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Research article

BRASSICA NIGRA AND *CUMINUM CYMINUM*: INHIBITORS OF FOOD BORNE PATHOGENS

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ABSTRACT : Dried seeds of spices namely *Brassica nigra* (mustard) and *Cuminum cyminum* (cumin) were screened independently, in culture media, in their different forms (aqueous extracts, essential oils and powders) against some bacterial strains of spoilage and health significance. Test microorganisms included one gram+ve bacterial strain *i.e. Bacillus cereus (*MTCC 430) and three gram-ve bacterial strains *viz. Enterococcus faecalis* (MTCC 439), *Psuedomonas aeruginosa* (MTCC 1688) and *Shigella sonnei* (MTCC 2957). Spice agar method was opted for screening antibacterial activities of powdered forms of aforementioned spices at their different concentration levels (0.0, 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)). *B.nigra* more effectively inhibited bacterial strains in culture media. Minimum inhibitory concentrations (MIC) of powdered form of *B.nigra* were also determined. It was the concentration of spice which arrested the growth of bacterial strain upto 80% level of the total incubation period of 30 days. Agar-well assay was followed for antibacterial screening of aqueous extracts and essential oils of test spices. Aqueous extracts of reference spice samples did not exhibit growth inhibitory zones towards any test bacterial strains. On the other hand, essential oils of *B.nigra* and *C.cyminum* showed distinct growth inhibitory zones against all the bacterial strains under observation. Results obtained from agar well assay revealed that essential oil of *B.nigra* was more potent in inhibiting bacterial strains followed by *C. cyminum* . It was also noticed that *B. cereus* (gram+ve) was inhibited at lower concentrations of test substances as compared to all the other three gram-ve bacterial strains under investigation.

Keywords: *Brassica nigra, Cuminum cyminum,* spices, essential oils, antibacterial.

INTRODUCTION

Food preservation is a continuous fight against microorganisms spoiling the food or making it unsafe for consumption. The use of synthetic chemicals for extending the shelf life of food commodities is rampant. Recently, these artificial preservatives are being challenged by the consumers due to their possible carcinogenecity, teratogenecity, neurotoxicity, long degradation periods, environmental pollution, accumulation in the food chain, disruption of ecosystem and their power to destroy useful microorganisms along with harmful pathogens (Alzamora et al.,1995; Satish et al.,1999). Moreover, due to the development of new physiological races of pathogens, many of these synthetic preservatives are gradually becoming ineffective (Delp 1980; Spotts et al., 1986). Thus, food industry and scientific community seek for the antimicrobials of natural origin, those are safe, socially more acceptable and ecofriendly as well. Spices need fewer introductions and for people throughout the world, they stimulate appetite, add flavor and texture to otherwise monotonous and insipid foods and create visual appeals in meals. Originally added to change and improve the taste, a number of spices have been well documented for their therapeutic, antioxidant and antimicrobial properties. Oil of *Brassica nigra* seeds is known to cleanse the blood and treat skin diseases because of its high sulphur content and have been used as preservatives in pickles and salads (Raghwan et al., 1971). *Cuminum cyminum* seeds have marked stomachic, diuretic, carminative, astringent, bactericidal and fungicidal properties (Farag et al., 1989a; Singh and Upadhyay 1991; Jain et al., 1992; Afifi et al., 1994; Iacobellis et al.,2005; Jirovetz et al.,2005, Singh et al., 2006; Gachkar et al.,2007). Present piece of work was undertaken to expand the spectrum of antibacterial agents from natural resources and in this context *Brassica nigra* and *Cuminum cyminum* were selected based on their traditional uses in medicine and domestic culinary practices in India.

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MATERIALS AND METHODS

Procurement of spice samples

The dried plant parts of the spices *i.e B. nigra* (seeds) and *C. cyminum* (seeds) 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 *B.nigra* and *C. cyminum* 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

Ethyl violet azide dextrose agar, Ethyl violet azide dextrose broth, MacConkey agar, MacConkey agar, 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 Pvt. Limited, India.

Bacterial cultures

All the pure bacterial cultures, *viz. Bacillus cereus* (MTCC 430), *Enterococcus faecalis* (MTCC 439), *Psuedomonas aeruginosa* (MTCC 1688) and *Shigella sonnei* (MTCC 2957) 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±1° C. Culture media ,incubation temperatures and duration of incubation of reference bacterial strains are presented in Table 1.

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 (Yin and Cheng,1998). 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 Whatman 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±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 (Bullerman and Azzouz, 1982). 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.

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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 microbial 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 *B.nigra*

MIC values of the powdered spices towards microbes under observation were determined from the observations of spice agar method (Rajkumar and Berwal, 2003). 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 (Iroegbu and Nkere 2005) was used. 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 four different concentration levels *i.e.* 30 ul, 50 ul, 80 ul and 100 ul) were introduced into the wells of agar plates. Sterile 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.

RESULTS AND DISCUSSSION

Antibacterial activities of powdered forms of spices

B.nigra, at different concentration levels, displayed growth inhibitory effect towards three bacterial strains namely *B. cereus, P. aeruginosa* and *S. sonnei*, whereas *E. faecalis* remained resistant and their growth was noticed on second day of incubation at all the used concentrations (0.1-6.0%), as in control petriplates (Table2). All the bacterial strains under investigation remained resistant towards powdered form of *B.nigra* upto its 2.0% concentration level. However, at a concentration of 2.5% , *B.cereus* (g+ve) was inhibited for 3 days, on the other hand all the other g-ve bacterial strains remained resistant. Growth of *P.aeruginosa* and *S. sonnei* was arrested at 3% level of *B.nigra.* Various concentrations of *B.nigra* gave different levels of inhibition towards reference bacterial strains and growth inhibitory effect produced by *B.nigra* was found to be concentration dependent (Table 3). At 6.0% level, it arrested *B.cereus* throughout the incubation period of 30days, while *P.aeruginosa* and *S. sonnei* were inhibited for 24 and 27 days respectively.

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Presented results have also been supported by a previous investigation in which *B.nigra* at 10 % level, significantly inhibited three strains of *Escherichia coli* in culture media (Rhee et al., 2002). Minimum inhibitory concentration values of *B.nigra* (concentration of spice which completely inhibited the growth of microorganisms upto the 80% level of the total incubation period of 30 days *i.e.* upto 24 days), towards *B.cereus, P.aeroginosa* and *S.sonnei* were 5.12%, 6.00% and 5.71% respectively (Table 3).

Table 1: Bacterial strains tested

Bacterial strains	Strain number	Media used	Temperature of incubation	Duration of incubation
B. cereus	MTCC 430	Nutrient agar, Nutrient broth	30ºC	24 h.
E. faecalis	MTCC 439	Ethylviolet azide dextrose agar, Ethylviolet azide dextrose broth	45ºC	24 h.
P. aeruginosa	MTCC 424	Nutrient agar, Nutrient broth	32ºC	24 h.
S. sonnei	MTCC 2957	Nutrient agar, Nutrient broth	32ºC	24 h.

MTCC:Microbial Type Culture Collection, Chandigarh, India.

Table 2 : Effect of different concentrations of dried and powdered seeds of *B. nigra* and *C. cyminum* (%, w/v) on the growth of bacterial strains

Spice Concentration (%,w/v)	Spices	Days of inhibition towards bacterial strains			
		B.cereus	*E.faecalis*	*P.aeruginosa*	*S.sonnei*
0.0, 0.1, 0.2	*B.nigra*	2	2	2	2
	C.cyminum	2	2	2	2
0.4, 0.6, 0.8	*B.nigra*	2	2	2	2
	C.cyminum	2	2	2	2
1.0, 1.5, 2.0	*B.nigra*	2	2	2	2
	C.cyminum	2	2	2	2
2.5	*B.nigra*	3	2	2	2
	C.cyminum	2	2	2	2
3.0	*B.nigra*	5	2	5	5
	C.cyminum	2	2	2	2
3.5	*B.nigra*	9	2	7	8
	C.cyminum	2	2	2	2
4.0	*B.nigra*	14	2	10	12
	C.cyminum	2	2	2	2
4.5	*B.nigra*	17	2	12	15
	C.cyminum	2	2	2	2
5.0	*B.nigra*	23	2	15	19
	C.cyminum	5	2	2	2
5.5	*B.nigra*	26	2	20	22
	C.cyminum	8	2	2	2
6.0	*B.nigra*	30	2	24	27

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Powdered seeds of *C.cyminum* upto the highest concentration of 6.0% remained ineffective in arresting all the bacterial strains of the present experiment except *B.cereues,* the growth of which was inhibited at a concentration of 5.0% for 5 days. At 6.0% level, *C.cyminum* inhibited *B.cereus* for 12 days (Table 2). Exactly why this differential inhibition of reference bacterial strains by *B.nigra* and *C.cyminum* occurred, is not clear but may be due to different constituents of essential oils of the test spices and their different modes of action. It has been reported that major functional component of essential oil of *B. nigra* is allylisothiocyanate (95-99%) (Han et al.,2005) and that of *C. cyminum* essential oil is cuminic aldehyde (40-65%) (Jirovetz et al., 2005). Allylisothiocyanate is a non phenolic sulphur compound and its activity is believed to be due to the inactivation of the extracellular enzymes through disulphide bonds (Delaquis and Mazza,1995). On the other hand, Cuminic aldehyde is thought to bind proteins and prevents the action of enzyme amino acid decarboxylases (Wendakoon and Sakaguchi,1993; Helander, 1998) in microorganisms.

Table 3: Different levels of inhibition produced by dried and powdered seeds of *B. nigra* and *C.cyminum* against bacterial strains

Level of Inhibition	Spices	Spice concentrations (%, w/v)			
		B. cereus	*E. faecalis*	*P. aeruginosa*	*S. sonnei*
40 %	*B.nigra*	3.81	ND	4.50	4.00
	C.cyminum	6.00	ND	ND	ND
60 %	*B.nigra*	4.62	ND	5.40	4.90
	C.cyminum	ND	ND	ND	ND
80 %	*B.nigra*	5.12 (MIC)	ND	6.00 (MIC)	5.71 (MIC)
	C.cyminum	ND	ND	ND	ND

MIC: Minimum inhibitory concentration ND: Not Detected

Among bacterial strains tested, g+ve *B. Cereus*, with more days of inhibition at a given concentration level and lower MIC value, was found to be more sensitive towards powdered forms of spices as compared to all the other g-ve bacterial strains under observation. The greater susceptibility of g+ve bacterial strains may be due to the absence of an outer membrane in their cell membrane which makes them more sensitive to external environmental changes such as temperature, pH, natural extracts, essential oils and other antimicrobial substances (Shelef et al., 1980). On the other hand, the lipopolysacharides in the cell membrane of g-ve bacteria could provide a barrier to many antimicrobial agents, rendering these bacteria more resistant to certain agents than g+ve bacteria.

Antibacterial activities of aqueous extracts and essential oils of spices

Aqueous extracts of powdered seeds of *B.nigra* and *C.cyminum*, at 30, 50, 80 and 100 (ul/well) levels did not exhibit growth inhibitory zones against any bacterial strain under observation (Table 4). 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 Whatman filter paper no. 1, which might have led to the removal of components, responsible for any antimicrobial activity.

During preliminary screening, essential oils of both the reference spices at a concentration level 10 ul/well, displayed distinct zones of inhibition towards all the bacterial strains. *B.nigra* produced wider growth inhibitory zones as compared to *C.cyminum* (Table 4). Reasons for higher antibacterial potential of *B.nigra* essential oil than *C.cyminum* essential oil and greater sensitivity of *B.cereus* are same as mentioned for the antibacterial potentials of powdered forms of reference spices.

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Table 4: Inhibitory activities of essential oils and aqueous extracts of spices towards bacterial strains

Bacterial strains	Zones of inhibition (mm)						
	DMSO (10 ul)	Spices	Essential oils (10 ul)	Aqueous extracts			
				(30ul)	(50ul)	(80ul)	(100ul)
B.cereus	0.00	*B.nigra*	33.80±0.22	0.00	0.00	0.00	0.00
		C.cyminum	28.00±0.51	0.00	0.00	0.00	0.00
E.faecalis	0.00	*B.nigra*	30.00±0.47	0.00	0.00	0.00	0.00
		C.cyminum	12.00±0.22	0.00	0.00	0.00	0.00
P.aeruginosa	0.00	*B.nigra*	30.20±0.17	0.00	0.00	0.00	0.00
		C.cyminum	20.00±0.23	0.00	0.00	0.00	0.00
S.sonnei	0.00	*B.nigra*	30.20±0.64	0.00	0.00	0.00	0.00
		C.cyminum	16.90±0.42	0.00	0.00	0.00	0.00

(Zones of inhibition: Mean±SD; n=3)

CONCLUSION

Findings of present *in vitro* study confirmed the folkloric claims of the antibacterial effectiveness of spices domestically consumed in India. Results suggested that essential oils of reference spices most effectively inhibited bacterial strains, followed by powdered forms, while aqueous extracts were found to be ineffective. Hence, essential oils of *B.nigra* and *C.cyminum* and powdered form of *B.nigra* may be considered for further *in vitro* and *in vivo* studies against other harmful pathogens and may be considered for food presevation.

REFERENCES

Afifi NA, Ramadan A, El-kashoury EA and El- banna HA (1994). Some pharmacological activities of essential oils of certain umbelliferous fruits. Veterinary Medical Journal Giza: Vol. 42:85- 92.

Alzamora SM, Cerrutti P, Gerrero S and Lopez-melo A (1995). In : Food preservation by moisture control. Fundamentals of Applications, Eds. G.V. Barbosa-canovas and Welti-chanes Technomic, Lancaster, PA, pp:463-492.

Bullerman LB and Azzouz MA (1982). Comparative antimycotic effects of selected herbs, spices, plant components and commercial antifungal agents. Journal of Food Protection: Vol. 45: 1298-1301.

Delaquis PJ and Mazza G (1995). Antimicrobial properties of isothiocyanates in food preservation. Journal of Food Technology: Vol. 49:73-84.

Delp CJ (1980). Coping with resistance to plant disease control agents. Plant Disease: Vol. 64: 652 – 657

Farag RS, Daw ZY, Hewedi FM and Ei-baroty GSA (1989a). Antimicrobial activity of some Egyptian spice essential oils. Journal of Food Protection : Vol. 52: 665-667.

Gachkar L, Davood Y, Mohammad BR, Masood T, Shakiba AA. and Iraj R (2007). Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* and essential oils. Food Chemistry: Vol. 102**:** 898-904.

Han JH, Holley RA and Nadarajah D (2005). Use of mustard flour to inactivate *Escherichia coli* 0157:H7 in ground beef under nitrogen flushed packaging. International Journal of Food Microbiology: Vol. 99: 257-267.

Helander IM, Alakomi HL, Latva-kala K, Mattile-sandholm T, Pol I, Smid EJ, Gorris LGM. and Von-wright A (1998). Characterization of the action of selected essential oil components on Gram-negative bacteria. Journal of Agricultural and Food Chemistry: Vol. 46: 3590-3595.

Iacobellis SN, Cantore LP, Capasso F and Sentore F (2005). Antibacterial activity of *Cuminum cyminum L.* and *Carum carvi L.* essential oils. Journal of Agricultural and Food Chemistry : Vol. 53: 57-61.

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Iroegbu CU and Nkere (2005). Evaluation of the antibacterial properties of *Picralima nitida* stem bark extracts. International Journal of Molecular Medicine and Advance Science :Vol. 1: 182-189.

Jain SC, Purohit M and Jain R (1992). Pharmacological evaluation of *Cuminum cyminum*. Fitoterapia: Vol. 63: 291-294.

Jirovetz L, Bushbauer G, Stoyanova AS, Georgiev EV and Damianova ST (2005). Composition, quality control and antimicrobial activity of the essential oil of the cumin seeds from Bulgaria that had been stored for up to 36 years. Journal of Flavor and Fragrance: Vol. 21: 186-191.

Raghwan B, Shankaranarayana ML, Nagalakshami S and Natarajan CP (1971). Volumetric determination of p-hydroxbenzyl in sinalbin and in mustard seeds. Journal of the Science of Food and Agriculture : Vol. 22: 523-525.

Rajkumar V and Berwal JS (2003). Inhibitory effect of clove (*Eugenia caryophyllus*) on toxigenic molds. Journal of Food Science and Technology: Vol. 40: 416-418.

Rhee MS, Dougherty RH and Kang DH (2002). Combined effects of mustard flour, acetic acid and salt against *Escherichia coli O157:H7* stored at 5 and 22°C. Journal of Food Protection: Vol.65:1632-1635.

Satish S, Raveesha KA and Janardhana GR (1999). Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris*. Letters in Applied Microbiology: Vol. 28: 145-147.

Shelef LA, Naglik OA and Bogen DW (1980). Sensitivity of some common food borne bacteria to the spices sage, rosemary and allspice. Journal of Food Science: Vol. 45:1042-1044.

Singh G and Upadhyay RK (1991). Fungitoxic activity of cuminaldehyde, main constituent of *Cuminum cyminum* oil. Fitoterapia: Vol. 62: 86.

Singh G, Marimuthu P and Lampasona MP (2006). *Cuminum cyminum* L. Chemical constituents, antioxidant and antifungal studies on its volatile oil and acetone extract. Indian Perfumer: Vol. 50:31-39.

Spotts RA and Cervanteos LA (1986). Populations, pathogenicity and benomyl resistance of *Botrytis sp.* , *Penicillium sp.* and *Mucor piriformics*. Journal of Plant Disease: Vol. 80: 106 -108.

Wendakoon CN and Sakaguchi M (1993). Combined effect of sodium chloride and clove on growth and biogenic amine formation of *Enterobacter aerogenes* in mackerel muscle extract. Journal of Food Protection: Vol. 56: 410-413.

Yin MC and Cheng WS (1998). Inhibition of *Aspergillus niger* and *Aspergillus flavus* by some herbs and spices. Journal of Food Protection : Vol. 61: 123-125.