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Antibacterial activity of some Indian medicinal plants

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Abstract Aqueous extracts of ten medicinal plants were examined for their antibacterial potential against some reference strains of human pathogenic bacteria. *Anethum graveolens*, *Elettaria cardamomum*, *Foeniculum vulgare*, *Trachyspermum ammi* and *Viola odorata* were found to be better/equally effective compared to standard antibiotics. *V. odorata* was the most effective antibacterial with minimum inhibitory concentration values ranging from 1 to 2%. The results provide a scientific basis for the centuries-old usage of aqueous extracts of these medicinal plants.

Keywords Antibacterial activity · Antibiotics · Bactericidal activity · Medicinal plants · Minimum inhibitory concentration

Introduction

Traditional medicinal practice has been known for centuries in many parts of the world for the treatment of various human ailments. The use of antibiotics has revolutionized the treatment of various bacterial infections. However, their indiscriminate use has led to an alarming increase in antibiotic resistance among microorganisms [1], thus necessitating the need for development of novel antimicrobials [2]. Recent years have witnessed a renewed interest in exploring natural resources for developing such compounds. Medicinal plants are relied upon by 80% of the world's population, and in India the use of plants as therapeutic agents remains an important component of the traditional medicinal system. A number of plants have been documented for their biological [3, 4] and antimicrobial properties [5–8]. *Anethum graveolens* (Dill), *Foeniculum vulgare* (Fennel), *Trachyspermum ammi* (Omum) and *Elettaria cardamomum*—queen of spices (Chotti elaichi)—have been commonly used to treat gastrointestinal disorders [9]; *Viola odorata* has been in use to treat respiratory diseases and as an anti-inflammatory agent [10], while *Syzygium aromaticum* (clove) is used for toothache due to its local anaesthetic activity [11]. Biological as well as antimicrobial activities of essential/volatile oils of these plants have been reported [12–14].

In an effort to expand the spectrum of antimicrobial agents from natural resources, ten medicinal plants belonging to seven families, have been selected based on their traditional uses in India to assess their antibacterial potential. To the best of our knowledge, no positive report is available on the antibacterial activity of aqueous extracts of some of these plants.

Experimental

Preparation of plant extracts

Different plants/parts were obtained from the local market in Amritsar (Punjab), India (Table 1). Plant parts were surface-sterilized with 1% mercuric chloride, crushed using a pestle and mortar and aqueous extracts prepared by taking weighed amounts of each sample (10, 20, 30, 40 and 50 g) in a known volume of

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Table 1 Plants and plant parts used

Sample number	Scientific name	Family	English name	Common name	Part used
P1	*Amomum subulatum*	Zingiberaceae	Greater cardamom	Badi elaichi	Fruit/seeds
P2	*Anethum graveolens*	Umbelliferae	Dill	Sowa	Seeds
P3	*Cinnamomum zeylanicum*	Lauraceae	Cinnamon	Dalchini	Bark
P4	*Elettaria cardamomum*	Zingiberaceae	Cardamom	Chotti elaichi	Fruit/seeds
P5	*Foeniculum vulgare*	Umbelliferae	Fennel	Saunf	Seeds
P6	*Glycyrrhiza glabra*	Leguminosae	Licorice	Mulathi	Root
P7	*Gnetum gnemon*	Gnetaceae	Melinjo	Magh	Fruit
P8	*Syzygium aromaticum*	Myrtaceae	Clove	Laung	Unopened bud
P9	*Trachyspermum ammi*	Umbelliferae	Omum	Ajowain	Fruit/seeds
P10	*Viola odorata*	Violaceae	Sweet violet	Banfshah	Flowers and twigs

water (50–250 ml) to obtain the desired concentration (10–50%). Different extracts were filtered through muslin cloth and centrifuged at 8,000 *g* for 15 min and the supernatants were used for antibacterial testing. Aqueous extracts (20%) were also used directly after adjusting their pH to 7.0 with 2.7 M HCl or 2.5 M NaOH. To assess the effect of method of preparation, extraction was carried out in three different ways: (1) the samples were allowed to soak in sterilized distilled water at ambient temperature for 15 min; (2) samples were allowed to soak in sterilized distilled hot water for 15 min; and (3) samples were boiled in sterilized distilled water for 10 min. To assess the effect of grinding on their antibacterial activity, aqueous extracts of shade-dried and powdered plant materials (20%) were prepared in hot water.

Bacterial cultures

All the bacterial cultures, viz. *Escherichia coli* (MTCC 119), *Klebsiella pneumoniae* 1 (MTCC 109), *K. pneumoniae* 2 (MTCC 530), *Pseudomonas aeruginosa* 1 (MTCC 647), *Ps. aeruginosa* 2 (MTCC 741), *Salmonella typhi* (MTCC 531), *Salmonella typhimurium* 1 (MTCC 98), *S. typhimurium* 2 (MTCC 1251), *Shigella flexneri* (MTCC 1457) and *Staphylococcus aureus* (MTCC 96) were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The reference strains of bacteria were maintained on nutrient agar slants, subcultured regularly (every 30 days) and stored at 4°C as well as at –80°C by preparing suspensions in 10% glycerol.

Inoculum preparation

A loopful of isolated colonies was inoculated into 4 ml peptone water and incubated at 37°C for 4 h. The turbidity of actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 McFarland units prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dihydrate with 99.5 ml 1% (v/v) sulphuric acid. This turbidity is equivalent to approximately $1–2 \times 10^8$ colony-forming units per millilitre (cfu/ml). This 4-h grown suspension was used for further testing.

Determination of antibacterial activity

The sensitivity of different bacterial strains to aqueous plant extracts was measured in terms of zone of inhibition using an agar diffusion assay [15]. Bacteria with a clear zone of inhibition of more than 12 mm were considered to be sensitive. Bactericidal activity of the selected plant parts was measured by the viable cell count method [16], and the results were expressed as number of viable cells as a percentage of the control. The minimum inhibitory concentration (MIC) of the effective plant extracts was determined by an agar dilution method using 1–10% of different plant extracts [17]. Experiments were performed in duplicate and repeated three times for each combination of extract and bacterial strain.

Sensitivity of bacteria to standard antibiotics

The sensitivity of the reference strains of bacteria to eight commonly employed antibiotics, viz. ampicillin (A 10 μg/disc), cefixime (Cfx 5 μg/disc), chloramphenicol (C 30 μg/disc), co-trimoxazole (Co 25 μg/disc), gentamicin (G 10 μg/disc), imipenem (I 10 μg/disc), pipericillin/tazobactam (Pt 10 μg/disc) and tobramycin (Tb 10 μg/disc) (Hi-Media Pvt., Mumbai, India) was assessed by the disc diffusion method.

Results and discussion

Initial screening of different plant extracts for their antibacterial activity carried out using Mueller-Hinton

and Nutrient agar media did not reveal any significant difference, thus further studies were carried out using nutrient agar medium only. The inhibitory effect of aqueous extracts increased with increasing concentration (10–50%). However, 20% aqueous extracts were selected for further testing. No significant differences were observed by testing the different plant extracts at their natural pH value, which ranged from 3.5 to 5.2, compared to when the pH was adjusted to 7.0. However, the extraction method did affect the antibacterial activity of plant extracts; extracts prepared in hot water gave maximum zones of inhibition compared to extracts prepared at ambient temperature or in boiling water. The observed difference in antibacterial activity might be attributed to incomplete leaching of the active substances at ambient temperature, and loss of active components during boiling. Grinding the plant parts resulted in the loss of their antibacterial activity. Thus, the data presented in this paper pertains to 20% aqueous extracts of crushed plant parts prepared in hot water without any pH adjustment (Table 2).

The results of the present study are encouraging as nine out of ten plants tested possessed antibacterial properties against two or more tested bacteria. No single plant was found to be equally effective against all the bacteria tested, which responded in a varied manner except *K. pneumoniae* 1, 2 and *Ps. aeruginosa* 1, which were completely resistant (data not shown). In the agar diffusion assay, five plants (*A. graveolens*, *E. cardamomum*, *F. vulgare*, *T. ammi* and *V. odorata*) demonstrated considerable inhibitory action. Similar studies reporting antimicrobial activity of these plants have also been conducted by other workers [13, 14, 18] using their essential oil/organic solvents. The resistant nature of most of the bacteria towards aqueous extracts of *S. aromaticum* is in line with an earlier study though the method of extract preparation varied [19]. In contrasts to the results reported here, another study [18] found no antibacterial activity using aqueous extracts of *E. cardamomum*, *F. vulgare*, *G. glabra*, *S. aromaticum*, *T. ammi* and *V. odorata*; the difference may be due to filtration of the extracts in the latter study through Whatman paper no. 1, which might have led to the removal of components responsible for any antibacterial effect. Thus the variation observed between earlier results and the present study could be attributed to the method of extraction or strain differences.

On the basis of results obtained by agar diffusion assay, five plants were selected to assess the bactericidal nature of their aqueous extracts using the viable cell count method with bacterial strains that had zones of inhibition of more than 12 mm in diameter. Incubation of different bacteria with aqueous plant extracts over a period of time resulted in a steady decline in viable cell count. The different plant extracts could lead to 100% cell death up to 14 h and no regrowth was observed up to 24 h. However, only the data pertaining to the most effective plant (*V. odorata*, P10) is presented (Fig. 1). *V. odorata* led to 100% killing of different bacteria in 4 h at a variable rate (*Staph. aureus* > *Ps. aeruginosa* > *Sh. flexneri* > *Salm. typhimurium* > *Salm. typhi* and *E. coli*). Aqueous extracts of *E. cardamomum* appeared to be the most effective as observed by agar diffusion assay, with zone sizes ranging from 15 to 28 mm, but *V. odorata* was found to be the most effective bactericidal agent in viable cell count studies. The effectiveness of *V. odorata* was further supported by MIC values, which ranged from 1 to 2%. The differences observed between the agar diffusion assay and viable cell count studies

Table 2 Antibacterial activity of hot water extracts of crushed plant parts and some of the standard antibiotics against human pathogenic bacteria. *P1 A. subulatum*, *P2 A. graveolens*, *P3 C. zeylanicum*, *P4 E. cardamomum*, *P5 F. vulgare*, *P6 G. glabra*, *P7 G. gnemon*, *P8 S. aromaticum*, *P9 T. ammi*, *P10 V. odorata*, *A 10* ampicillin, *Cfx 5* cefixime, *C 30* chloramphenicol, *Co 25* cotrimoxazole, *G 10* gentamicin, *I 10* imipenem, *Pt 10* pipericillin/tazobactam, *Tb 10* tobramycin

Bacteria	Zone of inhibition (mm)																	
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	A 10	Cfx 5	C 30	Co 5	G 10	I 10	Pt 10	Tb 10
E. coli	–[a]	12	–	19	15	–	–	–	17	–	20	20	19	21	18	28	20	16
Ps.aeruginosa 2	20	24	12	28	24	15	10	10	24	19	–	–	–	–	13	26	18	18
Salm. typhi	–	–	–	15	12	–	–	–	–	–	10	17	19	16	15	21	17	13
S.typhimurium 1	–	12	–	15	–	–	–	–	–	–	26	23	32	22	20	30	26	20
S.typhimurium 2	–	20	–	20	14	–	–	–	12	12	–	20	23	18	21	24	17	17
Sh. flexneri	16	14	–	22	18	12	–	13	15	20	23	23	25	18	27	32	23	17
Staph. aureus	21	25	13	28	24	14	10	14	23	21	30	21	22	21	17	39	33	18
Mean	19.0	17.8	12.5	21.0	17.8	13.6	10.0	12.3	18.2	18.0	21.8	20.6	23.3	19.3	18.7	28.6	22.0	17.0
±SD	2.65	5.95	0.71	5.42	5.15	1.53	0.00	2.08	5.17	4.08	7.56	2.25	4.84	2.34	4.57	5.88	5.89	2.16

[a] No zone of inhibition

could be attributed to the more viscous nature of *V. odorata* extract, which might not have diffused as effectively in the agar plates, thus giving no, or a smaller, zone of inhibition. MIC values (1–8%) for the extracts were plant- and strain-dependent (Table 3).

Sensitivity to standard antibiotics

The different cultures responded to standard antibiotics in a variable manner, resulting in zones of inhibition of 9–38 mm. Statistical analysis revealed that aqueous extracts of plants P2, P4, P5, P9 and P10 were better/equally effective against some of the bacterial strains as compared to standard antibiotics (Table 2). *Staph. aureus* was more susceptible to plant extracts (P2, P4, P5, P9 and P10) than to cefixime, chloramphenicol or co-trimoxazole. *Ps. aeruginosa*, which was resistant to cefixime, chloramphenicol, co-trimoxazole and ampicillin, was sensitive to eight plant extracts, five of which giving inhibition zones ranging from 20 to 28 mm. *Staph. aureus*, *Ps. aeruginosa*, *Sh. flexneri*, *Salm. typhi*, *Salm. typhimurium* and *E. coli* were all inhibited to a considerable extent by the five selected plants. Most of the above-mentioned microorganisms are developing resistance to commonly employed antibiotics and are a common cause of nosocomial infections [20, 21]. Thus the antibacterial activities of medicinal plants reported in the present study are noteworthy considering the importance of these microorganisms in nosocomial infections.

In conclusion, five of the plants tested here (*A. graveolens*, *E. cardamomum*, *F. vulgare*, *T. ammi* and *V. odorata*) exhibited broad-spectrum antibacterial activity. All these plants have been in use for many years as decoctions or infusions prepared in water to treat various ailments. This paper thus provides a scientific basis for the use of these aqueous plant extracts in home-made remedies and their possible application against microorganisms such as *Staph. aureus* and *Ps. aeruginosa*, etc., that cause nosocomial infections. Further studies may lead to their use as safe alternatives to synthetic antimicrobial drugs.

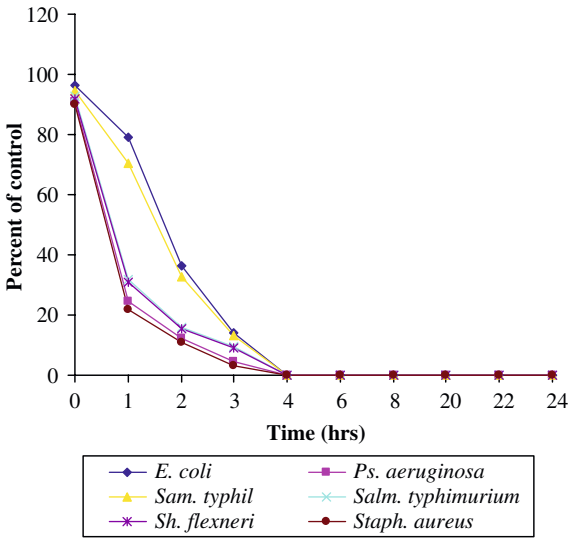

Fig. 1 Viable cell count of different bacteria with aqueous extract of *Viola odorata* (20%)

Table 3 Minimum inhibitory concentration (MIC) (percent) of aqueous plant extracts against human pathogenic bacteria. *P2 E. cardamomum*, *P4 A. graveolens*, *P5 F. vulgare*, *P9 T. ammi*, *P10 V. odorata*

Bacteria	Plants				
	P2	P4	P5	P9	P10
E. coli	5	4	6	8	2
Ps. aeruginosa 2	4	3	4	8	2
Salm. typhi	4	3	3	8	2
S. typhimurium 2	4	2	2	8	1
Sh. flexneri	4	5	2	8	1
Staph. aureus	4	3	6	7	1

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