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ANTIBACTERIAL ACTIVITY OF SOME ESSENTIAL OILS AGAINST FOOD BORNE PATHOGEN AND FOOD SPOILAGE BACTERIA

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ABSTRACT

Essential oils are well known in traditional medicine as antiseptic and antimicrobial agents. This study determined the antimicrobial effects of eleven spice essential oils using a disc diffusion method against four Gram-positive and eight Gram-negative bacteria of spoilage and health significance. Cassia oil showed the largest zones of inhibition (12 to 54 mm) and the widest antibacterial spectrum, followed by essential oil of allspice, clove and nutmeg. Essential oils of mace, celery, ginger, cardamom, black pepper, fennel and turmeric were the least effective spice oils. Gram-positive bacteria were shown to be more sensitive to the spice essential oils than Gram-negative bacteria. *Staphylococcus aureus* and *Bacillus subtilis* were the most sensitive bacterial strains tested; where as a strain of *Escherichia coli* (MTCC-118) was the least sensitive. These results showed that spice essential oils may prove useful in inhibiting bacteria of food spoilage and health significance.

KEY WORDS

Spices, Essential oils, Antibacterial activity, Gram-positive bacteria, Gram-negative bacteria

INTRODUCTION

Microbial activity by food spoilage bacteria is one of the primary causes of deterioration of many foods and is often responsible for food quality reduction, spoilage, and economic loss. In addition food industries have many safety concerns which have focused on particular foodborne pathogens, such as *Salmonella*, *E. coli,* and *S. aureus* etc which are recognized as one of the leading causes of food-borne bacterial diseases [1,2]. The problem of food preservation has grown to be more complex as new food products are frequently being introduced on the market, requiring long-term shelf life protection from microbial spoilage. The synthetic chemical preservatives require caution in handling since they are corrosive and some reported to convert ingested materials into toxic substances [3]. Brull and Coote [4] reported microbial resistance to antimicrobials used in food preservation.

Due to negative consumer perceptions of artificial preservatives, attention is shifting towards natural preservatives [1]. As such much interest has focused on utilization of plant-derived antimicrobials to control pathogens in foods. Consequently, alternative preservatives are needed which possess antimicrobial activity but cause no health problems.

Spices and condiments have been used for centuries to enhance the flavour and aroma of foods and their medicinal values. Spices are known for antimicrobial properties [5, 6] and are most commonly used natural antimicrobial agents in foods [2]. Additions of spices in foods not only enhance flavour and aroma of foods but natural antimicrobial compounds present in them also provides antimicrobial properties [5, 7]. Essential oils from different spices were found to possess antimicrobial activity [8, 9, 10, 11] and majority of the essential oils are classified by FDA as 'Generally Recognized as Safe' – GRAS [12].

Bacterial evolutionary responses to the antibiotics resulted in the development of bacterial strains resistant to antibiotics. The continuous exposure of bacteria to antimicrobial agents is the important cause of development of resistance among microorganisms. Among the resistant Gram-positive organisms, are the most feared pathogens [13]. Developing drug resistance between microorganism and undesirable effects of currently available drugs has necessitated a need for alternative safer, cheaper and effective antimicrobial agents for therapeutic management of bacterial infections.

The present investigation however, evaluates the antibacterial effects of various essential oils of commonly used spices against the bacteria of food spoilage and health significance, for future application as natural food preservative and chemotherapeutic agent.

MATERIALS AND METHODS

Essential Oils

Eleven samples of essential oils viz., Turmeric (*Curcuma longa*), Cassia (*Cinnamomum aromaticum*), Allspice (*Pimenta dioica*), Clove (*Syzygium aromaticum*), Nutmeg (*Myristica fragrans*), Ginger (*Zingiber officinale*), Fennel (*Foeniculum vulgare*), Cardamom (*Elettaria cardamom*), Mace (*Myristica fragrans*), Celery (*Apium graveolens*) and Black Pepper (*Piper nigrum*) were procured from Flavours & Essences Ltd, Mysore and Kancor Flavour & Extracts Ltd, Angamally, India. Essential oils were checked for sterility by observing no growth after spreading essential oil sample on nutrient agar (Hi-Media, Mumbai) and incubating at 37ºC.

Bacterial Strains

Fourteen bacterial strains obtained from Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh and National Collection of Industrial Microorganisms (NCIM), NCL, Pune, India was used in this study. The bacterial strains were *Bacillus cereus* (MTCC-430), *Bacillus subtilis* (NCIM-2117), *Enterobacter aerogenes* (NCIM-2340), *Escherichia coli* (MTCC-118), *Escherichia coli* (MTCC-119), *Escherichia coli* (NCIM-2066), *Klebsiella pneumoniae* (MTCC-109), *Proteus vulgaris* (NCIM-2027), *Proteus mirabilis* (NCIM-2388), *Pseudomonas aeruginosa* (NCIM-2074), *Salmonella typhi* (MTCC-734), *Salmonella paratyphi* A (MTCC-735), *Staphylococcus aureus* (NCIM-2492) and *Streptococcus pyogenes* (NCIM-2608). Bacterial cultures were maintained on Nutrient Agar (NA) slopes (Hi-Media, Mumbai), and tested for viability and purity before use.

Inoculum Preparation

A loopful of 24 hrs surface growth on a NA slope of each bacterial strain was transferred individually to 5 ml of Nutrient Broth (Hi-Media, Mumbai). The broth suspension was incubated at 37ºC till moderate turbidity was developed. The turbidity was matched with 0.5 McFarland standard[14] which corresponds to cell density approximately 1.5×10^8 CFU/ml.

Sensitivity Testing of Bacteria to Essential Oils

Antibacterial susceptibility testing of essential oils was performed by using the disc diffusion method [15]. For susceptibility testing, a sterile cotton swab was dipped into the standardized inoculum and rotated firmly against the upper inside wall of the test tube to remove excess inoculum from the swab. Entire sterile and dried Mueller Hinton agar (Hi-Media, Mumbai) surface of the plate was streaked with the swab three times, by turning the plate 60^0 between each streaking. Excess surface moisture was allowed to dry for not more than 15 minutes. A sterile disc of 6 mm diameter (SD067, Hi-Media, Mumbai) was impregnated with 20 µl of undiluted essential oil to be tested, with micropipette. The discs were then placed at center on the surface of seeded agar, aseptically. To allow diffusion of the essential oil into the agar, the plates were then left undisturbed for 30 min. The plates were incubated at 37^0C for 24 hrs and assessment of antibacterial activity was done by measuring the diameter of the zone of inhibition around each of the discs. The assay was performed in duplicate. The scale of measurement was the following (disc diameter included): $\geq$ 28 mm zone of inhibition is strongly inhibitory; $<$ 28 to 16 mm zone of inhibition is moderately inhibitory; $<$16 to 12 mm zone of inhibition is mildly inhibitory; and $<$12 mm is noninhibitory[16]. Antibiotic discs (Hi-Media, Mumbai) were used to evaluate the bacterial cultures for possible antibiotic resistance patterns that might affect sensitivity of assay. The antibiotics used were ciprofloxacin (5 µg/disc), chloramphenicol (30 µg/disc), ampicillin (10µg/disc), gentamycin (10 µg/disc) and tetracycline (30 µg/disc).

RESULTS

Antibacterial inhibition zones for essential oils against Gram-positive bacteria and Gram-negative bacteria are shown in Table 1. All the essential oils tested have shown the antibacterial activity but the degree of sensitivity varied with bacterial species. The essential oil of cassia inhibited all tested bacteria and thus exhibited highest activity, followed by essential oil of allspice and clove. Nutmeg oil, ginger oil, cardamom oil, mace oil, celery oil and black pepper oil showed inhibition rate ranging from 43 – 64%. The lowest inhibitory activity was recorded with fennel oil and turmeric oil. The percent bacterial inhibition by different essential oils is shown in Fig.1. Of the different bacterial species tested for their sensitivity, 93% bacterial species were inhibited by allspice oil.

Antibiotic sensitivity of the bacteria used in this study is shown in Table 2. No bacteria showed atypical antibiotics resistance patterns. *P. vulgaris* and *E. coli* MTCC118 were resistant to ampicillin, and *P. mirabilis* was resistant to tetracycline.

Table 1

Inhibition of growth of Gram-positive and Gram-negative bacteria by spice essential oils

Organism	Zone of Inhibition in mm										
	Essential oil										
	Cassia	Allspice	Clove	Nutmeg	Ginger	Fennel	Cardamom	Mace	Celery	Black pepper	Turmeric
Gram-positive bacteria											
B. cereus	37	19	19	14	14	11	15	12	16	16	12
B. subtilis	35	24	24	12	14	13	20	20	20	14	14
S. aureus	37	35	22	22	16	13	18	16	19	18	14
S. pyogenes	27	21	20	12	14	10	14	20	12	18	10
Gram-negative bacteria											
E. coli [1]	12	11	11	-	-	-	-	-	-	-	08
E. coli[2]	28	17	18	10	-	14	-	10	-	-	-
E. coli[3]	26	16	19	10	-	-	-	10	-	-	-
K.pneumoni.	20	15	14	08	-	11	-	08	-	-	08
S. typhi	54	32	24	14	-	-	-	-	12	-	-
S. paratyphi	28	17	17	13	-	-	-	-	-	-	-
E. aerogene	31	20	20	12	13	12	14	12	13	18	10
P. vulgaris	30	18	23	11	-	-	-	10	08	-	-
P. mirabilus	28	18	16	18	-	-	-	12	-	-	-
P. aeruginosa	27	20	24	15	12	11	12	15	16	17	14

E. coli[1] - *E. coli* MTCC-118, *E. coli*[2] - *E. coli* MTCC-119, *E. col* [3] - *E. coli* NCIM-2066,
– indicates no inhibition zone

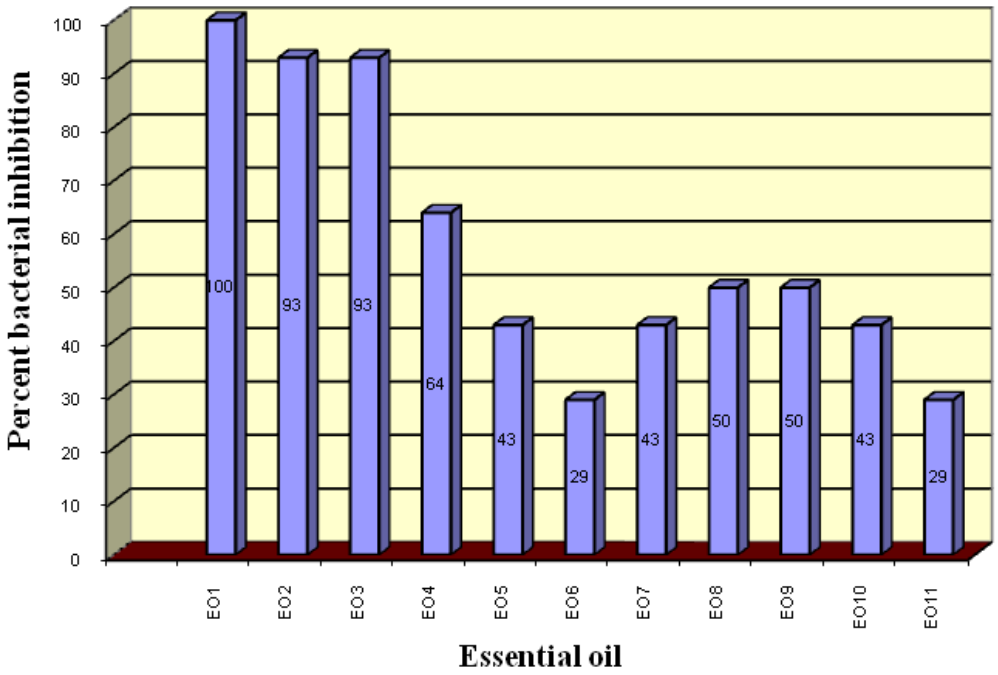
Table 2

Inhibition of growth of tested microorganisms by standard antibiotics

Organism	Zone of inhibition (mm)				
	Ciprofloxacin	Chloramphenicol	Ampicilin	Gentamycin	Tetracycline
B. cereus	38	32	22	30	30
B. subtilis	34	40	28	33	30
S. aureus	28	38	40	24	28
S. pyogenes	26	28	27	24	25
E. coli[1]	28	24	-	21	12
E. coli [2]	24	25	20	26	18
E. coli [3]	37	26	22	22	24
K. pneumoni.	22	24	15	19	15
S. typhi	38	24	30	28	20
S. paratyphi A	30	25	22	24	18
E. aerogenes	32	34	27	30	30
P. vulgaris	30	29	-	24	19
P. mirabilus	35	22	21	23	-
P. aeruginosa	29	39	25	29	20

E. coli[1] - *E. coli* MTCC-118,*E. coli*[2] - *E. coli* MTCC-119,*E. col* [3] - *E. coli* NCIM-2066,
– Indicates no inhibition zone.

Fig. 1
Percent bacterial inhibition shown by different spice essential oils



EO1- Cassia oil, EO2- Allspice oil, EO3- Clove oil, EO4- Nutmeg oil, EO5- Ginger oil, EO6- Fennel oil, EO7- Cardamom oil, EO8- Mace oil, EO9-Celery oil, EO10- Black pepper oil, EO11- Turmeric oil.

DISCUSSION

Cassia oil showed highest broad-spectrum inhibitory activity against both Gram-positive and Gram-negative bacteria. The strongest inhibition by cassia oil was recorded against *S. typhi* with largest inhibition zone (54 mm) of all, while MTCC118 strain of *E. coli* was weekly inhibited. Lis-Balchin and Deans [17], studied synergistic antibacterial agents in foods and reported inhibition zones for cassia oil against *B. subtilis*, *E. coli* and *S. aureus*. Similarly, in the present study, cassia oil inhibited *B. subtilis*, *E. coli* and *S. aureus* growth. A predominant food-borne pathogen *Listeria monocytogenes* has reported to be inhibited by cassia oil [18]. Cinnamic aldehyde, the main constituent of the cassia oil [7] may have inhibited growth of bacteria in the present study. The powerful antibacterial activity of cassia oil against Gram-negative pathogens, *S. aureus* and *S. pyogenes* suggests that cassia oil may be useful in some food formulations as an antimicrobial agent.

Zone of inhibition for tested bacteria exposed to the oil of allspice ranged from 11 to 35 mm. The oil exhibited strong antibacterial action only against *S. aureus* and *S. typhi* and moderate antibacterial action against all other bacterial strains except *E. coli* MTCC118. Rao et al. [19] reported strong antibacterial activity of essential oil from *Pimenta dioica* leaf against coagulase negative staphylococci and *Pseudomonas* spp. Allspice, a versatile condiment may aid in the control of bacterial growth and rancidity in food.

Clove essential oil also inhibited the growth of 93% bacterial species but was not strongly inhibitory to any of the tested microorganism. The antibacterial activity of clove is attributed to eugenol (2-methoxy-4-allyl phenol), which is considered as an

antiseptic and often employed as a preservative [20]. Clove bud oil contains high eugenol (85.3%) content [3]. Smith-palmer et al.[1] reported strong inhibitory activity of clove oil to food-borne pathogens including *E. coli* and *S. aureus*. Several other studies [3,8,9,21-24] recorded moderate to highest antimicrobial effect of clove oil against bacteria. In contrast, the present investigation showed substantial inhibitory activity of clove oil towards Gram-positive and Gram-negative bacteria.

Oil of celery had no inhibitory effect against three different strains of *E. coli*, *K. pneumoniae, S. paratyphi* A, *P. vulgaris* and *P. mirabilis* but moderately inhibitory against Gram-positive bacteria. Elgayyar et al. [16] reported moderate inhibitory activity of celery oil against *S. aureus*, and weak inhibitory activity to *E. coli* 0157:H7, *S. typhimurium* and *P. aeruginosa*. Misic et al.[25] also reported antibacterial activity of celery against *S. aureus*. Present investigation is in agreement with the previous reports [16,23] regarding inhibition of *B. cereus*, *S. aureus* and *P. aeruginosa* by celery oil.

Nutmeg oil did not strongly inhibit any of the bacteria tested in our study, whereas moderately inhibitory against *S. aureus* and *P. mirabilis* and had no inhibitory effect against *K. pneumoniae*, *P. vulgaris* and *E. coli*. Earlier studies[9] reported the inhibition of *B. subtilis, E. coli*, *K. pneumoniae*, *P. vulgaris* and *S. aureus* and no inhibition of *E. aerogenes* and *P. aeruginosa* by nutmeg essential oil. Smith-Palmer et al.[1] reported low inhibitory effect of nutmeg oil against Gram-positive and Gram-negative bacteria. Nutmeg oil contains monoterpenes[26] and it is concluded that combination of monoterpene hydrocarbons present in nutmeg oil are weakly effective against tested bacteria.

The present antimicrobial activity reveled that essential oil of fennel did not show any significant inhibitory activity against tested organisms. Smith-Palmer et al.[1] observed fennel oil to be slightly inhibitory to food borne pathogens. In contrast, others have reported the inhibitory activity of fennel oil at higher concentration[27] and in combination with known antimicrobial paraben [28].

The essential oil of ginger, cardamom & black pepper were totally ineffective against maximum Gram-negative tested organisms, whereas moderate to mild antimicrobial activity showed against Gram-positive bacteria. The major constituents of ginger are gingerone and gingerol, which have strong inhibitory activity against pathogenic bacteria[29]. Nanasombat and Lohasupthawee[2] reported that moderate antibacterial activity of ginger oil against enterobacterial pathogens and different strains of *Salmonella*. Lis-Balchin and Deans[17] reported no inhibition zone against gastroinstestinal bacteria. Similarly, present investigation showed that no inhibition zone against three different strains of *E coli* (Table 1). Essential oil of black pepper and cardamom, have been reported to be inactive against *E. coli* and *Salmonella* [2,30]. Present study is in agreement with the earlier reports regarding no inhibition effect against *E. coli* and *Salmonella* serotypes. Mace oil consist of monoterpenes (87.5%), monoterpene alcohol (5.5%) and other aromatics (7.0%) [31]. It was found that mace oil was moderately active against two strains of *E. coli*. In contrast Nanasombat and Lohasupthawee [2] reported no inhibition of *E. coli* by mace oil.

Comparison of inhibitory data in the present study reflected more sensitivity of Gram-positive bacteria to essential oils than Gram-negative bacteria. This is in agreement with previous reports [1, 3, 5]. However, there are some contradictory reports on sensitivity of Gram-positive and Gram-negative bacteria. Zaika [6] proposed that Gram-positive bacteria are more resistant than Gram-negative bacteria to the antibacterial properties of plant essential oils, while Dorman and Deans [9], hypothecated that susceptibility of bacteria to plant essential oils and the Gram reaction have little influence on growth inhibition. Lower susceptibility of Gram-negative bacteria may be related to the outer membrane of Gram-negative bacteria, which provides the bacterial surface with strong permeability barrier [32].

Comparing the correlation of zone of inhibition of tested essential oils and antibiotics, it was revealed that cassia oil showed much greater activity (54 mm) than

ciprofloxacin against *Salmonella typhi.* It suggests that cassia oil posse's higher potential in inhibiting *S. typhi* than all antibiotics tested.

CONCLUSION

It is evident from present data that all the spice essential oils showed varying inhibitions of the tested organisms of food spoilage and health significance. The results of present studies provide evidence that some essential oils might indeed be potential sources of new antibacterial agents in the food conservation system. Spices and essential oils are added as flavoring agents to foods, but are generally present in insufficient amount for their significant antimicrobial effects. Essential oils, which often contain the principal aromatic and flavoring component of spices, if added to food would prevent the onset of food spoilage.

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