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PHYTOCHEMICAL AND ANTIBACTERIAL PROPERTIES OF SPICES AGAINST FOOD BORNE BACTERIA WITH SPECIAL REFERENCE TO *PARMELIA PERLATA*

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ABSTRACT
Spices have effective antimicrobial potentials and in the present study, fourteen spices have been tested for their efficacy against food borne bacteria under in vitro conditions. Methanolic extracts were used to determine phytochemical and antibacterial properties of the spices. Phytochemical analysis of the spice extracts explored that glycosides, steroids and terpenoids as the major phytochemicals present. Activity of spice extracts was higher on Gram positive bacteria when compared to Gram negative ones. Well diffusion assay revealed that seven out of fourteen spices tested had effective antibacterial activity against the pathogens. Minimum inhibitory concentration (MIC) assays were performed and *Staphylococcus aureus* ATCC 6538 (20 mm) was found as the most sensitive organism for the spice extracts tested followed by *Bacillus subtilis* ATCC 6633 (18 mm). Moderate resistance was exhibited by *Escherichia coli* ATCC 8739 and *Salmonella typhimurium* MTCC 3224 (5-12 mm). In general, MIC in the range of 1-16 µg ml^{-1} for Gram positive bacteria and 8-16 µg ml^{-1} for Gram negative bacteria were observed. Cardamom, cinnamon and clove also exhibited significant antibacterial activity with the MIC values of 2-8 µg ml^{-1} against the Gram positive bacteria food borne bacteria. Significant antibacterial activity (MIC ≤ 2 µg ml^{-1}) of stone flower (*P. perlata)* against all the Gram positive food borne bacteria was observed. None of the extracts were able to control the growth of Gram negative bacteria except that of mace which has been found potential antibacterial activity against *S. flexneri* (MIC ≤ 1 µg ml^{-1}).

KEY WORDS: stone flower, antibacterial, food borne pathogens, biological control.

INTRODUCTION

Despite continuous advances in food safety and health, control and prevention of food borne pathogens are still remaining as a major concern. Controlling the growth of food borne microorganisms is achieved by physical (temperature, pH, osmotic pressure) and chemical (weak organic acids, hydrogen per oxide, organic biomolecules) methods (Ray, 1996, Brull and Coote, 1999). Food additives such as monosodium glutamate, aspartame, saccharin, sodium cyclamate, sulfites, nitrates, nitrites and antibiotics causes headache, nausea, weakness, mental retardation, seizures, cancer and anorexia (Rangan and Barceloux, 2009, Wroblewska, 2009). The increasing concern about the toxic chemical preservatives, demand for food with longer shelf life with no or less chemical preservatives put pressure to find alternatives for better healthcare. There is a considerable interest to stop the illness outbreaks caused by pathogenic and spoilage food microorganisms among food processors, food safety researchers and regulatory agencies. Antimicrobial agents from indigenous plant origin are quite effective and spices occupy a considerable attention as they have a great potential to be used as antimicrobial agents (Arora and Kaur, 1999). Spices have been recognized for their value of preserving foods and medicinal values due to the presence of bioactive antimicrobial compounds (Lai and Roy, 2004). It is well established that spice extracts have potential antimicrobial properties (Shelef, 1983, Sagdic *et al.,* 2003, Rahman and Kang, 2009, Rahman *et al.,* 2011). Ethno pharmacological studies on spices revealed its anti-oxidant (Madsen and Bertelsen, 1995), anti-inflammatory (Mueller *et al.,* 2010) and immunomodulatory (Cherng *et al.,* 2008) activities. In this study, we examined the phytochemical composition of fourteen spices and compared the inhibitory effects of extracts on the growth of food borne bacteria. Minimum inhibitory concentration values of methanolic extracts were studied in vitro against 6 food borne bacteria and the results are discussed.

MATERIALS AND METHODS

Spices

Fourteen samples of spices viz., *Laurus nobilis* (Bay leaf), *Capparis spinosa* (Caper), *Carum carvi* (Caraway), *Elettaria cardamomum* (Cardamom), *Cinnamomum verum* (Cinnamon), *Syzygium aromaticum* (Clove), *Coriandrum sativum* (Coriander), *Cuminum cyminum* (Cumin), *Foeniculum vulgare* (Fennel), *Myristica fragrans* (Mace) *Piper nigrum* (Pepper), *Papaver somniferum* (Poppy), *Illicium verum* (Star anise), *Parmelia perlata* (Stone flower) were used in this study.

Preparation of methanolic extracts

Samples of spices were pulverized and extracted twice in methanol (1:10 w/v) at room temperature for 48 hrs and filtered. The filtrates were concentrated to dryness under reduced conditions at room temperature. Dried extracts

were then suspended in dimethyl sulfoxide (DMSO) for further use.

Phytochemical analysis

Test for Alkaloids (Mayer's test)

0.5 ml of extract was added with a drop or two of Mayer's reagent by the side of test tube. Formation of white or creamy precipitate indicates positive test for alkaloids.

Test for Flavonoids (Ammonia test)

1 ml of the extract was taken in the test tube and ammonia solution was added (1:5) followed by the addition of conc. sulphuric acid. Appearance of yellow color and its disappearance on standing indicates the positive test for flavonoids.

Test for Glycosides (Keller Kiliani test)

5 ml of each extract was added with 2 ml of glacial acetic acid which was followed by the addition of few drops of ferric chloride solution and 1 ml of conc. sulphuric acid. Formation of brown ring at interface confirms the presence of glycosides.

Test for Phenols (Ferric chloride test)

0.5 ml of the extract was added with few drops of neutral ferric chloride (0.5%) solution. Formation of dark green color indicates the presence of the phenolic compounds.

Test for Saponins (Froth test)

1 ml of the extract was taken in a test tube and distilled water (2 ml) was added to it. The test tube was then kept in boiling water bath for boiling and was shaken vigorously. Existence of froth formation during warming confirms the presence of saponins.

Test for Steroids: (Libermann - Burchard's test)

2 ml of acetic anhydride was added to 0.5ml of the extract and then added 2 ml of conc. sulphuric acid slowly along the sides of the test tube. Change of colour from violet to blue or green indicates the presence of steroids.

Test for Tannins (Ferric chloride test):

1 ml of the extract was added with 5 ml of distilled water and kept for boiling in hot water bath. After boiling, sample was cooled down and to this 0.1% ferric chloride solution was added. Appearance of brownish green or blue black coloration confirms the presence of tannins.

Test for Terpenoids (Salkowski test)

5 ml of extract was taken in a test tube and 2 ml of chloroform was added to it followed by the addition of 3 ml of conc. sulfuric acid. Formation of reddish brown layer at the junction of two solutions confirms the presence of terpenoids.

Test organisms

Staphylococcus aureus ATCC 6538, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Salmonella typhimurium* MTCC 3224, *Bacillus cereus* ATCC 10876 *and Shigella flexneri* ATCC 12022 were obtained from American Type Culture Collection (ATCC) and Microbial Type Culture Collection (MTCC), Chandigarh. The bacteria were then standardized by adjusting the bacterial suspension to absorbance reading within the range of 0.08 to 0.10 at OD 625 nm which was equivalent to 1-2 × 10^8 CFU/mL

Antibacterial assay

The antibacterial activity of methanolic extract of spice samples were evaluated using well diffusion assay. 100µl of the appropriate bacterial suspension was inoculated on Mueller Hinton agar using sterile swabs. 20 µl of the extract was added into the 5 mm wells and the plates were allowed for pre-diffusion of the extract before incubation. The diameter of zone of inhibition mean of two replicates ± SD as indicated by clear area which was devoid of growth of microbes was measured to determine antibacterial activity. The experiment was replicated twice to confirm the reproducible results.

Minimum Inhibitory concentration (MIC)

Well diffusion method

Determination of MICs of the spice extracts was done by well diffusion and agar dilution techniques and the concentrations of the extracts used were 16, 8, 4, 2, 1 µg/ml. The zone diameter of inhibition was determined for the different concentrations tested. The data obtained were statistically analyzed by the variance analysis with a significance level (P) of 0.05.

Agar dilution method

MIC of spice extracts was tested by two fold dilution method described by Chandrasekaran and Venkateshulu (2004). In brief, the methanolic extracts were dissolved in DMSO and added into Luria-Bertani (LB) broth to obtain a concentration of 32 µg/ml and serially diluted to achieve 16, 8, 4, 2, 1 µg/ml. A 10 µl standardized suspension of each tested organism (10^7 CFU/ml) was transferred to each tube. The control tubes containing only bacterial suspension were incubated at 37°C for 24 hrs. The lowest concentration of the extract which did not show any growth of tested organism was determined as the MIC.

RESULTS AND DISCUSSION

Phytochemical analysis of the spice extracts explored that glycosides, steroids and terpenoids as the major phytochemicals universally present in the samples (Table-1). In addition to that, flavonoids, tannins, phenols were also found in most of the spices tested. Alkaloids were determined in four of the fourteen spices tested. Saponins were present only in clove extracts. Preliminary antibacterial studies of the 14 studied spice extracts are summarized in table-2. Well diffusion method revealed various degree of sensitivity by the pathogens against the extracts studied. From the preliminary screening, extracts exhibited the zone diameter of inhibition with more than 20 mm was considered as positive and further minimum inhibitory concentration assay was performed. In this case, bay leaf, cardamom, cinnamon, clove, mace, star anise, stone flower were selected and studied for their MIC values. Minimum inhibitory concentration (MIC) assay by well diffusion method had demonstrated *Staphylococcus aureus* ATCC 6538 (20 mm) as the most sensitive organism for the spice extracts tested followed by *Bacillus subtilis* ATCC 6633 (18 mm). Moderate resistance was exhibited by *Escherichia coli* ATCC 8739 and *Salmonella typhimurium* MTCC 3224 (5-12 mm) (Table- 3 and 4). *B. subtilis* was found to sensitive against cardamom and cinnamon (MIC: 2 and 4 µg ml^{-1}).

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TABLE - 1: Phytochemical analysis of spice extracts

Botanical name	Common name	Family	Part used	Phytochemical analysis							
				Alk	Fla	Gly	Phe	Sap	Ste	Tan	Ter
Laurus nobilis	Bay leaf	Lauraceae	Leaf	-	+	++	+	-	+	+	++
Capparis spinosa	Caper	Capparidaceae	Flower bud	-	+	+	++	-	+	++	++
Carum carvi	Caraway	Apiaceae	Seed	-	+	+	+	-	+	+	++
Elettaria cardamomum	Cardamom	Zingiberaceae	Seed	-	+	+	-	-	+	-	++
Cinnamomum verum	Cinnamon	Lauraceae	Bark	-	+	++	+	-	+	+	++
Syzygium aromaticum	Clove	Myrtaceae	Flower bud	+	+	+	++	+	+	++	++
Coriandrum sativum	Coriander	Apiaceae	Seed	-	+	+	-	-	+	-	++
Cuminum cyminum	Cumin	Apiaceae	Seed	-	+	++	++	-	+	++	++
Foeniculum vulgare	Fennel	Apiaceae	Seed	-	+	++	-	-	+	-	++
Myristica fragrans	Mace	Myristicaceae	Seed	+	+	++	-	-	+	+	++
Piper nigrum	Pepper	Piperaceae	Seed	+	+	++	-	-	+	-	++
Papaver somniferum	Poppy	Papeveraceae	Seed	-	-	+	-	-	+	-	++
Illicium verum	Star anise	Illiciaceae	Flower bud	-	-	+	+	-	+	+	++
Parmelia perlata	Stone flower	Parmeliaceae	Flower	+	-	+++	-	-	+	++	+

(+) presence; (-) absence

TABLE 2: Antibacterial activity of spice extracts as measured against food borne bacteria in well diffusion assay

Spices	Zone Diameter of Inhibition (mm)					
	B .cereus ATCC 10876	*B. subtilis* ATCC 6633	*S. aureus* ATCC 6538	*E. coli* ATCC 8739	*S. typhi* MTCC 3224	*S. flexneri* ATCC 12022
Bay leaf	20.1 ± 0.5	16.4 ± 1.0	25.0 ± 0.0	22.7 ± 0.1	15.6 ± 0.6	18.3 ± 1.2
Caper	10.2 ± 1.1	10.9 ± 0.9	18.1 ± 0.9	20.0 ± 0.6	10.0 ± 0.1	8.1 ± 0.0
Caraway	11.2 ± 0.3	19.3 ± 0.1	13.7 ± 1.6	18.1 ± 0.7	15.0 ± 0.9	10.2 ± 0.0
Cardamom	13.5 ± 0.7	28 .4± 0.6	13.5 ± 0.7	32.6 ± 0.7	15.0 ± 0.8	20.4 ± 1.3
Cinnamon	18.3 ± 0.6	25.5 ± 0.4	28.6 ± 1.3	35.8 ± 0.0	18.6 ± 0.3	35.0 ± 0.8
Clove	18.5 ± 1.4	23.7 ± 0.9	25.9 ± 1.8	30.6 ± 0.0	22.7 ± 0.7	28.9 ± 0.3
Coriander	NA	18.0 ± 0.7	13.0 ± 0.1	20.0 ± 0.2	10.1 ± 0.3	8.0 ± 0.4
Cumin	10.0 ± 0.8	NA	15.7 ± 0.9	19.1 ± 0.4	10.9 ± 0.7	19.6 ± 0.0
Fennel	NA	NA	10.8 ± 0.3	16.0 ± 0.6	10.3 ± 0.7	10.7 ± 0.8
Mace	23.1 ± 0.0	33.9 ± 0.8	22.6 ± 0.7	30.9 ± 0.6	NA	15.3 ± 0.1
Pepper	19.9 ± 1.0	18.7 ± 0.4	15.1 ± 0.9	NA	NA	10.1 ± 1.0
Poppy	NA	NA	10.5 ± 0.1	15.3 ± 0.1	12.7 ± 0.4	10.1 ± 0.9
Star anise	15.4 ± 0.1	15.6 ± 0.0	15.1 ± 0.6	30.2 ± 0.0	22.7 ± 0.3	20.8 ± 0.0
Stone flower	30.6 ± 0.7	14.1 ± 0.0	30.0 ± 0.7	23.1 ± 0.2	NA	12.1 ± 0.5

Note: NA- no activity

TABLE 3: Minimum inhibitory concentrations of spice extracts against Gram positive bacteria in well diffusion assay (µg/ml)

	B. cereus ATCC 10876					*B. subtilis* ATCC 6633					*S. aureus* ATCC 6538				
	1	**2**	**4**	**8**	**16**	**1**	**2**	**4**	**8**	**16**	**1**	**2**	**4**	**8**	**16**
Bay leaf	-	-	-	-	8	-	-	-	8	10	8	8	8	18	20
Cardamom	-	-	-	15	18	8	10	13	15	18	-	-	5	13	15
Cinnamon	-	-	5	8	15	-	-	10	13	15	-	5	10	12	15
Clove	-	-	10	12	12	-	-	-	10	15	8	10	11	13	15
Mace	-	5	8	12	20	-	5	5	13	15	8	10	10	13	20
Star anise	-	-	-	8	12	-	-	-	8	10	-	-	-	5	8
Stone flower	8	15	20	22	22	10	13	15	18	18	10	12	15	15	15

TABLE 4: Minimum inhibitory concentrations of spice extracts against Gram negative bacteria in well diffusion assay (µg/ml)

	E. coli ATCC 8739					*S. typhi* MTCC 3224					*S. flexneri* ATCC 12022				
	1	**2**	**4**	**8**	**16**	**1**	**2**	**4**	**8**	**16**	**1**	**2**	**4**	**8**	**16**
Bay leaf	-	-	-	5	8	-	-	-	-	8	-	-	-	10	13
Cardamom	-	-	-	5	8	-	-	-	5	8	-	-	-	10	20
Cinnamon	-	-	-	5	8	-	-	5	9	12	8	8	8	18	20
Clove	-	-	-	8	10	-	-	-	8	12	-	-	-	15	18
Mace	-	-	-	-	5	-	-	-	-	5	12	15	18	20	20
Star anise	-	-	-	8	12	-	-	-	8	10	-	-	-	8	10
Stone flower	-	-	-	-	10	-	-	-	5	8	-	-	-	15	18

TABLE 5: Minimum inhibitory concentrations of spice extracts against food borne bacteria in agar dilution assay

	Minimum Inhibitory Concentration (µg/ml)					
Spices	***B. cereus*** **ATCC 10876**	***B. subtilis*** **ATCC 6633**	***S. aureus*** **ATCC 6538**	***E. coli*** **ATCC 8739**	***S. typhi*** **MTCC 3224**	***S. flexneri*** **ATCC 12022**
Bay leaf	≥ 16	16	≤ 8	≥ 16	≥ 16	8
Cardamom	≤ 8	2	≤ 8	≥ 16	≥ 16	8
Cinnamon	16	4	4	≥ 16	≥ 8	8
Clove	8	8	2	16	≥ 8	≤ 8
Mace	8	8	2	≥ 16	≥ 16	≤ 1
Star anise	16	16	≥ 16	16	16	16
Stone flower	≤ 2	≤ 1	≤ 1	16	≥ 16	≤ 8

However, significant antibacterial activity was observed with mace against the Gram positive bacteria followed by clove and cardamom (MIC 8-2 µg ml^{-1}). *S. flexneri* was the only Gram negative bacteria exhibited significant sensitivity against mace extract (MIC ≤ 1 µg ml^{-1}) (table-5). Strains of resistant food borne pathogens to a variety of antimicrobials have become a major health concern (Kiessling *et al.,* 2002). In general, the activity of spice extracts is high on Gram positive bacteria when compared to Gram negative ones which were in accordance with previous findings (Russel, 1991, Shan *et al.,* 2007). This may be due to the composition and concentration of spices and kind of microorganism (Souza *et al.,* 2005). Further, phospholipidic membrane and lipopolysaccharide components of Gram negative bacteria make its cell wall impermeable to high molecular mass compounds. Agar dilution assay indicated that relationship between zone diameter inhibition and MIC values were far from evident. This could be due to some of the crude plant extract constituents may influence the diffusion properties of the active compound (Rios *et al.,* 1988)Antimicrobial activity of stone flower was reported in previous studies (Thippeswamy *et al.,* 2012, Vidyalakshmi and Kruthika, 2012, Momoh and Adikwu, 2008). In this study, antibacterial activity of stone flower (*P.perlata)* against food borne bacteria is well established with Gram positive bacteria as the most susceptible ones (MIC ≤ 2 µg ml^{-1}). Mace has been reported to possess antibacterial activity (Narasimhan and Dhake, 2006) and our findings revealed the significant antibacterial effect against *S. flexneri* by the mace extract (MIC ≤ 1 µg ml^{-1}).

CONCLUSION

The use of natural antibacterial agents will be suitable for applications in the food industry because spices are known to be non toxic as it has been consumed by mankind for centuries. In our study, potential antibacterial activity of stone flower (*P.perlata*) and mace (*M. fragrans*) against food borne and pathogenic bacteria were established. A further study is in the pipeline to evaluate and isolate the bio-active compounds present in various organic extracts of those spices.

REFERENCES

Aboaba, O. and Efuwape, B. M. (2001) Antibacterial Properties of Some Nigerian Species. Bio Res Comm. **13**,183-188.

Arora, D. S. and Kaur, J. (1999) Antimicrobial activity of spices. Int J Antimicrob Agents. **12**, 257-262.

Brull, S. and Coote, P. (1999) Preservative agents in foods: mode of action and microbial resistance mechanisms. Int J Food Microbiol. **50**, 1-17.

Chandrasekaran, M. and Venkatesalu, V. (2004) Antibacterial and antifungal activity of *Syzygium jambolanum* seeds. Journal of Ethnopharmacology. **91**, 105-108.

Cherng, J., Chiang, W. and Chiang, L. (2008) Immunomodulatory activities of common vegetables and spices of umbelliferae and its related coumarins and flavonoids. Food Chemistry. **106**, 944-950.

Kiessling, C. R., Cutting, J. H., Loftis, M. K., Kissling, W., Data, A. R. and Sofos, J. N. (2002) Antimicrobial resistance of food retailed Salmonella isolates. Journal of Food Protection. **65**, 603-608.

Lai, P. K. and Roy J. (2004) Antimicrobial and chemopreventive properties of herbs and spices. Current Medicinal Chemistry. **11**, 1451-1460.

Souza, E. L., Guerr, N. B., Stamford T. L. M. and Lima, E. O. (2006) Spices: alternative sources of antimicrobial compounds to use in food conservation. Rev Bras Farm. **87**, 22-25.

Madsen, H. L. and Bertelsen, G. (1995) Spice as antioxidants. Trends in Food Science and Technology. **6**, 271–277.

Momoh, M. A. and Adikwu, M. U. (2008) Evaluation of the effect of colloidal silver on the antibacterial activity of ethanolic extract of the lichen *Parmelia perlata*. Afr J Pharm Pharmacol. **2**, 106-109.

Mueller, M., Hobiger S. and Jungbauer, A. (2010) Anti-inflammatory activity of extracts from fruits, herbs and spices. Food Chem. **122**, 987-996.

Narasimhan, B. and Dhake, A. S. (2006) Antibacterial principles from *Myristica fragrans* seeds. J Med Food. **9**, 395-399.

Rahman, A. and Kang, S. C. (2009) In vitro control of food borne and food spoilage bacteria by essential oil and ethanol extracts of *Lonicera japonica* Thunb. Food Chem. **116**, 670-675.

Rahman, S., Parvez, A. K., Islam, R. and Khan, M. H. (2011) Antibacterial activity of natural spices on multiple drug resistant *Escherichia coli* isolated from drinking water, Bangladesh. Ann Clin Microbiol Antimicrob. **10**, 10.

Rangan, C. and Barceloux, D.G. (2009) Food additives and sensitivities. Dis. Mon. **55**, 292-311.

Ray, B. (1996) Fundamental Food Microbiology. New York: CRC Press.

Rios, J.L., Recio M. C. and Villar A. (1988) Screening methods for natural products with antimicrobial activity: a review of the literature. J Ethnopharmacol. **23**, 127-149.

Russel, A.D. (1991) Mechanisms of bacterial resistance to non-antibiotics: food additives and pharmaceutical preservatives. J Appl Bacteriol. **71**, 191-201.

Sagdic, O., Karahan, A.G., Ozcan, M. and Ozkan G. (2003) Effect of some spice extracts on bacterial inhibition. Food Sci. Technol. Int. **9**, 353-356.

Shan, B., Cai, Y. Z., Brooks J. D. and Corke, H. (2007) The in vitro antibacterial activity of dietary spice and medicinal herb extracts. Int J Food Microbiol. **117**, 112-119.

Shelef, L. A. (1983) Antimicrobial effects of spices. J. Food Safety. **6**, 29-44.

Sofowora, A. (1993) Screening plants for bioactive agents. In: Medicinal Plants and Traditional Medicinal in Africa, 2nd ed., Spectrum Books Ltd., Sunshine House, Ibadan, Nigeria, pp. 134-156.

Souza, E.L., Stamford, T. L. M., Lima, E. O., Trajona V. N. and Filho J. M. B. (2005) Antimicrobial effectiveness of spices: an approach for use in food conservation systems. Braz Arch Biol Technol. **48**, 549-558.

Thippeswamy, B., Sushma, N. R. and Naveenkumar, K. J. (2012) Antimicrobial property of bioactive factor isolated from *Parmelia perlata*. Int Multidisc Res J. **2**, 1-5.

Trease, G.E. and Evans, W. C. (2002) Pharmacognosy, 15th ed. Springer, Berlin.

Vidyalakshmi, A. and Kruthika, K. (2012) Antibacterial activity of *Parmelia perlata*. Asian Pacific J Trop Med. S892-S894.

Wroblewska, B. (2009) Influence of food additives and contaminants (nickel and chromium) on hypersensitivity and other adverse health reactions - a review. Pol J Food Nutr Sci. **59**, 287-294.