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Antibacterial activity of certain spice extracts

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Abstract

Seventeen raw spice samples were examined for phytochemical constituents and antimicrobial properties. Qualitative phytochemical analysis of crude spice extracts revealed the occurrence of alkaloids, coumarins, flavonoids, saponins, terpenes and tannins. Turmeric, clove and bay leaf showed the highest frequency of occurrence of these plant components among others. Terpenes were present in 94.12% of the samples evaluated. Ethanol extracts of spice samples were *in vitro* evaluated for their antimicrobial properties using well diffusion assay against six Gram positive and Gram negative bacteria. Results showed that all the spice extracts, except black cardamom, possess biological activity on one or more of the test bacteria. Clove extracts displayed the highest antibacterial activity (19.5 mm) against *Escherichia coli*, followed by bay leaf (19 mm) against the same bacteria and cumin (19 mm) against *Pseudomonas aeroginosa*, at 1000 µg/100 µl. Extracts of galangale, turmeric and fennel also exhibited a broad spectrum biological activity. The most susceptible bacteria, based on frequency values, were *E. coli* (76.4%), *P. aeruginosa* and *Bacillus subtilis* (58.82%) and the least susceptible species were *Salmonella arizonae* (23.52%) and *Enterobacter aerogenes* (17.64%) at 1000 µg/100 µl. Overall, the presence of biologically active compounds and potent antimicrobial properties elucidate the potential use of spices in small amounts, individually or in combination in human therapy or folk medicine and as food preservative.

Keywords: antimicrobial properties, spices, phytochemicals, plant composition

Introduction

The word "spices" was derived from the Latin word "species" that indicates plants used both as quack medicaments and flavouring essences or as preservatives (Giese 1994). Some other uses of spices include; antioxygenic properties, preserving action, antimicrobial activity, perfumery and cosmetics. The constituents responsible for the spice properties in plants are mostly secondary metabolites.

Although there are large number of classes of plant constituents, most plants contain only few of them. It is frequently observed that botanically related plants contain similar or even the same constituents; thus also explains why spices appear clustered in some plant families, while other families do not contain any aromatic plant (Chopra *et al.* 1992).

Bahraini people have a very long known standing history in the use of ethnomedicine from local plant sources or some imported spices that

also served as an alternative therapeutic agents (Abbas & Al- Saleh 2002). The practice of traditional medicine is still very strong in the treatment of minor ailments. Ginger, clove, turmeric and galangale are examples of widely used spices for the treatment of common cold, sore throat and as antiseptic agent.

Food preparations containing synthetic antibiotics (e.g. griseofulvin, pimarcin) and chemical preservatives (e.g. sorbic, acetic benzoic acids) are restricted in many countries. Moreover, with the large and diverse increase in food consumption, the known biological preservatives approved for use in food preparation remain rather limited and unfit for all foodstuffs. The search for cheap, non-toxic and natural food preservatives which could be used safely and effectively is an important factor for future food industry. Inhibitory activity of spices against microbes has long been reported (Shelef 1983; Zaika 1988; El-Kady *et al.* 1993). Moreover, little work was done to match their ethnobotanical information with analytical research to identify active chemical compounds.

Therefore, a study was conducted to evaluate the inhibitory effects of certain spice extracts against some known Gram positive and Gram negative bacteria and to qualitatively screen spice samples for the presence of phytochemical constituents.

Materials and methods

Collection and preparation of spices

Fresh spice and condiment samples imported from India, Pakistan, Sri Lanka, Iran and USA, were collected from spice market of Bahrain during the months of March and April 2000. Sampling and processing of spice samples was performed according to Kneifel & Berger (1994). Spice samples were usually stored in metal, plastic containers, wooden boxes or in gunny bags or on bare ground. In the laboratory, materials were further sorted out, based on their purities and country of origin, rinsed twice in sterilized distilled water and dried in an oven at 50° C on a clean sterilized paper towel for 24 hours. The well-dried materials were finely ground and sieved through No. 50 mesh, before storing at 5°C for further analysis.

Ethanol extraction

For each spice sample, 20 g of fine dry powder was extracted with 200 ml of 96% ethanol in a soxhlet apparatus. The suspension was slowly heated in a metal heater at 60-70°C for about 4-6 hours. The ethanol extracts were passed through Whatman filter paper embedded in Buchner fennel. At the end of the extraction, the ethanol filtrate was evaporated using a rotary evaporator for about 15 minutes and the precipitated extract was concentrated as necessary and stored at room temperature for later antimicrobial assays.

Preparation of samples

The spice extracts (500 mg) individually transferred in to small vials and suspended in 1 ml dimethylsulphoxide (DMSO) were considered as stock solutions of the extracts. Dilution with further DMSO were obtained to achieve 500 and 1000 µg /100 µl.

Test bacteria and growth conditions

The following six bacterial species strains were used as test microorganisms for the antimicrobial activity assay. All bacteria were obtained from the American Type Culture Collection (ATCC) as lypholysed in ampoules. Gram positive species included *Bacillus subtilis* (ATCC 6051), *Enterobacter aerogenes* (ATCC 13048) and *Staphylococcus aureus* (ATCC 6538). The Gram negative species were *Escherichia coli* (ATCC 11775), *Salmonella arizonae* (ATCC 13314) and *Pseudomonas aeruginosa* (ATCC 10145). Cultures of these bacteria were grown in 25 ml nutrient agar (Oxoid) plates, except for *E. coli*, which was grown on LB plates, and were incubated for 24 hours at 37 ± 0.2°C.

Assay for antibacterial activity

The diluted crude extract in DMSO (at 500 and 1000 µg/100µl concentrations) was employed to screen for antimicrobial activity against the above bacterial species. The procedure used is

essentially the modified well diffusion method (Nimiri *et al.* 1999). Overnight broth cultures of bacteria (10^5 per 0.5 ml) were aseptically mixed with 20 ml nutrient agar cooled to 50°C in petri dishes. Plates were allowed to stand for 10 minutes at room temperature and then wells of 9 mm in diameter were made in the solidified agar medium with sterilized cork borer. One hundred µl of each spice extract concentration was slowly added to the wells using micropipettes and then incubated for 24 hours at 37°C. Each experiment was repeated at least twice and diameters of inhibition zone surrounding the agar well for each plate was averaged and expressed in mm. Appropriate treatments with positive and negative controls were included in the assay.

Phytochemical screening

Phytochemical analysis of spice extracts was performed employing standard qualitative methods as described by different investigators (Rizk & Bashir 1980; Fadeyi *et al.* 1989). The oven-dried materials were qualitatively screened for the presence of biologically active compounds including alkaloids, anthraquinones, coumarins, fiavonoids, saponins, terpenes and tannins (Table 3).

Results and discussion

Antibacterial activity of seventeen spices against *E. coli, P. aeruginosa, S. arizonae, B. subtilis, E. aerogenes* and *S. aureus* is presented in Table 1. All the examined extract samples demonstrated varying degrees of antibacterial activity against the tested bacteria. Moreover, inhibitory effects of extracts were usually higher at spice concentration level of 1000 µg/100 µl than at 500 µg/100 µl. The highest inhibitory antibacterial activities were observed against *E. coli* (15.30 mm) followed by *P. aeruginosa* (14.35mm) and *S. arizonae* (14.00 mm) at 1000 µg/100 µl concentration, and the lowest were detected against *B. subtilis* (11.18 mm) followed by *S. aureus* (12.25 mm) and *S. arizonae* (13.87 mm) at 500 µg/100 µl. Quantitatively, the highest susceptibility towards all spice extracts were also noted with *E. coli* (76.4%), followed by *B. subtilis* (58.82%) and *P. aeruginosa* (52.82%) at 1000 µg/100 µl concentration level and the lowest were detected with *E. aerogenes* (11.76%), followed by *S. aureus* (23.52%) and *S. arizonae* (23.52%) at 500 µg/100 µl concentration (Table 1).

In general, the highest inhibitory effect of spice extracts among Gram negative bacteria was exhibited by cloves against *E. coli* (19.5 mm inhibition zones followed by bay leaf (19 mm inhibition zone) against the same bacterium and cumin (19 mm inhibition zone) against *P. aeroginosa* at 1000 µg/100 µl concentration. Antibacterial susceptibility among Gram-positive bacteria varied considerably, with the highest recorded in *S. aureus* (17.5 mm inhibition zone) against galangale and the lowest detected in *B. subtilis* (10.25 mm inhibition zone) against coriander at 1000 µg/100 µl concentration. However, black cardamom showed no biological activity against all bacteria, even in the highest concentration used.

Results of *in vitro* evaluation of 14 various standard antibiotic disks against six Gram positive and Gram negative bacteria are given in Table 2. Among Gram negative bacteria, *E. coli* showed the highest susceptibility level against oxytetracyclin and tetracyclen (22.5 mm). Other bacteria showed variable inhibitory reactions. In addition *E. coli* displayed strong resistance against penicillin, cloxacillin and erythromycin (6mm). Among Gram positive bacteria chloramphenicol revealed the highest activity (33 mm) against *B. subtilis* followed by neomycin (32 mm) and erythromcin (31.3 mm).

Occurrence of some phytochemical constituents among spices determined by qualitative methods are shown in Table 3. Results were expressed as + or - based on visual chemical reactions. Anthraquinones were not detected in all spice samples. In general, no clear pattern can be inferred from those phytochemical constituents in spices. With the exception of caraway, terpenes revealed the highest frequency

Table 1. Antibacterial activity of spice extracts against some selected Gram-negative and Gram-positive bacteria

Spice	Diameter of inhibition zone (mm)											
	E. coli		*P. aeruginosa*		*S. arizonae*		*B. subtilis*		*E. aerogenes*		*S. aureus*	
	500	1000	500	1000	500	1000	500	1000	500	1000	500	1000
Galangale	14.0	14.5	14.50	14.50	0.00	0.00	0.00	11.00	14.0	14.00	14.00	17.50
Green cardamom	14.5	14.5	15.00	15.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
'Black cardamom'	0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Black pepper	15.0	15.0	15.00	15.00	0.00	0.00	11.00	11.00	0.00	0.00	0.00	0.00
Red chilli	16.0	18.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.00	15.00
Lemon, Dry	0.0	13.0	0.00	0.00	13.50	13.50	11.00	11.00	0.00	0.00	11.00	12.00
Turmeric	12.0	12.0	0.00	0.00	0.00	0.00	11.50	12.00	0.00	0.00	0.00	0.00
Ginger, Dry	0.0	0.0	0.00	0.00	0.00	0.00	10.40	10.50	0.00	0.00	0.00	0.00
Cinnamon	0.0	0.0	0.00	0.00	12.50	13.00	0.00	10.50	0.00	0.00	0.00	0.00
Cloves	18.5	19.5	12.00	12.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fennel	14.5	15.0	12.00	12.00	0.00	0.00	0.00	10.60	0.00	12.00	0.00	14.00
Nutmeg	15.5	16.0	0.00	12.00	0.00	0.00	12.00	14.50	0.00	0.00	12.00	13.00
Coriander	0.0	0.0	13.00	13.00	14.50	14.50	0.00	10.25	0.00	0.00	0.00	12.50
Caraway	0.0	12.0	0.00	0.00	15.00	15.00	0.00	0.00	0.00	0.00	0.00	0.00
Bay leaf	16.0	19.0	14.50	15.00	0.00	0.00	0.00	11.00	14.00	14.00	0.00	0.00
Cumin	15.5	15.5	12.00	19.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.00
Black cumin	0.0	15.0	16.00	16.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total average	15.5	15.3	13.77	14.35	13.87	14.00	11.18	11.23	14.00	13.33	12.25	13.71

of occurrence among all spices (94.12%). This was followed by saponins and coumarins (41.17%), alkaloids (29.41%), flavonoids (23.52%) and tannins (17.65%). The highest frequency of occurrence of phytochemical constituents for each spice sample were observed in turmeric, cloves, and bay leaf (57.14%) and the lowest were found in galangale, green and black cardamom, caraway and black cumin (14.28%). Tannins showed very high reaction in cinnamon, cloves, and bay leaf, whereas other phytochemicals showed variable intensity of chemical reactions.

With the increase in consumption of spices in our daily diet to enhance flavour, aroma and to some extent as natural preservative, it was believed to be useful to evaluate their antimicrobial properties at different concentration levels. The results obtained indicate a fairly good correlation between ethnomedicinal information and microbial activity (Table 2), as some of these spices (cinnamon, ginger, galangale, lemon, cloves) are commonly used in local folk medicine (Nimiri *et al.* 1999). Compounds extracted from plant sources, unlike synthetic preparation, have broad effects against microbes, safe with little or no side effects and comparatively cheaper and readily available.

The findings of the present work clearly showed that in general spice extracts possessed antibacterial properties. Extracts of galangale and fennel displayed a broad range effects against Gram-positive and Gramnegative bacteria as determined by inhibition zones. However, the strongest microbial activity was observed in cloves (19.5 mm inhibition zone) at the concentration of 1000 µg/100 µl (Table 1). Bay leaf and cumin displayed also high inhibitory level (19 mm inhibition zone) at higher concentration. These biological activities are comparable with several standard antibiotics used as a positive control (Table 2). Other spices, for example, coriander, cinnamon, black pepper and cumin gave various degrees of inhibitions. Dry lemon, turmeric and ginger had relatively low inhibitory effects against bacteria, while black cardamom revealed no biological activity under similar experimental conditions of the current study.

The results obtained in this study concerning the biological activity of spice extracts against wide range of microbes are in agreement with others (Zaika 1988; Shelef *et al.* 1980; Shelef 1983). El-Kady *et al.*, (1993) compared *in vitro* antibacterial properties of essential oils of several spices against species of Grampositive and Gramnegative bacteria. They grouped the inhibitory activity of essential oils into highly effective as in thyme, cinnamon and cardamom; moderately effective as in peppermint, marjoram, and rosemary and low or not effective as in cloves, Chinese cassia, cumin and eucalyptus.

Table 1 revealed that Gramnegative bacteria were somewhat more susceptible to spice extracts in contrast to Grampositive bacteria. Similar finding were not reported when essential spice oils were used (Farag *et al.* 1989; El-Kady *et al.* 1993). The extent of the inhibitory effect of some of these essential oils was attributed to the presence of aromatic nucleus containing a polar function group (El-Kady *et al.* 1993). Nevertheless, the differences in each study could be due to the differences in the extraction procedures and biological testing for which our extracts contain several other compounds and impurities compared to essential oils (Farag *et al.* 1989).

Terpenes, beside other compounds, were found to be a major component of nearly all spice samples that were analysed, except in caraway, (Table 3). Cinnamon contains 0.5 to 1.0% volatile oil composed mainly of cinnamaldehyde, eugenol cinnamic acid, and O-methoxycinnamaldehyde. Morozumi (1978) studied the inhibition of bacterial growth by O-methoxycinnamaldehyde and concluded that out of eight different isolates of pathogenic bacteria tested, the compound was effective only against *S. aureus* and *Colstridium botulinum*. Cinnamylphenol compounds have been recog-

Table 2. Antibacterial activity of some standard antibiotic disks against tested microorganisms

Antibiotic	Concentration	Diameter of inhibition zone (mm)					
		E. coli	*P. aeruginosa*	*S. arizonae*	*B. subtilis*	*E. aerogenes*	*S. aureus*
Nystatin	100 units	0.0	16.0	0.0	0.0	10.0	0.0
Penicillin	10 units	6.0	0.0	13.0	22.5	15.0	15.0
Vancomycin	30 µg	7.0	19.0	0.0	23.5	0.0	16.0
Neomycin	30 µg	7.0	15.0	20.0	32.0	19.0	20.0
Chloramphenicol	30 µg	20.0	0.0	0.0	33.0	25.0	28.0
Streptomycin	10 µg	12.0	20.0	15.0	16.0	22.0	15.0
Tobramycin	10 µg	8.0	20.0	20.0	29.5	21.0	18.0
Ampicillin	10 µg	0.0	0.0	0.0	25.0	0.0	29.5
Oxytetracyclin	30 µg	22.5	0.0	20.0	17.0	12.0	20.0
Tetracycline	30 µg	22.5	12.0	13.5	20.0	10.0	15.0
Cloxacillin	1 µg	6.0	16.0	0.0	17.0	14.0	0.0
Chlortetracyclin	30 µg	17.5	0.0	22.0	0.0	16.0	0.0
Novobiocin	30 µg	7.5	0.0	0.0	25.0	15.0	0.0
Erythromycin	15 µg	6.0	0.0	0.0	31.3	15.0	0.0

Table 3. Phytochemical constituents* of spices

Spice	Alkaloids	Flavonoids	Terpenes	Tannins	Saponins	Coumarins	Anthraquinones
Galangale	-	-	+++	-	-	-	-
Green cardamom	-	-	++	-	-	-	-
'Black cardamom'	-	-	+++	-	-	-	-
Black pepper	+++++	+++++	+++++	-	-	-	-
Red chilli	++++	+++	+++++	-	-	-	-
Lemon, Dry	++++	-	+++	-	-	++++	-
Turmeric	++	-	++++	-	+	+++	-
Ginger, Dry	++	-	+++++	-	+++	-	-
Cinnamon	-	-	+++	+++++	++++	-	-
Cloves	-	++++	++++	+++++	++++	-	-
Fennel	-	-	++	-	+	+++	-
Nutmeg	-	+++	+++++	-	-	+++	-
Coriander	-	-	+++	-	-	++	-
Caraway	-	-	-	-	-	+++	-
Bay leaf	-	-	++	+++++	+	+++	-
Cumin	-	-	+++++	-	+	-	-
Black cumin	-	-	++	-	-	-	-

* Chemical reaction of spices was expressed as (- to +++++) where: (-) = no reaction; (+)=scarce reaction; (++)= weak reaction; (+++)=moderate reaction; (++++)= high reaction and (+++++)= very high reaction.

nized as a new group of natural products occurring in species of *Dalbergia* and *Macharium* (Gregson *et al.* 1968). Dupuis *et al.* (1972) tested the antibacterial activity of cinnamylpyrogallol and found strong antibacterial activity in tests with *Streptomyces griseus* and *B. mycoides*.

El-Kady *et al.* (1993) and Farag *et al.* (1989) studied the effect of spices and their essential oils on growth of several prokaryotic and eukaryotic organisms and concluded that spices used in normal amount for ordinary food were insufficient as preservative. However, when used in larger amount, cinnamon, cloves and allspice retarded microbial growth. Paster *et al.* (1995) have shown that essential oils from thyme (which contain carvacrol and thymol) are effective as fumigants against fungi on stored grain. These investigators have proposed using them as alternative to chemicals for preserving stored grains.

Phytochemical analysis revealed the presence of several bioactive plant constituents, like tannins, phenolics, glycosides, alkaloids, flavonoids, steroids and saponins, which are in agreement with findings of previous workers (Chopra *et al.* 1992; Hamsaveni & Purushothaman 1986; Scalbert 1991). Synergistic action of the above plant constituents may be responsible for enhanced activity in these plant extracts, especially in clove and bay leaf which showed high potency.

Antimicrobial assay on spice extracts are valuable in screening and detecting the presence of antimicrobial activities. However, such assays do not provide true quantitative measure of the activities of some components present in the extract such as the polar and large molecules which have lower mobility in the water-agar medium (Kumar *et al.* 1997). The biologically active components in the tested spice extracts are not known and needs further analysis.

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