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A comparative *in vitro* antibacterial activity study on Indian medicinal plants

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Abstract

Objective: The aim of the study was to find the anti bacterial activity of five Indian medicinal plants namely, *Abutilon indicum, Azadirachta indica, Leucas aspera, Ocimum sanctum* and *Vitex negundo* against human pathogens *Bacillus subtilis, Escherichia coli* and *Klebsiella pneumonia.*
Methods: The methanol, acetone, and ethanol leaf extracts were studied for their activity against human pathogens using well diffusion method.
Results: The results of this study showed that acetone extracts of all the five medicinal plants used against *Bacillus subtilis, Escherichia coli* and *Klebsiella pneumonia* revealed better antibacterial activity compared to those of methanol and ethanol extracts. Further, the acetone leaf extract of *Vitex negundo* exhibited maximum inhibition zone against *K. pneumonia* and *B. subtilis* than the other acetone leaf extracts of medicinal plants experimented in the study. The maximum zone of inhibition (20 mm and 19 mm respectively) of acetone leaf extract of *Vitex negundo* was against *Klebsiella pneumonia* and *Bacillus subtilis.* The acetone leaf extract of *Leucas aspera* showed minimum inhibitory antibacterial activity (7 mm inhibitory zone) against *E. coli*. Whereas, the leaf extracts of *Abutilon indicum, Azadirachta indica* and *Ocimum sanctum* showed moderate antibacterial activity (between 8 and 13 mm).
Conclusion: The study records the *in vitro* validation for the traditional use of these herbals in the treatment of bacterial infections. However, further clinical studies are required for assessing the real potentials and safety aspects of these herbal drugs.

Keywords: Antibacterial, Indian medicinal plants, human pathogens, acetone leaf extracts, well diffusion method.

1. Introduction

As the microorganism showing resistance to antibiotics keeps escalating, the need for the mankind to develop new antimicrobial compound arises. Plants, since time immemorial, have been used to treat common infectious diseases and the healing potential of many plants has been used in the same or related manner as the traditional ethnomedicines [1-8] and in Indian traditional medicines like Siddha, Ayurveda and Unani [9-15] found that many of the herbs and species used by humans yielded useful medical compounds and the screening of plant extracts and their products for antibacterial activity has shown that higher plants represent a potential source of novel antibiotic prototypes. Hence, the present investigations on the following five popular medicinal plants in the Indian systems of medicine (Table 1) have been taken up for comparing their antibacterial potentials against three known human pathogens.

Table 1. Medicinal plants investigated for their antibacterial activity

Sl. No.	Botanical name and family	Tamil name	Useful part(s)	Mainly used for/as
1	*Abutilon indicum* (Malvaceae)	Thuththi	Leaf, Root, flower	Anthelmintic and cough
2	*Azadirachta indica (*Meliaceae)	Neem	Leaf, Root, flower, seed	Antiseptic, Worm infestations and cough
3	*Leucas aspera* (Lamiaceae)	Thumbai	Leaf, flower	Fever, and cough
4	*Ocimum sanctum* (Lamiaceae)	Tulsi	Leaf, seed	Urinary disorders and cough.
5	*Vitex negundo* (Verbenaceae)	Nochi	Leaf, root, bark, flower	Vermifuge and cough

The medicinal uses of five Indian medicinal plants as given in Table-1 share a common therapeutic character of acting against cough and so a preliminary study on their effect as antibacterial drug in Siddha and Ayurveda for various human ailments is undertaken. The comprehensive medicinal uses and the chemical constituents of the plants experimented [16] are presented below:

Abutilon indicum

Uses: Whole plant is used as a febrifuge, anthelmintic and anti-inflammatory, cough, also in urinary troubles and lumbago. Root nervine tonic and antipyretic, also used in piles. Roots with Chalmogra oil is used for curing leucoderma. Bark astringent and diuretic. Leaf extract is used as diuretic and demulcent. Seeds are used as laxative and demulcent.

Chemical constituents: Plant contains fructose, galactose, n-alkane mixture, an alkanol fraction, β- sitosterol, vanillic acid, p-coumaric acid, p-hydroxybenzoic acid, caffeic acid, fumaric acid, p-b-D-glycoxyloxybenzoic acid, leucine, histidine, theronine, serine, glutamic acid, aspartic acid and galacturonic acid, gossypetin-8 and 7-glucosides and cyanidin-3-rutinoside. Petals contain cyanidin-3-rutinoside, gossypetin-8-glucoside and gossypetin-7-glucoside. Caryophyllene and its oxide, cineole, pinene, geraniol, gerany acetate, eudesmol, farnesol and borneol are identified in oil [17].

Azadirachta indica

Uses: Bark used in skin troubles. Leaves antiseptic, applied to boils in the form of poultice, leprosy, cough, asthma, urinary disorder, diabetes, wounds and skin diseases [18]. Neem has been extensively used in ayurveda unani and homeopathic medicine [19]. It is also very good for treating worms [20]. Decoction given for ulcers and eczema. Flowers stomachic and tonic. Berries purgative, emollient. Seed oil used in skin troubles. Fresh tender twigs used to clean teeth particularly in pyorrhoea.

Chemical constituents: Root bark contains nimidol, morgocin, morgocinin, morgocilin, nimbilin,nimbolide, 28-deoxonimbold. Leaves contain flavonone, meliacin – solannolide, solannin, triterpenoid nimocinol, azadirachtanin, tetranortriterpenoid isoazaditolide, nimbocinolide.

Leucas aspera

Uses: Juice of leaves applied externally in psoriasis, cough, chronic skin eruptions and painful swellings. Flowers given with honey in coughs and colds. Herb is used as an antipyretic. The methanol extract of *Leucas aspera*, the alkaloidal residue tested showed the potential of antibacterial activity [21].

Chemical constituents: Plant contain sterols, alkaloids, galactose, oleanolic acid, ursolic acid and β- sitosterol. Aerial parts contain α—sitosterol. Shoots contain long chain compounds – 1- hydroxytetratriacontan-4-one and 32-methyl-tetratriacontan-8-ol, dotriacontanol.

Ocimum sanctum

Uses: Leaves stimulant, diaphoretic, anti-periodic and expectorant, used in cough, catarrh and bronchitis, ringworm bronchial asthma, diarrhea, dysentery, arthritis and painful eye diseases, skin diseases, chronic fever, insect bite etc., [22]. Infusion used as a stomachic. Decoction of roots given as a diaphoretic in malarial fevers. Seeds mucilaginous and demulsent used in genitor-urinary disorders. The *Ocimum sanctum* has also been suggested to possess antifertility, anticancer, antibiabetic, antifungal, antimicrobial, hepatoprotective, cardioprodtective, anticmetic, antisparmodie, analgesie, adaptogenic and diaphoretic actions [23].

Chemical constituents: Plant contains alkaloids, glycosides, saponins and tannins. Stem bark contains stigmasterol, β – sitosterol and triacontanol ferulat. Leaves contain volatile oil, ascarpic acid. Seeds contain fixed oil. Leaf wax contains n-alkanes. Essential oil consists of methyl chavicol, camphor, β-caryophyllene, eugenol, caryophyllene, camphene, α-pinene etc.

Vitex negundo

Uses: Roots tonic, febrifuge, diuretic, used in rheumatism and dyspepsia and as an anthelmintic; also used as a demulsent in dysentery and piles. Leaves tonic and vermifuge ; smoked for relief in catarrh, cough and headache; their decoction employed in medicinal baths for catarrhaland rheumatic affections. Leaves and roots possess tranquilizing effects and form a constituent of many ayurvedic preparations. Flowers also used in diarrhea, fever and liver complaints. . It acts as a astringent, vermifuge, expectorant, insecticide and repellent [24].

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Chemical constituents: Plant contains hydroxybenzoic acids, flavonoids, aromatic acids, iridoid. Bark contains vanillic and p- hydroxybenzoic acids. Stem contains flavones glycosides. Leaves contain oil consisting of caryophyllene, camphene, α-pinene and citral, 2 alkaloids nishidine and hydrocotylene, acids,amorphous glucoside, iridoid glucoside, phenolic acids, flavonoids casticin, chrysophenol-D, luteolin, iso-orientin. P-hydroxybenoic acid, sugar. Seeds contain triacontanes.

2. Materials and methods

2.1. Plant material

The collected fresh and healthy leaves were washed thoroughly in tap water, rinsed in distilled water and shade dried for 3-4days. Dried leaves were powdered in mixer grinder and stored in sterile polythene covers separately.

2.2. Preparation of plant extracts

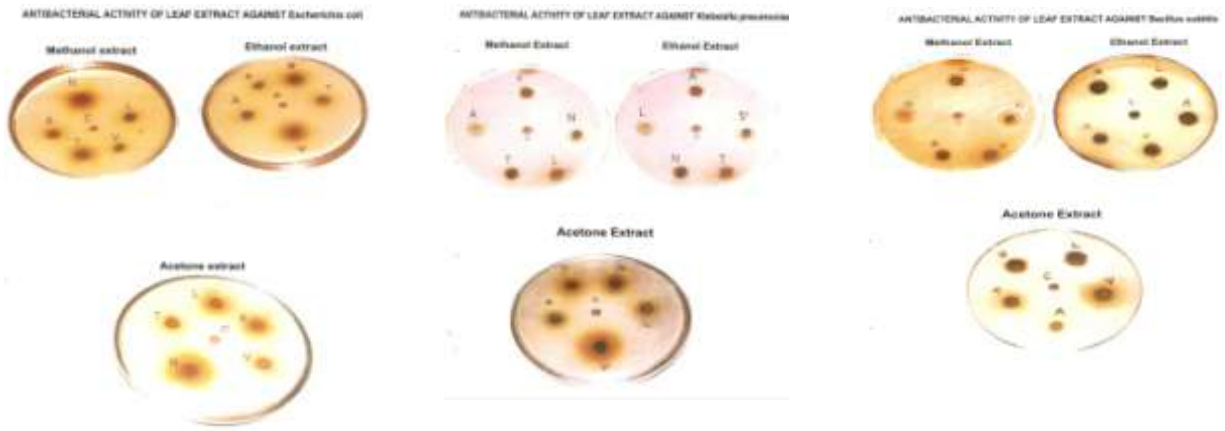
The finely grained leaves were extracted with methanol, acetone and ethanol by following the method [25]. Here the extraction of the leaf powder was done with solvents in the ratio of 1: 10 under shaking condition. The extracts were collected in different in different conical flasks and the same was repeated thrice to attain maximum extraction. Then the solvents were evaporated and condensed to concentrate the extracts obtained. The concentrated residues were weighed and re-dissolved in respective solvents to yield 10mg/ml solutions for further analysis.

2.3. Antibacterial activity

The crude extracts were subjected to antibacterial screening against (1) *Escherichia coli* (MTCC 1687), (2) *Klebsiella pneumonia* (MTCC 109), and (3) *Bacillus subtilis* (ATCC 9372).

Nutrient agar was prepared and poured in the petridish. The 24 hrs growing culture (*Escherichia coli, Klebsiella pneumonia* and *Bacillus subtilis*) were swabbed on it. The wells (10 mm diameter) were made by using cork borer and the crude extract were loaded in the wells. The plates were then incubated at 37^0 C for 24 hrs. The inhibition diameter was then measured [26].

Figure 1. The antibacterial activities exhibited by the leaf extracts of the three solvents for the five medicinal plants



(a) methanol extract; (b) acetone extract; (c) ethanol extract

(N, L, V, A and T are the first alphabet of Neem , Leucas , vitex , abutilon and Tulsi are given in Fig.1).

3. Results and discussion

The antibacterial property of the leaf extracts of three solvents for each of *Azadirachta indica* [Neem], *Ocimum sanctum* [Tulsi], *Abutilon indicum* [Thuththi], *Leucas aspera* [Thumbai], *Vitex negundo* [Nochi] were investigated by well diffusion method assay. The acetone leaf extract exhibited higher activity against the test pathogens when compared to other extracts (Fig 1). The maximum zone of inhibition of acetone leaf extract of *Vitex negundo* was found to be 20 mm and 19 mm respectively against *Klebsiella pneumonia* and *Bacillus subtilis* (Table 2).

Table 2. In vitro antibacterial activity of ethanol, methanol and acetone leaf extracts of five Indian medicinal plants (values indicate the zone of inhibition in mm).*

	A.indicum			*L.aspera*			*V.negundo*			*A.indica*			*O.sanctum*		
Organism	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)
B. subtilis	10	9	10	7	11	9	11	19	10	13	11	10	11	10	9
E.coli	7	12	7	7	7	7	7	12	8	9	10	9	9	12	7
K. pneumonia	13	16	10	11	14	10	9	20	12	11	16	10	10	11	10

*****All the values are mean of three replicates.

From the results obtained in this study, it is evident that the leaves of these five Indian medicinal plants are effective against all the three pathogens, *Klebsiella pneumoniae, Bacillus subtilis* and *Escherichia coli.* Comparatively, the acetone leaf extract of *Vitex negundo* showed maximum inhibition zone against *K. pneumoniae* and *B.subtilis* than the other leaf extracts. The leaf extracts of *Leucas aspera* showed minimum inhibitory antibacterial activity against *E.coli*. The leaf extracts of *Abutilon indicum, Azadirachta indica* and *Ocimum sanctum* showed moderate antibacterial activity. These results revealed that all these five plants could be a potential source of herbal medicine for infections caused by *Bacillus subtilis,* and *Escherichia coli* and *Klebsiella pneumonia and* underline the necessity to elucidate the exact bioactive compounds in them responsible for the destined antibacterial action.

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