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ANTIBACTERIAL ACTIVITY OF SOME SELECTED INDIAN MEDICINAL PLANTS

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ABSTRACT

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants. In the present study aqueous- ethanol extract of ten plants each belonging to different families was evaluated for antibacterial activity against medically important bacteria viz. *S. aureus* (MTCC 3160), *B. subtilis* (MTCC441), *E. coli* (MTCC40), *K. pneumoniae* (MTCC3384), *P.mirabilis* (MTCC425), *P.aeruginosa* (MTCC741). The *in vitro* anti-bacterial activity was performed by agar disc diffusion and agar well diffusion method. *P. mirabilis* was the most resistant bacterium while *S. aureus* was the most susceptible bacteria. Amongst the plant species studied, *Terminalia chebula* showed best antibacterial activity.

INTRODUCTION: Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization [1]. They usually contain many biologically active ingredients and are used primarily for treating mild or chronic ailments. According to World Health Organization (WHO), about 80% of the world population relies chiefly on the plant based traditional medicine especially for their primary healthcare needs. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs [2, 3].

Infectious diseases are a major cause of morbidity and mortality worldwide. The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agents, intravenous catheters, organ transplantation and ongoing epidemics of human immunodeficiency virus (HIV) infections [4, 5]. This situation provided the impetus to the search for new antimicrobial substances from various sources like medicinal plants. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects. Therefore, there is a need to search for new infection-fighting strategies to control microbial infections [6].

Plant medicines are used on a worldwide scale to prevent and treat infectious diseases[7]. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, terpenoids and flavonoids having been found *in vitro* since they have antimicrobial properties and may serve as an alternative, effective,

cheap and safe antimicrobial for the treatment of microbial infections [8]. Plant based antimicrobial compounds have great therapeutic potential as they have lesser side effects as compared with synthetic drugs and also little chance of development of resistance. Therefore an attempt has been made to study the antibacterial activity of ten medicinally important plants viz. *Artocarpus heterophyllus, Berberis aristata, Chromolaena odorata, Embelia ribes, Jasminum angustifolia, Mahonia leschenaultii, Pluchea lanceolata, Plumbago indica, Terminalia chebula, Vitex negundo.*

MATERIALS AND METHODS:

Plant materials: Fresh plant/ plant parts were collected randomly from Kerala and Tamilnadu region, India. Some were obtained from the herbal garden (Arya Vaidya Sala, Kottakkal, Kerala, India) and raw drug museum of Centre for Medicinal Plant Research (Arya Vaidya Sala, Kottakkal, Kerala, India). The plants selected for the study include *Artocarpus heterophyllus* Lam., *Berberis aristata DC, Chromolaena odorata* L., *Embelia ribes* Burm.f., *Jasmine angustifolia* L., *Mahonia leschenaultii* Wall.ex.wt & Arn, *Pluchea lanceolata* DC., *Plumbago indica* L., *Terminalia chebula* Retz, *Vitex nigundo* L. The details of the plant/plant parts were studied - their families, vernacular names and their therapeutic uses are given in **Table 1** [9, 10, 11]. The plant materials were identified and authenticated and the voucher specimens were deposited in the raw drug museum of Centre for Medicinal Plant Research (CMPR, Arya Vaidya Sala, Kottakkal, Kerala, India).

TABLE 1: ETHNOBOTANICAL INFORMATION ON SOME TRADITIONALLY USED INDIAN MEDICINAL PLANTS SELECTED FOR ANTIBACTERIAL SCREENING

Plant species	Family	Common name	Parts used	Therapeutic use
Artocarpus heterophyllus Lam.	*Moraceae*	Jack fruit tree	Roots, leaves, fruits, seeds	Antidiarrhoeal, boil, wound, skin diseases, dyspepsia, ulcer, convulsions, dyspepsia ophthalmitis, pharyngitis
Berberis aristata, DC.	*Berberidaceae*	Indian barberry	Root bark	Skin disease, menorrhagia, diarrhoea, jaundice
Chromolaena odorata L.	*Asteraceae*	Eupatorium	Leaf	Leaf juice used as antiseptic in cuts and wounds
Embelia ribes Burm.f.	Myrsinaceae	Embelia	Fruit, root, bark	Antihelmintic, diuretic, carminative, contraceptive dyspepsia, skin diseases, piles, leprosy, pruritis.
Jasminum angustifolia L.	Oleacea	Wild jasmine	Leaf, root	Poison, herpes, ophthalmology, leprosy, pruritis, wounds, ring worm.
Mahonia leschenaultii Wall.ex.wt & Arn.	*Berberidaceae*	Mahonia	Root	Diuretic, demulcent
Pluchea lanceolata, DC.	Asteraceae	Rasna	Stem, root	Thermogenic, carminative, rheumatic disorders, nervous diseases, anorexia, cough, asthma, bronchitis.
Plumbago indica L.	Plumbaginaceae	Fire plant	Root	Leprosy, oedema. Piles, worm infestation, anaemia, anorexia, fever, bronchial asthma, leucoderma, diabetes.
Terminalia chebula, Retz.	Combretaceae	Black myrobalan	Fruit	Promote digestive power, heals wounds and ulcers, skin &eye disease, diabetes, chronic & recurrent fever, anaemia, cardiac disorders, diarrhoea, spleen enlargement, piles.
Vitex negundo L.	Verbenaceae	Chaste tree	Leaf, root	Nervous & eye, ear diseases, cough, intestinal worms, rheumatoid arthritis, leprosy, wounds, oedema, bronchial asthma

Preparation of Extract: The plant parts were washed thoroughly, dried under shade and powdered. The powdered material (100 g) was extracted with 50% aqueous-ethanol using water bath- shaker at 40^0C for 72 h. After 72 hours, the supernatant was filtered and the solvent completely evaporated using vacuum. The residue obtained was stored at 4^0C for further studies.

Bacterial cultures: Bacterial cultures were obtained from Microbial Type Culure Collection, Chandigarh, India. The microorganims used for the present study include *Staphylococcus aureus* (MTCC 3160)*, Bacillus subtilis* (MTCC441)*, Escherichia coli* (MTCC40)*, Klebsiella pneumonia* (MTCC3384)*, Pseudomonas aeruginosa* (MTCC741)*, Proteus mirabilis,* (MTCC425). All the bacterial cultures were maintained in nutrient agar and stored at 4^oC.

Preparation of inocula: Several colonies were transferred to sterile peptone water (5 ml) from the sub cultured organism. The suspensions were mixed for 15 seconds to ensure homogeneity and subsequently diluted to match the turbidity of a 0.5 McFarland standard (i.e. OD = 0.12–0.15 at k = 530 nm, corresponding to 1–5 x 106 CFU/ml).

The antimicrobial assay was performed by two methods viz. agar disc diffusion method 12 and agar well diffusion method 13. Mueller Hinton agar (MHA) was prepared in plates as the media for test bacteria. The bacterial inoculum was spread evenly on the surface of the MHA plates using a sterilized cotton swab. For agar disc diffusion method, sterile filter paper discs (6mm) were saturated with different concentrations of the test compound, allowed to dry and introduced on the upper layer of the seeded agar plate.

For agar well diffusion method, a well was prepared in the plates with the help of a cork-borer (0.6cm). 100 µl of the test compound was introduced into the well. The plates were incubated overnight at 37 °C For each bacterial strain controls were maintained where pure solvents were used instead of the extract. Sterile distilled water served as negative control. The result was obtained by measuring the zone diameter. The experiment was done thrice and the mean values are presented.

The results were compared with the standard antibiotics nitrofurantoin (300mg/disc), chloram-phenicol (30mg/disc), cephalxein (30mg/disc) and gentamicin (10 mg/disc).

RESULTS AND DISCUSSION: Antibiotic resistance has become a global concern [14]. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced scientists to search for new antimicrobial substances from various sources like the medicinal plants.

Nature has been a source of medicinal agents since times immemorial. Plants have provided a source of inspiration for novel drug compounds as plant derived medicines have made significant contribution towards human health. Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components [15, 16]. The presence of antibacterial substances in the higher plants is well established [17].

The study reveals the profound antimicrobial activity of aqueous-ethanol extract of ten different plant species. **Table 2 and 3** shows the results of antibacterial potential of different plant extracts. All the tested extracts showed varying degrees of strain specific inhibitory action. Among the plants screened, *Terminalia chebula* exhibited maximum growth inhibitory activity and *Mahonia leschenaultii* exhibited minimum growth inhibitory activity towards the tested strains. In disc diffusion method the higher dose of *M. leschenaultii* extract (100µg) inhibited the growth of *B.subtilis* with a zone mean diameter of 12mm.

In well diffusion method the extract inhibited the growth of *B.subtilis, E.coli* and *K.pneumoniae* with a zone diameter 15, 13 and 14 respectively. *Proteus mirabilis* is found to be the least sensitive organism, which only showed sensitivity towards *Embelia ribes*, *Jasminum angustifolia, Terminalia chebula and Vitex negundo.*

TABLE 2: ANTIMICROBIAL ACTIVITY OF AQUEOUS- ETHANOL EXTRACT OF SCREENED MEDICINAL PLANTS – DISC DIFFUSION METHOD

Plants	Zone of Inhibition (mm)																							
	S. aureus MTCC 3160				*B. subitilis* MTCC 441				*E. Coli* MTCC40				*K. pneumonae* MTCC3384				*P. mirabilis* MTCC 425				*P. aeruginosa* MTCC 741			
	20	40	80	100	20	40	80	100	20	40	80	100	20	40	80	100	20	40	80	100	20	40	80	100
Artocarpus heteropyllus	8	10	13	16	-	-	-	-	-	-	-	-	7	10	11	12	-	-	-	-	17	20	21	22
Berberis aristata	19	28	29	30	6	9	12	13	7	13	15	19	-	11	12	13	-	-	-	-	-	14	16	17
Chromolaena orderata	12	20	21	23	11	15	16	18	5	6	6	7	-	-	-	10	-	-	-	-	-	5	6	7
Embelia ribes	7	10	14	17	-	-	-	-	8	10	13	15	9	11	15	18	5	9	10	11	6	8	12	15
Jasminum angustifolia	28	33	34	36	7	9	13	20	-	-	10	15	-	-	7	13	9	10	13	16	-	-	-	-
Mahonia leschenaultii	-	-	-	-	-	-	10	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pluchea lanceolata	10	13	16	19	-	-	-	-	9	13	14	17	9	11	13	17	-	-	-	-	9	10	13	16
Plumbago indica	9	14	17	20	-	-	-	-	9	10	11	13	7	10	12	13	-	-	-	-	7	8	12	13
Terminalia chebula	20	22	24	26	15	17	22	25	20	25	28	30	10	11	13	15	13	16	20	21	11	15	18	20
Vitex negundo	11	14	16	20	-	-	-	-	-	-	-	-	-	15	18	20	9	13	14	16	-	14	18	20

TABLE 3: ANTIMICROBIAL ACTIVITY OF AQUEOUS- ETHANOL EXTRACT OF SCREENED MEDICINAL PLANTS – WELL DIFFUSION METHOD

Plants	Zone of Inhibition (mm)																							
	S. aureus MTCC 3160				*B. subitilis* MTCC 441				*E. Coli* MTCC40				*K. pneumonae* MTCC3384				*P.mirabilis* MTCC 425				*P.aeruginosa* MTCC 741			
	20	40	80	100	20	40	80	100	20	40	80	100	20	40	80	100	20	40	80	100	20	40	80	100
Artocarpus heteropyllus	10	12	15	17	-	-	-	-	-	-	-	-	6	12	15	18	-	-	-	10	15	19	22	25
Berberis aristata	16	19	24	26	-	-	10	12	-	-	11	12	-	-	-	13	-	-	-	-	-	-	-	-
Chromolaena orderata	14	16	20	22	12	15	17	20	8	10	12	15	-	10	15	19	-	-	-	-	-	8	8	10
Embelia ribes	12	14	18	20	-	-	-	-	10	13	16	20	10	12	16	20	6	12	14	16	9	11	13	15
Jasminum angustifolia	30	32	34	38	8	10	12	18	-	10	14	16	-	-	9	17	8	12	15	18	-	-	-	-
Mahonia leschenaultii	-	-	-	-	7	10	13	15	-	-	11	13	-	10	12	14	-	-	-	-	-	-	-	-
Pluchea lanceolata	18	22	23	26	-	-	-	-	10	17	20	23	12	14	23	25	-	-	-	-	10	12	15	19
Plumbago indica	15	18	23	24	-	-	-	-	9	10	12	13	10	13	14	15	-	-	-	-	9	10	12	14
Terminalia chebula	20	22	24	26	15	17	22	25	20	25	28	30	10	11	13	15	13	16	20	21	11	15	18	20
Vitex negundo	13	15	18	22	-	-	-	-	-	-	-	-	-	19	23	23	10	13	17	18	10	14	20	24

Among these four extracts, *P.mirabilis* showed maximum sensitivity towards *T.chebula* with a zone diameter of 21 for disc diffusion and 24 for well diffusion at a concentration of 100 µg of the extract. *S.aureus* is found to be the most susceptible organism to all the tested extracts except *M. leschenaultii.* In both methods maximum zone of growth inhibition against *S.aureus* was shown by *Jasminum angustifolia*

and the zone diameter was found to be 36 for disc diffusion (100 µg) and 38 for well diffusion method (300 µg).

Various researchers have shown that Gram positive bacteria are more susceptible towards plant extracts as compared to Gram negative bacteria [18, 19]. These differences may be attributed to fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is multilayered structure [20].

The antibacterial activity of the plant extracts studied was compared with standard antibiotics. The zone of growth inhibition formed by the standard antibiotics was given in Table 4. The results signify that most of the plant extracts possess more growth inhibitory activity than the standard antibiotics against all the tested organisms except for P.mirabilis. The growth of P.mirabilis is significantly inhibited by all the standard antibiotics tested.

TABLE 4: ANTIBACTERIAL ACTIVITY OF STANDARD ANTIBIOTICS

Bacterial strains	Zone of Inhibition (mm)			
	FU	CP	CK	GM
S. aureus	10	R	35	15
B. Subitilis	12	R	20	13
E. coli	14	R	R	8
K. pneumoniae	9	R	14	9
P.mirabilis	22	25	21	20
P.aeruginosa	R	R	R	R

FU: Nitrofurantoin (300mg), CK: Chloramphenicol (30mg), CP: Cephalexin (30mg), GM: Gentamicin (10 mg)

The study thus reveals the effectiveness of the tested plant extracts against some pathogenic bacteria commonly associated with various human infections. They can be used as potential source for the development of a phytomedicine to act against infectious bacteria. The antimicrobial activities can be enhanced if the phytoactive components are purified and adequate dosage determined for proper administration. As the global scenario is now changing towards the use of non toxic plant products having traditional medicinal use, development of modern drugs from plants should be emphasized for the control of infectious diseases.

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