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Screening of spices for antimicrobial activity

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Abstract

Thirty five spices were screened for their antimicrobial activity against *Bacillus subtilis, Escherichia coli* and *Saccharomyces cerevisiae.* The results indicated that clove, cinnamon, bishop's weed, chilli, horse raddish, cumin, tamarind, black cumin, pomegranate seeds, nutmeg, garlic, onion, tejpat, celery and cambodge had potent antimicrobial properties.

Key words: spices, antimicrobial activity.

Introduction

Spices have been used since ancient times not only for flavouring foods, but also for their preservative and medicinal properties. Spices have also been reported to prevent microbial spoilage of foods (Daw 1994; Curtis *et al.* 1996; Hosono 1996). This preservative property of spices has been attributed to the presence of some antimicrobial principles contained in their oils (Sreenivasamurthy & Krishnamurthy 1959). The antimicrobial properties of some of the important spices are well documented (Web & Tanner 1945; Anderson *et al.* 1953; Conner & Beachut 1984; Tripathy *et al.* 1986; Karapinar 1990; Meena & Sethi 1994; Tewari 1994; Kandil *et al.* 1994; Kano *et al.* 1995; Tombe *et al.* 1995; Thomas *et al.* 1996; Cichewicz & Thorpe 1996; Jones *et al.* 1997; Ji *et al.* 1997)

An attempt has been made in this study to investigate the antimicrobial properties of 35 Indian spices for their antibacterial and antifungal properties. Spices with already established antimicrobial activities were also included, in order to make a comparative study with the presently included spices and also to standardize a method for screening. The study will help to establish the traditional role of spices as preservatives, antiseptics and disinfectants.

Materials and methods

Sources of spices

The spices (refer Table 1 for list of spices) used for this study were either bought fresh from the local market or were supplied by Spices Board, Cochin.

Test organisms

The test organisms used were *Bacillus subtilis* (ATCC 6633), a gram positive

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Table 1. Effect of spices on growth of test organisms

Spice	Dose (mg/ml)	Test organism		
		B. subtilis	*E. coli*	*S. cerevesiae*
Cumin	1	+	+	+
(*Cuminum cyminum* L.)	2	+	+	+
	5	+	+	+
	10	-	+	+
	25	-	+	+
	50	-	+	+
	100	-	+	+
Cinnamon	1	+	+	+
(*Cinnamomum zeylanicum* Blume)	2	+	+	+
	5	+	+	+
	10	+	+	+
	25	+	+	+
	50	+	+	+
	100	-	+	+
Black cumin	1	+	+	+
(*Nigella sativa* L.)	2	+	+	+
	5	+	+	+
	10	+	+	+
	25	+	+	-
	50	+	+	-
	100	+	+	-
Clove	1	+	+	+
(*Syzygium aromaticum*	2	+	+	+
(L.) Merr. et. Perry)	5	-	+	+
	10	-	+	+
	25	-	-	-
	50	-	-	-
	100	-	-	-
Onion	1	+	+	+
(*Allium cepa* L.)	2	+	+	+
	5	+	+	+
	10	+	+	+
	25	+	+	+
	50	+	+	+
	100	-	+	+

+ = Growth; - = No growth

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Table 1. (Continued)

Spice	Dose (mg/ml)	Test organism		
		B. subtilis	*E. coli*	*S. cerevesiae*
Bishop's weed	1	+	+	+
(*Trachyspermum ammi*	2	+	+	+
(L.) Sprague)	5	+	+	+
	10	-	-	-
	25	-	-	-
	50	-	-	-
	100	-	-	-
Chilli	1	+	+	+
(*Capsicum annuum* L.)	2	+	+	+
	5	+	+	-
	10	+	+	-
	25	-	+	-
	50	-	-	-
	100	-	-	-
Garlic	1	+	+	+
(*Allium sativum* L.)	2	+	+	+
	5	+	+	+
	10	+	+	+
	25	+	+	+
	50	-	+	+
	100	-	+	+
Celery				
(*Apium graveolens* L.)	1	+	+	+
	2	+	+	+
	5	+	+	+
	10	+	+	+
	25	+	+	+
	50	-	+	+
	100	-	+	+
Basil	1	+	+	+
(*Ocimum sanctum* L.)	2	+	+	+
	5	+	+	+
	10	+	+	+
	25	+	+	+
	50	+	+	-
	100	+	+	-

+ = Growth; - = No growth

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Table 1. (Continued)

Spice	Dose (mg/ml)	Test organism		
		B. subtilis	*E. coli*	*S. cerevesiae*
Tejpat	1	+	+	+
Cinnamomum tamala Nees	2	+	+	+
	5	+	+	+
	10	+	+	+
	25	+	+	+
	50	+	+	-
	100	+	+	-
Nutmeg	1	+	+	+
(*Myristica fragrans* Houtt.)	2	+	+	+
	5	-	+	+
	10	-	+	+
	25	-	+	+
	50	-	+	+
	100	-	+	+
Small cardamom	1	+	+	+
(*Elettaria cardamomum* Maton)	2	+	+	+
	5	+	+	+
	10	+	+	+
	25	+	+	+
	50	+	+	+
	100	+	+	-
Caraway	1	+	+	+
(*Carum carvi* L.)	2	+	+	+
	5	+	+	+
	10	+	+	+
	25	+	+	+
	50	+	+	-
	100	+	+	-
Turmeric	1	+	+	+
(*Curcuma longa* L.)	2	+	+	+
	5	+	+	+
	10	+	+	+
	25	-	+	+
	50	-	+	+
	100	-	+	+

+ = Growth; - = No growth

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Table 1. (Continued)

Spice	Dose (mg/ml)	Test organism		
		B. subtilis	*E. coli*	*S. cerevesiae*
Tamarind	1	+	+	+
(*Tamarindus indica* L.)	2	+	+	+
	5	+	+	+
	10	+	+	+
	25	-	+	+
	50	-	-	+
	100	-	-	+
Aniseed	1	+	+	+
(*Pimpinella anisum* L.)	2	+	+	+
	5	+	+	+
	10	+	+	+
	25	+	+	+
	50	+	+	+
	100	+	+	-
Black pepper	1	+	+	+
(*Piper nigrum* L.)	2	+	+	+
	5	+	+	+
	10	+	+	+
	25	+	+	+
	50	+	+	+
	100	+	+	+
Horse raddish	1	+	+	+
(*Armoracia rusticana*)	2	+	+	+
	5	-	-	+
	10	-	-	+
	25	-	-	+
	50	-	-	-
	100	-	-	-
Pomegranate seeds	1	+	+	+
(*Punica granatum* L.)	2	+	+	+
	5	+	+	+
	10	+	+	+
	25	-	+	+
	50	-	+	+
	100	-	-	-

+ = Growth; - = No growth

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Table 1. (Continued)

Spice	Dose (mg/ml)	Test organism		
		B. subtilis	*E. coli*	*S. cerevesiae*
Cambodge	1	+	+	+
(*Garcinia cambogia*	2	+	+	+
(Gaertn.) Desr.)	5	-	+	+
	10	-	+	+
	25	-	+	+
	50	-	+	+
	100	-	+	-
Mustard	1	+	+	+
(*Brassica juncea* L.)	2	+	+	+
	5	+	+	+
	10	+	+	+
	25	+	+	+
	50	+	+	+
	100	+	+	+

(Growth of all test organisms also observed at all doses for the following spices)
Coriander (*Coriandrum sataivum* L.)
Fennel (*Foeniculum vulgare* Mill.)
Ginger (*Zingiber officinale* Rosc.)
Large cardamom (*Amomum subulatum* Roxb.)
Curry leaf (*Murraya koenigii* (L.) Sprengel)
Mint (*Mentha piperita* L.)
Parsley leaf (*Petroselinum crispum* Miller)
Pepper long (*Piper longum* L.)
Dill (*Anethum graveolens* L.)
Mace (*Myristica fragrans* Houtt.)
Sweet flag (*Acorus calamus* L.)
Fenugreek (*Trigonella foenum graecum* L.)
Poppy seed (*Papaver somnifeum* L.)

+ = Growth; - = No growth

organism, *Escherichia coli* (ATCC 10536), a gram negative organism and *Saccharomyces cerevisiae* (ATCC 9763), an yeast. These organisms were obtained from Central Drug Laboratory, Calcutta and were maintained at 4°C after subculturing every 2 months in nutrient agar media. For the yeast subculture (YEPD media-yeast extract, peptone, dextrose), the method of Gupta & Banerjee (1970) was followed where benzyl penicillin and streptromycin sulfate were added to the yeast media to inhibit bacterial contamination.

Solvent extraction

The fresh spices were dried in shade and powdered. About 5 g of the powdered mass was extracted with ethyl alcohol of 90% purity. The powdered

spice was mixed with the solvent (5 times the mass of the spice taken) and placed on a rotary shaker for 12 h. The process was repeated several times till the extract was colour and odour free. It was then cold centrifuged at 4°C and concentrated to a fixed volume and stored in sterile vials at 10°C. The pH of the extracts was adjusted to 6.7.

A series of test tubes were then taken, which contained 5 ml of sterile molten nutrient agar media with its temperature being maintained at 45-50°C. A fixed volume of 0.05 ml extract of spice was added to each of the tubes so that the final concentration of spice varied from 1-100 mg/ml in the tubes. The whole process was done asceptically and was repeated six times for each of the spices.

The media was then allowed to solidify in the form of a slant and were then seeded with the test organisms. Nutrient agar media was used for *B. subtilis* and *E. coli* and YEPD media was used for *S. cerevisiae*. Control tubes were run simultaneously with identical solvent concentrations in order to check their

Table 2. Minimum Inhibitory Concentration (MIC) values of spices with antimicrobial activities

Spice*	MIC (mg/ml)		
	B. subtilis	*E. coli*	*S. cerevisiae*
Nutmeg	2-5	+	+
Cambodge	2-5	+	+
Cumin	5-10	+	+
Turmeric	10-25	+	+
Garlic	25-50	+	+
Celery	25-50	+	+
Cinnamon	50-100	+	+
Onion	50-100	+	+
Clove	2-5	10-25	10-25
Bishop's weed	2-5	10-25	10-25
Chilli	10-25	25-50	2-5
Horse raddish	2-5	2-5	25-50
Pomegranate seed	10-25	50-100	50-100
Tamarind	10-25	25-50	+
Black cumin	+	+	10-25
Basil	+	+	25-50
Caraway	+	+	25-50
Small cardamom	+	+	50-100
Aniseed	+	+	50-100

* Alcohol extracts; + Indicates growth (no inhibition)

possible growth inhibitory effects. The concentration of the spice at which no growth of the organism was observed was considered as the Minimum Inhibitory Concentration (MIC) of the spice for that organism. The results were expressed in terms of growth of organisms (+) or inhibition of growth (-) after addition of the spices. The values were expressed as means of six replications.

Results and discussion

The results (Table 1) indicated that nutmeg, cambodge, cumin, turmeric, garlic, celery, cinnamon and onion had antibacterial properties, while clove, bishop's weed, chilli, horse radish, pomegranate seed and cambodge had both antibacterial and antifungal proerties. Black cumin, basil, tejpat, small cardamom and aniseed had only antifungal properties.

From the range of MIC doses given in Table 2, it is also clear that clove, bishop's weed, chilli, horse radish and pomegranate seed had more antimicrobial (both antibacterial and antifungal) properties than others and needs to be investigated thoroughly. Some work already done in this regard include, inhibition of food borne pathogens by eugenol from clove and thymol from bishop's weed (Karapinar & Aktug 1987), antioxidant and antimicrobial activities of capsaicin from *Capsicum frutescens* (De & Ghosh 1993; Chowdhury & De 1996), inhibitory effects of crushed horse raddish on growth of microorganisms (Chemical Abstracts 1939) and antibacterial properties of the oil of pomegranate seed (Heftman *et al.* 1996).

Hence, the possible reason for the antimicrobial properties of clove, bishop's weed, horse radish, chilli and pomegranate seed may be due to the presence of eugenol, thymol, allyl isothiocyanate, capsaicin and punicic acid, respectively. However, the exact mode of action of the active principles is to be studied in detail.

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