-	-	-	-	-	-	-	-	-
	Methanol	-	-	-	-	+	-	-	+	-	-
Anacardium occidentale L.	PE	-	-	-	-	+	+	-	-	-	-
	Chloroform	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
	E. acetate	-	-	-	-	+	+	-	-	-	-
	Methanol	+	+	-	+	+	+	-	-	-	+
Duranta repens L.	PE	+	-	-	-	-	-	-	-	-	-
	Chloroform	-	+	+	-	+	+	+	-	-	-
	E. acetate	-	+	+	+	-	+	+	-	-	-
	Methanol	-	+	+	+	+	+	+	-	-	-
Euphorbia cotinifolia L.	PE	-	-	-	-	+	NT	-	+	-	-
	Chloroform	-	-	-	-	-	NT	-	-	-	-
	E. acetate	-	+	+	+	+	NT	-	-	-	-
	Methanol	-	+	+	+	+	NT	-	-	-	-
Piper betel L.	PE	+	+	-	-	+	+	-	-	+	-
	Chloroform	+	-	-	-	-	+	-	-	+	-
	E. acetate	-	+	+	-	-	+	-	+	+	-
	Methanol	-	+	+	-	+	+	+	+	+	-
Eucalyptus camaldulenis Dehnh.	PE	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
	Chloroform	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
	E. acetate	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
	Methanol	+	+	-	-	-	-	-	-	+	-
Foeniculum vulgare Mill.	PE	+	-	-	+	+	+	-	-	-	-
	Chloroform	+	-	-	+	+	+	-	-	-	-
	E. acetate	+	-	-	+	+	+	-	-	-	-
	Methanol	+	+	-	+	+	+	-	+	-	-

+ = present;- = absent; NT=Not Tested

CONCLUSION

The obtained results support the use of these plants in traditional medicine. The potential for developing antimicrobials from higher plants appears rewarding as it leads to the development of new drugs which is needed today. Further research is necessary to find the active compounds within these plants with their full spectrum of efficacy. However, the present study of *in vitro* antibacterial activity of some plants forms primary platform for further phytochemical and pharmacological studies.

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