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Inhibitory activities of *Brassica nigra* , *Cinnamomum cassia (Blume)* and *Cuminum cyminum* towards *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

Three Indian spices, namely Brassica nigra, Cinnamomum cassia (Blume) and Cuminum cyminum were examined independently, in their different forms (aqueous extracts, essential oils and powdered), for antibacterial potentials against Escherichia coli (MTCC1687) and Staphylococcus aureus (MTCC5021). Spice agar method was opted for investigating antibacterial potentials of powdered spice samples. Minimum inhibitory concentrations of the powdered spice samples were also determined. It was the concentration of spice sample that arrested the growth of test bacteria upto 80 % level of the total incubation period of 30 days. Agar well assay was followed for screening of antibacterial potentials of aqueous extracts and essential oils Results revealed that essential oils of spices arrested bacterial strains most effectively followed by powdered forms, while aqueous extracts were found ineffective. Among powdered spice samples tested, C. cassia (Blume) most effectively arrested test bacterial strains and among essential oils under investigation B. nigra displayed widest growth inhibitory zones towards microbes under observation. It was also noticed that S. aureus was more susceptible towards test substances as compared to E.coli.

Keywords: Antimicrobial, *Brassica nigra*, *Cinnamomum cassia*, *Cuminum cyminum*, spices.

INTRODUCTION

Food borne illness resulting from the consumption of foods contaminated with pathogenic bacteria or their toxins have been of great concern in the public health [1]. There is, however, a strong demand for the reduction of synthetic chemicals in food preservation sector. This opinion is based on the increased concern about the health risks associated with the use of artificial preservatives. Moreover, interest in improving health and fitness through natural products is also gaining momentum. At present it has been estimated that about 80% of the world population rely on botanical preparations as medicine and preservative to meet the needs as they are considered safe [2]. Spices are vital culinary adjuncts and beside masking taint odors, these have been used as preservatives, perfumes, aphrodisiacs and for the treatment of a variety of ailments, since time immemorial.

Brassica nigra (mustard) is of the family *'Cruciferae'*, and whole seeds of *B. nigra* have been used in pickles and salads as a spice and preservative [3]. *Cinnamomum cassia (Blume)*, commonly known as cassia, is a member of *'Lauraceae'* family, and is among the earliest, most popular spice used by mankind. Its dried, scented stem barks sold as whole sticks are called 'quills'. Powdered quills are an important ingredient of various spice blends and are used as tonic, stomachic and carminative. *Cuminum cyminum* (cumin) is native of Mediterranean region and

belongs to family '*Apiaceae*' . Its seeds mainly find their application where spicy foods are prepared and thus commonly used in Indian, Middle Eastern, Maxican, Portuguese and Spanish cookery. In the present study, three spices viz. *Brassica nigra*, *Cinnamomum cassia (Blume)* and *Cuminum cyminum* were investigated for their antibacterial effects against two food borne pathogens namely *Escherichia coli* and *Staphylococcus aureus.*

MATERIALS AND METHODS

Procurement of spice samples

Dried seeds of *B. nigra* and *C. cyminum* and dried stem barks of *C. cassia (Blume)* were procured in a single lot, in the amounts of 500 g each, from a wholesaler spice-seller, local market, Hisar, India. These spice samples were cleaned manually for extraneous material, ground to powdered form and were kept in airtight containers till further use.

Essential oils of all the three test spices were procured from Aroma Chemicals, Delhi, India. Procured essential oils were stored in the dark amber colored, screw capped glass bottles and were kept away from light to avoid physicochemical changes in their compositions. These bottles were closed tightly to check the loss of volatiles and were opened only for a shortwhile, whenever required. Purity of the spice essential oils was assured by the company to be more than 99.0 %.

Chemicals and culture media

MacConkey agar, MacConkey broth, Nutrient agar and Nutrient broth were obtained from Hi-Media Pvt. Ltd, India. Dimethylsulphoxide (DMSO) and Sodium chloride (NaCl) were purchaged from Central drug house (CDH) Pvt. Limited, India.

Bacterial cultures

Pure bacterial cultures of *Escherichia coli* (MTCC1687) and *Staphylococcus aureus* (MTCC5021), were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The reference bacterial strains were maintained on their respective media slants, subcultured bimonthly to maintain their viability and were stored at 4$\pm$1° C. Culture media viz. MacConkey agar and MacConkey broth were used for *E.coli*, while Nutrient agar and Nutrient broth were utilized for *S. aureus.* Temperatures of incubation for *E. coli* and *S. aureus* were 45^{o}C and 37^{o} C respectively. Culture media and incubation temperatures of bacterial strains followed in present study were as per MTCC recommendations.

Inoculum preparation

A flamed sterile wire loop was used to dislodge the lawns of test bacterial strains from their respective pure culture slants (24h. old) with 10 ml of sterilized normal saline (NaCl, 0.85% (w/v)) solution under aseptic conditions. Bacterial suspensions were adjusted with the same solution to contain approximately 1×10^{7} cfu /ml and were utilized the same day.

Prepearation of aqueous spice extracts

Aqueous extracts of powdered spice samples of *B. nigra and C.cyminum* were prepared [4]. Powdered spice samples were steeped overnight (temperature: 24-27°C) in sterilized distilled water in a ratio of 1:1 (w: v), followed by their homogenization in a blender at high speed for 2 min. The homogenized spice mixtures were filtered through Whatmann No. 1 filter paper. Filtrates thus obtained, were sterilized by passing through syringe filters containing 0.45 um pore size membrane filters under aseptic conditions, collected in sterilized glass vials and were stored at 4$\pm$1° C. These stored aqueous extracts were further used within the 2 h. of their preparation.

Screening antibacterial activities of powdered forms of spices

Antibacterial activities of powdered forms of spice samples were examined in culture media using spice agar method [5]. Erelenmeyer flasks (100 ml capacity) containing 20 ml of appropriate media (containing agar) and powdered spices at different concentration levels (0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 (%,w/v)), were autoclaved at 121° C for 20 minutes. After autoclaving, spice agar mixtures (cooled but still molten) were poured into sterilized petriplates under aseptic conditions and these plates were kept undisturbed for 30 min. for proper setting of agar. Freshly prepared inoculum of each test microbe at 100 ul level was evenly spread over the entire surface of the respective solidified media in petriplate using a sterile bent glass rod. Seeded petriplates were incubated in incubator at appropriate temperatures and were examined for bacterial growth at 12 h.

intervals, throughout the incubation period of 30 days. A similar experiment was carried out without any spice sample that served as control. The time for initiation of bacterial growth on control (without spice samples) and media supplemented with different concentration of spices were recorded.

Determination of minimum inhibitory concentration (MIC) values of powdered spices

MIC values of the powdered spices towards microbes under observation were determined from the observations of spice agar method [6]. For determining MIC values, concentrations of spices (%, w/v) were plotted on x-axis and days of inhibition on y-axis of the graph. The days elapsed prior to initiation of microbial growth were substracted from the days taken by the test mold to grow in the control samples (without spice).The 80% level of incubation period was calculated first i.e. 24 days, and then a horizontal straight line was drawn from this level to intersect the curve. From the point of intersection a perpendicular line was drawn indicating the minimum inhibitory concentration of the reference spice samples.

Screening of antibacterial activities of aqueous extracts and essential oils of spices

Agar-well diffusion technique was used [7]. Freshly prepared inoculum (100 ul) of each reference bacterial strain was poured in plates with 20 ml of appropriate media . The petriplates seeded with bacterial strains were kept undisturbed for 30 min. for proper solidification and setting of agar to facilitate uniform digging of wells. Sterile cork borer (diameter, 8 mm) was used to bore wells in the solidified media plates previously seeded with bacterial inocula. Subsequently, different volumes of test substances (10 ul of essential oils of *Brassica nigra and Cuminum cyminum*; Aqueous extracts of reference spices were used at three different concentration levels *i.e.* 50 ul, 80 ul and 100 ul) were introduced into the wells of agar plates. Sterilized dimethylsulphoxide (DMSO) instead of test samples of spices served as negative control. These plates were allowed to stand at room temperature for at least 1 h. for the even diffusion of poured components and were incubated without inversion at their respective incubation temperatures in incubator for 24 h. After incubation, zones of inhibition formed around the wells were measured in millimeters (mm) and results were expressed as the net zone of inhibition which represented the subtraction of the diameter of the well (8 mm) from the measured zone.

Statistical analysis

All the experiments were performed in triplicates with two independent trials and the results obtained were highly reproducible. Values of growth inhibitory zones are mean ±SD (standard deviation) of three replicates i.e. n=3.

RESULTS AND DISCUSSION

Antibacterial activities of powdered spices

The data pertaining to antibacterial activities of powdered forms of spices are presented in Table 1. *B.nigra* upto its highest concentration level of 6.0%, remained ineffective in arresting the growth of *E. coli*, and visible growth of reference bacterial strain in was noticed on 2nd day of incubation, as in control set of petriplates. However, at 2.5% level, powdered seeds of *B.nigra* delayed the growth of *S.aureus* by 4 days. The inhibition increased with the increase in the concentration of spice, and at a cocentration of 6.0% , *B.nigra* arrested the growth of *S. aureus* upto 28 days of the total incubation period of 30 days. *C. cyminum* did not produce any growth inhibitory towards *E.coli* and *S. aureus*. On the other hand, *C. cassia* arrested both the test microbes effectively in culture media. *S. aureus* was inhibited by 4 days at 0.8% level of *C. cassia*, while *E.coli* was arrested upto 3 days at 1.0% level of reference spice. A direct and positive relationship was noticed between the concentration of *C.cassia* and level of inhibition produced, and at a concentration level of 6.0%, it inhibited *E.coli* and *S.aureus* for 26 and > 30 days respectively. It is quite obvious from the results that all the three spices under investigation produced different growth inhibitory effects towards bacterial strains under investigation. Exactly why this differential inhibition occurred is not clear, but may be due to differing compositions of microbial membranes and their relative permeability to antimicrobial components of spices. Antimicrobial activity of the spices is known to be due to their volatile aromatic secretions known as essential oils. Considering the large number of different groups of chemical compounds present in the essential oils of spices, it is most likely that antibacterial potential is not attributable to one specific mechanism but that there are several targets in the cell [8]. Hypothesis have been proposed by different workers which involve: hydrophobic and hydrogen bonding of active compounds to membrane proteins and partition in the lipid bilayer [9], perturbation of membrane permeability consequent to its expansion and increased fluidity causing the leakage of ions and other cell contents, inhibition of membrane embedded enzymes, destruction of electrons transport systems, disruption of proton motive force (PMF) and coagulation of cell contents [10]. Not

all of these mechanisms are separate targets; some are affected as a consequence of another mechanism being targeted.

Results suggest that *S. aureus* was inhibited for greater number of days at lower concentrations of spices as compared to *E. coli*. The greater susceptibility of *S.aureus* may be due to the absence of an outer membrane in their cell membrane which makes this g+ve bacteria more sensitive to external environmental changes such as temperature, pH , natural extracts, essential oils and other antimicrobial substances [11]. On the other hand, the lipopolysacharides in the cell membrane of *E.coli* could provide a barrier to many antimicrobial agents, rendering this g-ve bacteria more resistant to certain agents than g+ve *S.aureus*.

The growth inhibitory efficacy of powdered spices in their descending order towards bacterial strain on the basis of MIC values (Table 3) and days of inhibition produced may be presented as: *C. cassia (Blume)> B.nigra> C.cyminum.*

Table 1: Inhibitory efficacies of powdered spice samples towards *E.coli* and *S.aureus*

Spice Concentration (%,w/v)	Days of inhibition					
	B. nigra		*C. cassia*		*C. cyminum*	
	E.coli	*S.aureus*	*E.coli*	*S.aureus*	*E.coli*	*S.aureus*
0.0	2	2	2	2	2	2
0.1	2	2	2	2	2	2
0.2	2	2	2	2	2	2
0.4	2	2	2	2	2	2
0.6	2	2	2	2	2	2
0.8	2	2	2	4	2	2
1.0	2	2	3	5	2	2
1.5	2	2	5	7	2	2
2.0	2	2	7	11	2	2
2.5	2	4	9	14	2	2
3.0	2	8	10	17	2	2
3.5	2	11	12	21	2	2
4.0	2	15	15	24	2	2
4.5	2	19	17	27	2	2
5.0	2	22	20	30	2	2
5.5	2	26	22	>30	2	2
6.0	2	28	26	>30	2	2

Table 2: Different levels of inhibition produced by dried and powdered spices against *E.coli* and *S. aureus*

Level of Inhibiton	Spice concentration (%, w/v)					
	B. nigra		***C. cassia (Blume)***		***C.cyminum***	
	E.coli	***S. aureus***	***E.coli***	***S. aureus***	***E.coli***	***S. aureus***
40 %	ND	3.60	3.50	2.08	ND	ND
60 %	ND	4.89	4.72	3.18	ND	ND
80 %(MIC)	ND	5.25	5.71	4.00	ND	ND

ND: Not detected ; MIC: Minimum inhibitory concentration

Antibacterial activities of aqueous extracts and essential oils

Aqueous extracts of reference spices during agar well assay (Table 3) did not exhibit growth inhibitory zones against any bacterial strain under observation. The passive nature of aqueous extracts may be due to the non extraction of the lipophilic antimicrobial components of spices in aqueous phase or may be due to the high volatility of essential oil components and their subsequent losses during the grinding and extraction procedure. Moreover, filteration of extracts in the present *in vitro* study was done through Whatmann filter paper no. 1, which might have led to the removal of components, responsible for any antimicrobial activity.

Zone inhibition assay results show that essential oils of all the spices at 10 ul/well exhibited distinct zones of inhibition towards bacterial strains under observation, while in control samples with DMSO no growth inhibitory zones were detected (Table 3). The diameter of growth inhibitory zones varied with the type of microbial strain and essential oil implicated in the study. Essential oil of *B. nigra* gave widest inhibitory zones towards test microbes followed by *C. cassia* and *C. cyminum.* The antimicrobial properties of essential oils of *B.nigra, C. cassia* and

C.cyminum are chiefly attributed to allylisothiocyanate [12], cinnamic aldehyde [13] and cuminic aldehyde [14], respectively, however, the extact mechanisms of their modes of action are still not well understood. It is worth mentioning here that all the essential oils under investigation produced wider inhibitory zones towards *S.aureus* than *E.coli*. Reasons for greater susceptibility of *S. aureus* are same as mentioned for the antibacterial potentials of powdered forms of reference spices.

Table 3: Inhibitory effect of aqueous extracts and essential oils of test spices towards *E.coli* and *S.aureus*

Spices	Bacterial strains	Zones of inhibition (mm)				
		Aqueous extracts			Essential oils	DMSO
		50 ul	80 ul	100 ul	10 ul	100 ul
B.nigra	***E. coli***	0.00	0.00	0.00	25.00±0.20	0.00
	S.aureus	0.00	0.00	0.00	41.00±0.62	0.00
C. cassia	***E. coli***	0.00	0.00	0.00	17.10±0.22	0.00
	S.aureus	0.00	0.00	0.00	39.00±0.34	0.00
C. cyminum	***E. coli***	0.00	0.00	0.00	15.10±0.78	0.00
	S.aureus	0.00	0.00	0.00	20.20±0.24	0.00

DMSO: Dimethylsulphoxide

CONCLUSION

Our present results indicate that powdered form of *C.cassia* and essential oils of all the spices effectively arrested food borne pathogens in culture media and may be further considered for their possible applications in food processing and preservation sector. Further studies must be undertaken to elucidate the safety, stability and organoleptic aspects of essential oils in different food systems before these substances can be reliably used in commercial applications. Furthermore, the exact mechanism of modes of action of spice essential oils is still not so well understood, thus, it would be the next line of research.

REFERENCES

[1] JH Meng; SH Zhao; MP Doyle; SW Joseph*; J. Food Prot*.,**1998**,61,1511-1514.
[2] H Hashim; EL Kamali; Y Mohammed; *Cur. Res. J. Bio. Sci*.,**2010**, 2,143-146.
[3] B Raghwan; ML Shankaranarayana; S Nagalakshami; CP Natarajan*; J.Sci. Food Agri*., **1971**, 22,523-525.
[4] MC Yin; WS Cheng; *J. Food Prot*., **1998**,61,123-125.
[5] LB Bullerman; MA Azzouz ; *J. Food Prot*., **1982**, 45,1298-1301.
[6] V Rajkumar; JS Berwal; *J. Food Sci. Tech*.,**2003**,40,416-418.
[7] CU Iroegbu; Nkere; *Int. J. Mol.Med. Adv. Sci*.,**2005,**1,182-189.
[8] CF Carson; BJ Mee; TV Riley ; *Antimicro. Agents Chemother*.,**2002**,46,1914-1920.
[9] IM Helander; HL Alakomi; K Latva-kala; T Mattile-sandholm; I Pol; EJ Smid; LGM Gorris; A Von-wright; J. *Agr. Food Chem*.,**1998**,46,3590-3595.
[10] RJW Lambert; PN Skandamis; PJ Cotte; GJE Nychas; *J. Appl. Micro*.,**2001**, 91, 453-562.
[11] LA Shelef; OA Naglik; DW Bogen; *J. Food Sci*.,**1992**,45,1042-1044.
[12] JH Han; RA Holley; D Nadarajah; *Int. J. Food Micro*., **2005**, 99, 257-267.
[13] B Krishnamoorthy; TJ Zachariah; J Rema; PA Mathew; *J. Spi. Aroma.Crops*.,**1999**,8, 193-195.
[14] L Jirovetz; G Bushbauer; AS Stoyanova; EV Georgiev; ST Damianova; *J. Flav. Frag*.,**2005**, 21, 186-191.