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Antimicrobial activity of some herbal oils against common food-borne pathogens

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In this study we screened ten herbal oils, which were purchased from the local market of Meerut region to study their role as inhibitors of food-borne pathogens. Of the ten essential oils, only cinnamon and clove oil exhibited a broad range of antimicrobial activity, followed by peppermint and *Eucalyptus* oil. Cinnamon oil exhibited the lowest minimum inhibitory concentration (MIC) of 1.25% (v/v), followed by clove oil with an MIC of 2.5% (v/v). These oils therefore possess potential to be used as food bio-preservatives.

Key words: Herbal oils, agar well diffusion, minimum inhibitory concentration (MIC), two-fold dilution.

INTRODUCTION

The demand for safer and more natural foods has been increasing since consumers have become more concerned over the presence of chemical residues in their food stuff. Spices and herbal oils offer a promising alternative to the chemical preservatives used in the food products. The antimicrobial activity of herbal oils is well documented (Lis-Balchin and Deans, 1997) and reviewed (Rios et al., 1988; Janssen et al., 1987; Garg and Dengre, 1986; Inouye et al., 1983; Jain and Kar, 1971). This study, part of a comprehensive project, evaluated the antimicrobial potential of some Indian herbal oils, with a view to exploring their potential to application in food industries as botanical preservatives.

MATERIALS AND METHODS

Collection of materials

All chemicals used were of analytical reagent-grade and obtained from E. Merck (Mumbai, India). The ready-made herbal oils of cinnamon bark (*Cinnamomum zeylanicum*), clove bud (*Syzygium aromaticum*), peppermint leaf (*Mentha piperita*), nutmeg fruit (*Myristica fragrans*), olive fruit (*Olea europaea*), almond fruit (*Prunus dulcis*), mustard seeds (*Brassica juncea*), *Eucalyptus* leaf (*Eucalyptus globulus*), anise seed (*Pimpinella anisum*) and lemon fruit (*Citrus limonum*) were purchased from the local market in Meerut (Uttar Pradesh, India). Dr. C. M. Govil, Botany Department, CCS University, Meerut, India, confirmed the origin of the respective oils. These oils were stored in amber-coloured bottles at 4°C until use.

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Test bacteria

The test microorganisms were *Staphylococcus aureus* (CCSUB1), *Staphylococcus epidermidis* (CCSUB2), *Bacillus subtilis* (CCSUB3), *Bacillus cereus* (CCSUB4), *Bacillus* sp. (CCSUB5), *Listeria monocytogenes* (CCSUB6), *Micrococcus luteus* (CCSUB7), *Escherichia coli* (CCSUB8), *Pseudomonas aeruginosa* (CCSUB9) and *Klebsiella* sp. (CCSUB10). These bacteria were isolated previously from the spoiled food products (milk, sweets, spoiled vegetables, fruits and tomato sauce). The identity of the cultures was confirmed by standard bacteriological methods (Cheesbrough, 1984; Collins et al., 1970). The cultures were plated on nutrient agar medium (Hi-Media, Mumbai, India) and incubated for 24 h at 37°C. The agar plates were stored at 4°C until required.

Screening of the herbal oils for antimicrobial activity

The herbal oils were screened for their antimicrobial activity using an agar well diffusion technique (Okeke et al., 2001). Each bacterium was first subcultured in nutrient broth at 37ºC for 24 h. One hundred microlitres (100 µl) of standardized inoculum (10^6 CFU/ml; 0.5 MacFarland) of each test bacterium was spread with the help of sterile spreader onto sterile Muller-Hinton Agar (MHA) (Hi-Media) to achieve confluent growth. The plates were allowed to dry and a sterile cork borer (6 mm diameter) was used to bore wells in the agar. Subsequently, a 50 l volume of the oil was introduced in triplicate wells of the agar plates. Sterile DMSO served as negative control. The plates were allowed to stand for at least 1 h for diffu-

Table 1. Zone of inhibition (mm) by different herbal oils against test bacteria on Mueller-Hinton agar medium (each value is an average of three independent replicates).

S. No.	Test bacteria	Cinnamon oil	Clove oil	Nutmeg oil	Peppermint oil	Eucalyptus oil	Negative control
CCSUB1	*Staphylococcus aureus*	20.0	18.0	14.0	15.0	24.0	0.0
CCSUB2	*Staphylococcus epidermidis*	15.0	20.0	16.0	14.0	28.0	0.0
CCSUB3	*Bacillus subtilis*	16.0	18.0	0.0	12.0	19.0	0.0
CCSUB4	*Bacillus cereus*	29.0	24.0	19.0	32.0	28.0	0.0
CCSUB5	*Bacillus* sp.	15.0	17.0	0.0	13.0	19.0	0.0
CCSUB6	*Listeria monocytogenes*	18.0	21.0	14.0	28.0	21.0	0.0
CCSUB7	*Micrococcus luteus*	18.0	16.0	14.0	16.0	18.0	0.0
CCSUB8	*E .coli*	16.0	17.0	15.0	10.0	0.0	0.0
CCSUB9	*Pseudomonas aeruginosa*	16.0	0.0	0.0	0.0	11.0	0.0
CCSUB10	*Klebsiella* sp.	14.0	15.0	13.0	10.0	0.0	0.0

Table 2. Zone of inhibition (mm) by different herbal oils against test bacteria on Mueller-Hinton agar medium (each value is an average of three replicates).

S.No.	Test bacteria	Anise oil	Lemon oil	Almond oil	Olive oil	Mustard oil	Negative control
CCSUB1	*Staphylococcus aureus*	0.0	16.0	0.0	0.0	0.0	0.0
CCSUB2	*Staphylococcus epidermidis*	0.0	16.0	0.0	0.0	0.0	0.0
CCSUB3	*Bacillus subtilis*	0.0	13.0	0.0	0.0	0.0	0.0
CCSUB4	*Bacillus cereus*	0.0	29.0	0.0	0.0	0.0	0.0
CCSUB5	*Bacillus* sp.	0.0	14.0	0.0	0.0	0.0	0.0
CCSUB6	*Listeria monocytogenes*	11.0	13.0	0.0	0.0	0.0	0.0
CCSUB7	*Micrococcus luteus*	0.0	21.0	0.0	0.0	0.0	0.0
CCSUB8	*Escherichia coli*	0.0	11.0	0.0	0.0	0.0	0.0
CCSUB9	*Pseudomonas aeruginosa*	0.0	Partly	0.0	0.0	0.0	0.0
CCSUB10	*Klebsiella* sp.	0.0	12.0	0.0	0.0	0.0	0.0

sion to take place and then incubated at 37°C for 24 h. The zone of inhibition was recorded to the nearest size in mm (Norrel and Messely, 1997). The results were expressed in terms of the diameter of the inhibition zone: <9 mm, inactive; 9 - 12 mm, partially active; 13 - 18 mm, active; >18 mm, very active (Junior and Zanil, 2000).

Determination of Minimum Inhibitory Concentration (MIC) of the selected oils

The MIC was defined as the lowest concentration that completely inhibited the growth for 24 h (Thongson et al., 2004). The MIC for the cinnamon, clove, lemon, peppermint and nutmeg oils was determined by the agar well diffusion technique. A two-fold dilution series was prepared to achieve a decreasing concentration range of 10 to 0.625% (v/v). A 50 µl volume of each dilution was added aseptically into the wells of Mueller Hinton agar plates that were already seeded with the standardized inoculums (10^6 CFU/ml) of the test bacteria. Sterile DMSO, without oil, served as negative control. All experiments were performed in triplicate. The agar plates were incubated at 37°C for 24 h. The lowest concentration of oil showing a clear zone of inhibition was considered as the MIC.

RESULTS AND DISCUSSION

The data pertaining to the antimicrobial potential of the herbal oils are presented in Tables 1 and 2, respectively. About 50% of the oils were effective against both Gram-positive and Gram-negative bacteria. Cinnamon oil was equally effective against both the groups of bacteria. It produced the widest zone of inhibition against *B. cereus* with diameter of 29.0 mm, followed by *S. aureus* (20 mm) . It also inhibited growth of the Gram- negative bac-teria *E. coli, P. aeruginosa* and *Klebsiella* sp. The inhi-bition by cinnamon oil could be due to the presence of active constituents such as cinnamaldehyde and cinna-mic acid. These are terpenoids in nature. Their activity is a function of the lipophilic properties of the constituent terpenes, the potency of their functional groups and their aqueous solubility (Knobloch et al., 1989; Elgayyar et al., 2001).

Clove, *Eucalyptus* and peppermint oil were also effective against the majority of the test bacteria. Clove oil showed good antibacterial activity against all Gram-positive and Gram - negative bacteria, except *P.* pinene, α-phellandrene, 1,8 cineole, limonene, terpinen- 4-ol, aromadendrene, epiglobulol, piperitone and globulol and they are all tannins in nature. Their mode of antimicrobial action is related to their ability to inactivate microbial

Table 3. The MIC (minimum inhibitory concentration) values in (%, v/v) of selected herbal oils on Mueller-Hinton Agar Medium.

S.NO	Test bacteria	Cinnamon oil	Clove oil	Lemon oil	Peppermint oil	Nutmeg oil
CCSUB1	*Staphylococcus aureus*	2.5%	5%	6.25%	6.25%	12.5%
CCSUB2	*Staphylococcus epidermidis*	2.5%	2.5%	25%	6.25%	6.25%
CCSUB3	*Bacillus subtilis*	2.5%	5%	50%	50%-	-
CCSUB4	*Bacillus cereus*	2.5%	5%	12.5%	3.12%	6.25%
CCSUB5	*Bacillus* sp.	1.25%	5%	12.5%	3.12%	12.5%
CCSUB6	*Listeria monocytogenes*	1.25%	5%	50%	6.25%	25%
CCSUB7	*Micrococcus luteus*	5%	10%	6.25%	6.25%	50%
CCSUB8	*Escherichia coli*	1.25%	5%	50%	50%	50%
CCSUB9	*Pseudomonas aeruginosa*	5%	-	-	-	-
CCSUB10	*Klebsiella* sp.	1.25%	5%	50%	50%	50%

adhesion, enzymes and cell envelope proteins (Ya et al., 1988). Peppermint oil showed exceptionally high activity against *B. cereus*, producing the widest inhibition zone (32 mm) (Table 1). However, the test Gram-negative bacteria were found to be resistant. The antimicrobial activity of peppermint oil is due to the presence of terpenoids-menthol, menthone, 1,8-cineole, methyl acetate, metho-furan, isomenthone, limonene, b-pinene, α-pinene, germacrene-d, trans -sabinene hydrate and pulegone (Ahmed et al., 2001).

Nutmeg oil showed good antibacterial activity only against *B. cereus, S. epidermidis* and *E. coli* (Table 1). Nutmeg oil contains monoterpenes such as -pinene, camphene, -pinene, sabinene, myrcene, α-phellan-drene, α-terpinene, limonene, 1,8-cineole, γ-terpinene, linalool, terpinen-4- ol, safrole, methyl eugenol and myris-ticin, as their active principles.

Lemon oil, like peppermint oil, also demonstrated prefe-rentially more activity against Gram- positive bacteria as compared to Gram- negative bacteria (Table 2). The anti-microbial activity of lemon oil is due to the presence of terpenoids- a-pinene, camphene, b-pinene, sabinene, myrcene, α-terpinene, linalool, b- bisabolene, limonene, trans-a- bergamotene, nerol and neral (Ahmed et al., 2001).

The Gram -negative test bacteria were found to be resistant against the majority of the herbal oils, except the cinnamon and clove oil. Table 3 shows the minimum inhibitory concentration (MIC) of the most effective herbal oils. Cinnamon oil showed the lowest MIC of 1.25% (v/v), while the MIC for clove varied from 2.5 - 5% (v/v). The MIC of peppermint, lemon and nut-meg oil was higher than the cinnamon and clove oils. Their MIC ranged from 3.12 - 50% (v/v).

In conclusion, based on the above findings, both cinna-mon and clove oil appears to have potential for use as botanical preservative. Both clove and cinnamon oil con-tain eugenol. Clove oil has 79.2% eugenol, while cinna-mon oil from bark and leaf has 4.7 and 76.9% eugenol, respectively (Ranasinghe et al., 2002). It can thus also be concluded that components with a phenolic structure, such as eugenol, are highly active against the test micro-organisms.

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