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Comparative Evaluation of Antimicrobial Activities of Commonly Used Indian Spices Against Microbes Associated with Juices

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ABSTRACT

In this study, comparison of the antimicrobial activities of various extracts of commonly used Indian spices against microbes associated with juices such as *Bacillus cereus*, *Serratia* and *Rhodotorula mucilaginosa* by agar well diffusion method and Minimum Inhibitory Concentration (MIC)/Minimum Bactericidal Concentration (MBC) values were determined through the macrodilution broth method. Different extraction solvent such as acetone, methanol, ethanol and aqueous (hot and cold) were used. The type of solvent has great influenced on the antimicrobial activity of the spices. In general the spice extracts with antimicrobial activity were more effective against gram positive than gram negative bacteria and yeast. Methanol extract of *Syzygium aromaticum* showed greater diameter zone of inhibition against *B. cereus* followed by methanolic extract of *Cinnamomum tamala*. This study has demonstrated that extracts of spices have potential antimicrobial activity against microbes associated with juices. These findings established the potential of selected extracts of spices as effective natural food preservative in juices.

Key words: Spices, antimicrobial activity, preservative, agar well diffusion

INTRODUCTION

The practice of using chemical or synthetic antimicrobial agents is one of the oldest methods for controlling infections and microbial food spoilage. Consumers are more aware about the safety of foods containing chemical preservative. There is growing evidences about adverse effects of chemical preservatives on human health, so continuous pressure has been developed to reduce the amount of added preservatives in foods (Bukvicki *et al*., 2014; Tyagi *et al*., 2014). Therefore, there has been great interest in the development of effective and nontoxic antimicrobial compounds from natural sources, such as extracts of plants, for food preservation (Shan *et al*., 2007). The first scientific evidence of the preservation potential of spices, describing antimicrobial activity of cinnamon oil against spores of anthrax bacilli were reported in 1830. A variety of plant and spice based antimicrobials is used for reducing or eliminating pathogenic microorganisms and increase the shelf life of food (Tajkarimi *et al*., 2010). In India, natural herbs and spices are consumed either in food or used as medicine in order to maintain proper sanitation, health and hygiene and to increase longevity of life (Sofia *et al*., 2007). Several spices such as ajowan, clove, ginger, black pepper, cumin and asafetida are commonly used in the Indian diet (Arora and Kaur, 1999). Herbs and spices are used as one of the safest and effective remedies in curing various diseases and long term consumption is not known to produce any side effects. They do not exhibit toxicity at levels consumed (Sunilson *et al*., 2009). National food standard agencies of various countries, US Food

and Drug Act, the European Union standards and the Codex Alimentarius which constitutes the FAO/WHO joint, Food safety and standards authority of India, published the list of food additives. According to these regulations, the majority of natural antimicrobials are Generally Recognized As Safe (GRAS) (Raybaudi-Massilia *et al*., 2009b). The regulatory status of the ten spices which are used in this study is described in Table 1.

The extracts of many plant species contain many bioactive molecules which gain momentum for pharmaceutical and food processing sectors. The antimicrobial activity of plant form the basis for many applications including raw and processed food preservation, pharmaceuticals, alternative medicines and natural therapies (Shan *et al*., 2007). In the last few years, numerous studies have been conducted on the antimicrobial activities of plant extracts against different types of microbes (Negi, 2012).

Juice is defined as unfermented but fermentable juice, intended for direct consumption, obtained by the mechanical process from sound, ripe fruits (Aneja *et al*., 2014a). Juices contain water, sugars, organic acids, vitamins and trace elements create an ideal environment for microbial spoilage. Like most acidic foods, juices become regularly spoiled by aerobic acid tolerant bacteria together with yeasts and moulds (Vantarakis *et al*., 2011; Bukvicki *et al*., 2014). Fruit juice spoilage bacteria include *Acetobacter*, *Alicyclobacillus*, *Bacillus*, *Gluconobacter*, *Lactobacillus*, *Leuconostoc*, *Zymomonas, Zymobacter*, *Propionibacterium* and members of Enterobacteriaceae (*Klebsiella* sp., *Citrobacter* sp. and *Serratia* sp.). Among yeasts *Pichia, Candida, Saccharomyces* and *Rhodotorula* are frequently encountered genera responsible for spoilage of juices (Raybaudi-Massilia *et al*., 2009a; Lawlor *et al*., 2009; Bevilacqua *et al*., 2011). There is also rise in food borne outbreaks associated with consumption of fruit juices caused by *Escherichia coli* and different serovars of *Salmonella* (Raybaudi-Massilia *et al*., 2009a; Aneja *et al*., 2014a).

The main objective of present study was to evaluate the in vitro antimicrobial activity of different spice extracts and to compare the effect of different solvents in the extraction method for antimicrobial activity.

MATERIALS AND METHODS

Plant materials: Ten Indian spices such as Badi elaichi, choti elaichi, dhania, tejpatta, asafetida, cumin, fennel, clove, jaiphal and ajowan were procured from local market in Yanunanagar, Haryana, India. The taxonomic identity of these plants was confirmed by Dr. B.D. Vashishta, plant taxonomist, Chairman of Botany Department, Kurukshetra University, Kurukshetra. The scientific name and tested parts of the 10 plants are detailed in Table 1.

Extraction of plant material: Four different solvents, namely ethanol, methanol, acetone and aqueous (hot and cold), were used for extraction and plant extracts prepared according to the methods described by Sharma *et al*. (2012).

Test microorganisms: In the previous study (Aneja *et al*., 2014b) microbiological analysis of fruit juices was done by serial dilution agar plate technique. On the basis of percentage of occurrence of microorganisms in juice samples, one gram positive bacteria, one gram negative bacteria and one yeast was selected for examining the antimicrobial activity of spices. Bacterial strains were identified on the basis of gram staining, biochemical and molecular characteristics (16S rRNA sequencing) (Lawlor *et al*., 2009). Yeast was identified on the basis of staining, morphological, cultural characteristics and molecular characteristics (28S rRNA sequencing).

Table 1: Ethanobotanical description, phytochemical composition, regulatory status and part of plants used in antimicrobial study

Common name	Scientific names	Family	Plant part tested	Phytoconstituents of part used	Traditional uses	Regulatory status	References
Badi elachi	*Amomum subulatum*	*Zingiberaceae*	Fruit/seeds	Carbohydrates, flavonoids, amino acids, steroids, triterpenoids, glycosides, tannins, alkaloids, 1,8-cineole, limonene	Curative for throat trouble, Congestion of lungs, inflammation of eyelids, digestive disorders and in the treatment of pulmonary tuberculosis, flavouring agent In confectionery, hot or sweet pickles and in beverages	FSSAI 2.9.9.4	Madhusoodanan and Rao (2001) and Bisht *et al.* (2011)
Tejpatta	*Cinnamomum tamala*	*Lauraceae*	Leaves	Phellandrene, eugenol, linalool and some traces of α-pinene, pcymene, β-pinene and limonene, phenylpropanoids	Use in treatment of rheumatism, colic, diarrhoea, nausea	-	Shah and Panchal (2010) and Panday *et al.* (2012)
Dhania, Coriander	*Coriandrum sativum*	*Apiaceae*	Fruits	Flavonoids, isocoumarins, fatty acids, sterols and coriandrones, coumarins, catechins, polyphenolic compounds	Used for indigestion, against worms, rheumatism, pain in the joints, against intestinal parasites, seeds in sweet vodka, ingredient of pickles	FSSAI 2.9.7, GRAS, 21 CFR182.10	Asgarpanah and Kazemivash (2012)
Jeera	*Cumin cyminum*	*Apiaceae*	Fruits	Diverse flavonoids, isoflavonoids, flavonoid glycosides, monoterpenoid glucosides, lignins and alkaloids and other phenolic compounds	Used in the treatment of mild digestive disorders, diarrhea, dyspepsia, flatulence, morning sickness, colic, dyspeptic headache and bloating, flavouring agent in confectionery, meat, sausage and bread manufacturing and as a preservative in food processing	FSSAI 2.9.8, GRAS, 21 CFR182.10	Amin (2001) and Johri (2011)
Chhoti elachi	*Elettaria cardamomum*	Zingiberaceae	Fruits/seeds	α-terpineol, 1,8-cineole, with smaller amounts of borneol, camphor, limonene, α-terpenyl acetate and α-pinene	Used in aromatherapy to stimulate energy, aphrodisiac and remedy in case of digestive problems, asthma, bronchitis and urinary complaints and several other human ailments, in flavouring pickles, meat and canned soups	FSSAI 2.9.2.1,	Korikanthimath (2001) and Kaushik *et al.* (2010)
Hing	*Ferula asafoetida*	*Apiaceae*	Gum resin	Sesquiterpene coumarins, 2-butyl 1-propenyl disulfide, 1-(methylthio)propyl 1-propenyl disulfide and 2-butyl 3-(methylthio)-2-propenyl disulfide	Used for Flatulence, hysteria and nervous disorders, asthma, flavoring spice in a variety of foods	FSSAI 2.9.29	Iranshahy and Iranshahi (2011)

Table 1: Continue

Common name	Scientific names	Family	Plant part tested	Phytoconstituents of part used	Traditional uses	Regulatory status	References
Fennel/Saunf	*Foeniculum vulgare*	*Apiaceae*	Fruit	Anethole, fenchone	Essence in cosmetics and perfumes industry	FSSAI 2.9.9.2, 21 CFR182.10	Oktay *et al*. (2003)
Jaiphal	*Myristica fragrans*	*Myristicaceae*	Fruit	Myristicin, lignans, monoterpene hydrocarbons pinene and sabinene	Used for flatulence, nausea and vomiting, for convalescents, as an ointment for piles, for leucorrhoea and as a local stimulant to the gastro-intestinal tract, flavouring agent for food products and liquors	FSSAI 2.9.14, GRAS 21, CFR182.10	Krishnamoorthy and Rema (2001) and Chatterjee *et al*. (2007)
Clove, Laung	*Syzygium aromaticum*	*Myrtaceae*	Dry flower buds	Eugenol, eugeniin, acetyl eugenol, quercetic acid, gallic acid, vanillin	Used in toothache, particularly to aid digestion, cure stomach disorders and in pain relief, antiseptic, for topical anesthesia in dentistry	FSSAI 2.9.6	Arora and Kaur (1999), Nurdjannah and Bermawie (2001) and Negi (2012)
Ajowan	*Trachyspermum copticum*	*Apiaceae*	Fruits	Thymol, terpinene, p-cymene, pinene	Used as a digestive stimulant or to treat liver disorders	FSSAI 2.9.22	Shankaracharya *et al*. (2000) and Murthy *et al*. (2009)

Fssai: Food safety and standards authority of India, GRAS: Generally recognized as safety, CFR: Title 21 of the US code of federal regulations

Two bacteria, namely *Serratia marcescens* (KC67407*), *Bacillus cereus* KRC1 (KC67408) and one yeast, *Rhodotorula mucilaginosa* (KC67409) were identified. The bacterial isolates were subcultured on nutrient agar and *R. mucilaginosa* on potato dextrose agar and incubated aerobically at 37 and 25°C, respectively. The media were procured from Hi Media Laboratory Pvt. Ltd., Bombay, India (*Nucleotide sequence of all microoraganisms has been submitted to GenBank database which provided the GenBank accession number, KC67407-KC67409).

Screening for antimicrobial activity: The acetone, methanol, ethanol, hot and cold aqueous extracts of different plants were used for evaluation of antimicrobial activity by the agar well diffusion method. In this method, a pure isolate of bacteria and yeast was grown on NA and PDA plates and incubated at 37 and 25°C for 24 and 72 h, respectively. One plate of each microorganism was taken and colonies were transferred into normal saline (0.85%) under aseptic conditions. Density of each microbial suspension was adjusted to be equal to that of 10^6 CFU mL^{-1} (standardized by 0.5 McFarland standard) and used as the inoculum for performing an agar well diffusion assay. One hundred microliter (100 μL) of the inoculum of each test organism was spread onto the agar plates so, as to achieve a confluent growth. The agar plates were allowed to dry and 8 mm wells were made with a sterile borer in the inoculated agar plates. The lower portion of each well was sealed with molten agar medium. The dried extracts were reconstituted to 20% in dimethylsulphoxide (DMSO) to the final concentration of 100 mg mL^{-1} for the bioassay analysis. A 100 μL volume of each extract was propelled directly into the wells (in triplicate) of the inoculated agar plates for each test organism. The plates were allowed to stand for 1 h at room temperature (40°C) for diffusion of the extract into agar and incubated at 37 and 25°C for 24 and 72 h, respectively. Sodium benzoate (100 mg mL^{-1}) was used as positive reference standards to determine the sensitivity of each microbial species tested. Sterile DMSO served as the negative control. The antimicrobial activity, indicated by an inhibition zone surrounding the well containing the extract, was recorded if the zone was greater than 8 mm. The experiments were performed in triplicate and the mean values of the diameter of inhibition Zones±Standard deviations were calculated. The experiments were performed in triplicate and the mean values of the diameter of inhibition Zones±Standard deviations were calculated (Aneja *et al*., 2010).

Determination of minimum inhibitory concentration: Minimum Inhibitory Concentration (MIC) for each test organism was determined by the macrodilution broth method (Das *et al*., 2010).

Determination of minimum bactericidal concentration: Minimum Bactericidal Concentration (MBC) is the lowest concentration of antimicrobial agent that will not allow the growth of an organism after subculturing on antibiotic free media. MBC was determined by subculturing the preparations that did not show any bacterial growth in the MIC determination (Ncube *et al*., 2008).

Determination of minimum fungicidal concentration: A loopful of culture from each set of tubes that did not show any visible growth of the yeast in MIC determination was subcultured on to fresh plates of PDA and incubated at 25°C for 72 h. Minimum fungicidal concentration for each plant extracts against the tested yeast was recorded as the lowest concentration that did not yield any fungal growth on the solid medium (Aneja *et al*., 2011).

Statistical analysis: The experimental results were repeated thrice in triplicate each time and expressed as Mean±SD and results were statistically evaluated using SPSS software version 16 at 5% significant level. Means were compared using Tukey's simultaneous test set at $p<0.05$.

RESULTS

Antimicrobial activities: In the present study, the antimicrobial activity of the ten spice extracts in different solvents was examined. The mean diameters of the inhibition zones of all spice extracts against three microbes associated with juices are given in Table 2. There was significant variation ($p<0.05$) observed between acetone, methanol, ethanol, cold aqueous and hot aqueous solvents for the antimicrobial activities of each tested spices. Hot and cold aqueous extracts of plants possessed less antimicrobial activities in comparison to organic extracts (Table 2). Acetonic extract of *S. aromaticum* exhibited maximum zone of inhibition (29.6 mm) against *B. cereus* followed by *C. tamala* (26.6 mm).

The DIZ values of 39 extract (accounting for 78% of the 50 extracts) for *B. cereus* was between 12.3-29.6 mm. However, 11 extracts did not exhibit inhibitory activity. For *Serratia* sp., 13 extracts (26%) exhibited high inhibitory activity (DIZ = 17.3-25.6 mm) and 12 extracts had low activity (11.3-15.3 mm). The remaining 25 extracts (50%) showed no inhibitory activity. Out of 50 extracts, only 16 extracts showed antimicrobial activity against *R. mucilaginosa* with DIZ values between 11.3-21.6 mm.

Table 2: Antimicrobial activity of different plants

Plants	Type of microorganisms	Antimicrobial activity of different extracts (DIZ) (mm)				
		Acetone	Methanol	Ethanol	Cold aqueous	Hot aqueous
Amomum subulatum	*B. cereus*	21.6±0.57^{bx}* **	22.3±0.57^{cx}	25.6±0.57^{dx}	16.3±0.57^{ex}	12.3±0.57^{fx}
	Serratia	17.3±0.57^{by}	19.6±0.57^{cy}	18.3±0.57^{dy}	-	-
	R. mucilaginosa	-	-	-	-	-
Cinnamomum tamala	*B. cereus*	26.6±0.57^{bx}	22.6±1.52^{cx}	20.3±0.57^{dx}	18.3±0.57^{ex}	12.3±0.57^{fx}
	Serratia	18.6±0.57^{by}	13.3±0.57^{cy}	12.3±1.52^{dy}	-	-
	R. mucilaginosa	12.3±0.57^{bz}	-	-	-	-
Coriandrum sativum	*B. cereus*	24.3±1.52^{bx}	19.6±1.52^{cx}	16.3±0.57^{dx}	13.6±0.57^{ex}	-
	Serratia	20.6±0.57^{by}	15.3±0.57^{cy}	14.3±0.57^{dy}	-	-
	R. mucilaginosa	17.6±0.57^{bz}	-	-	-	-
Cumin cyminum	*B. cereus*	19.6±1.52^{bx}	22.3±1.52^{cx}	20.3±1.52^{dx}	-	-
	Serratia	13.6±1.52^{b}	17.6±1.52^{c}	15.3±1.52^{d}	-	-
	R. mucilaginosa	12.3±1.52^{bz}	14.3±1.52^{cz}	12.6±1.52^{dz}	-	-
Elettaria cardamomum	*B. cereus*	16.6±0.57^{bx}	12.3±0.57^{cx}	13.6±0.57^{dx}	-	-
	Serratia	-	-	-	-	-
	R. mucilaginosa	-	-	-	-	-
Ferula asafoetida	*B. cereus*	24.6±0.57^{bx}	22.3±0.57^{cx}	17.6±0.57^{dx}	16.3±0.57^{ex}	12.3±0.57^{fx}
	Serratia	23.6±0.57^{by}	18.6±0.57^{cy}	12.3±0.57^{dy}	-	-
	R. mucilaginosa	14.3±0.57^{bz}	12.6±0.57^{cz}	11.3±0.57^{dz}	-	-
Foeniculum vulgare	*B. cereus*	21.6±0.57^{bx}	18.6±0.57^{cx}	26.3±0.50^{dx}	-	-
	Serratia	15.3±0.57^{by}	17.6±0.57^{cy}	19.6±0.50^{dy}	-	-
	R. mucilaginosa	11.3±0.57^{bz}	12.3±0.57^{cz}	15.6±0.57^{dz}	-	-
Myristica fragrans	*B. cereus*	18.6±0.57^{bx}	-	-	-	-
	Serratia	-	-	-	-	-
	R. mucilaginosa	-	-	-	-	-
Syzygium aromaticum	*B. cereus*	29.6±0.57^{bx}	24.3±0.57^{cx}	23.6±0.57^{dx}	19.3±0.57^{ex}	12.6±0.57^{fx}
	Serratia	25.6±0.57^{by}	22.3±0.57^{cy}	20.6±0.57^{dy}	12.3±0.57^{ey}	-
	R. mucilaginosa	19.3±0.57^{bz}	21.6±0.57^{cz}	17.3±0.57^{dz}	16.6±0.57^{ez}	-
Trachyspermum copticum	*B. cereus*	16.6±0.57^{bx}	13.6±0.57^{cx}	14.3±0.57^{dx}	-	-
	Serratia	13.6±0.57^{by}	12.3±0.57^{cy}	11.3±0.57^{dy}	-	-
	R. mucilaginosa	12.6±0.57^{bz}	-	-	-	-
Sodium benzoate	*B. cereus*			20.6±0.57^{ax}		
	Serratia			16.6±0.57^{ay}		
	R. mucilaginosa			14.6±0.57^{az}		

*Values, including diameter of the well (8 mm), are means of three replicates, **Standard deviation, within five extracts and control of the same spice with three different microorganisms tested different letters are significantly ($p<0.05$) different

Table 3: Minimum inhibitory concentration and minimum bactericidal concentration of plant extracts against juice associated bacteria and yeast

		MIC and MBC value of different extracts							
		Acetone		Methanol		Ethanol		Cold aqueous	
Plants	Type of microorganisms	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Amomum subulatum	*B. cereus*	3.12	6.25	3.12	6.25	1.56	3.12	50	100
	S. marcescens	6.25	12.50	12.50	25.00	6.25		-	
	R. mucilaginosa	-	-	-		-		-	
Cinnamomum tamala	*B. cereus*	1.56	3.12	6.25	12.50	6.25		12.5	25
	S. marcescens	12.50	25.00	50.00	100.00	---		-	
	R. mucilaginosa	--		-		-		-	
Coriandrum sativum	*B. cereus*	3.12	6.25	12.50	25.00	25.00	50.0	50	100
	S. marcescens	6.25	12.50	25.00	50.00	50.00	100.0	-	
	R. mucilaginosa	-	--	-		-		-	
Cumin cyminum	*B. cereus*	12.50	25.00	3.12	6.25	6.25	12.5	-	
	S. marcescens	50.00	100.00	12.50	25.00	25.00	50.0	-	
	R. mucilaginosa	Nt	Nt	25.00	50.00	50.00	100.0	-	
Elettaria cardamomum	*B. cereus*	25.00	50.00	50.00	100.00	50.00	100.0	Nt	
	S. marcescens	-		-		-		-	
	R. mucilaginosa	-		-		-		-	
Ferula asafoetida	*B. cereus*	1.56	3.12	3.12	6.25	25.00	50.0	25	50
	S. marcescens	3.12	6.25	6.25		Nt		-	
	R. mucilaginosa	12.50	25.00	50.00		Nt		-	
Foeniculum vulgare	*B. cereus*	3.12	6.25	12.50		1.56		-	
	S. marcescens	25.00	50.00	25.00		12.50		-	
	R. mucilaginosa	Nt		---		25.00		-	
Myristica fragrans	*B. cereus*	25.00	50.00	-		-		-	
	S. marcescens	-		-		-		-	
	R. mucilaginosa	-		-		-		-	
Syzygium aromaticum	*B. cereus*	0.78	1.56	1.56	3.12	1.56	3.12	Nt	Nt
	S. marcescens	1.56	3.12	3.12	6.25	3.12	6.25	Nt	Nt
	R. mucilaginosa	6.25	12.50	3.12	6.25	12.50	25.00		
Trachyspermum copticum	*B. cereus*	50.00	100.00	50.00	100.00	25.00	50.00	-	
	S. marcescens	--		--		--		-	
	R. mucilaginosa	--		-		-		-	

In general, a total of 6 spices, *C. tamala*, *Cumin cyminum*, *F. asafetida*, *Foeniculum vulagre*, *S. aromaticum* and *T. copticum* possessed antimicrobial activity against all tested microbes. *A. subulatum* and *Coriandrum sativum* exhibited antibacterial activity not antiyeast and *E. cardamom* and *Myristica fragrans* showed antibacterial activity against *B. cereus*.

MIC, MBC and MFC values of different extracts: The results of the MIC, MBC and MFC of spices extracts are presented in Table 3. Gram positive bacteria are more sensitive to the spice extracts than the Gram negative bacteria and yeast. The results revealed that MBC and MFC values are twofold higher than the MIC values against the corresponding microbes. Hot aqueous extracts did not show MIC values within the tested concentration and cold aqueous extracts of *A. subulatum*, *Cinnamomum tamala, Coriandrum sativum* and *Ferula asafetida* showed MIC values ranges between 12.5-100 mg mL^{-1} against *B. cereus*.

The acetonic extract of *S. aromaticum* displayed the best antimicrobial activity with MIC value 0.78 mg mL^{-1} and MBC of 1.56 mg mL^{-1} against *B. cereus* that increased to MIC value of 1.56 mg mL^{-1} against *Serratia* sp.

DISCUSSION

Of the 50 spice extracts tested in this study, sixteen exhibited broad spectrum activity against the tested microbes. Previous studies showed the similar results up to some extent

(Ahmad and Beg, 2001; Arora and Kaur, 1999; Sofia *et al*., 2007; Shan *et al*., 2007; Sunilson *et al*., 2009; Weerakkody *et al*., 2010; Negi, 2012). In literature, various studies have been published on the antimicrobial activities of plant extracts against different microorganisms. However, there is difficulty in comparison of the results obtained in these studies because of the adoption of different methods including solvents, concentrations, microbial strains and antimicrobial test methods (Thongson *et al*., 2004; Shan *et al*., 2007; Weerakkody *et al*., 2010). For example in previous study *Cumin cyminum* methanolic extract did not show any activity against *B. cereus*, *Listeria monocytogenes*, *S. aureus*, *E. coli* and *S. anatum* in a agar well diffusion method at a concentration of 100 mg mL^{-1} (Shan *et al*., 2007). In this study, methanolic extract of *C. cyminum* showed 22.3, 17.6 and 14.3 mm DIZ against *B. cereus*, *Serratia* sp. and *R. mucilaginosa*. All the extracts of *S. aromaticum* except hot aqueous showed best antimicrobial activity against all the tested microbes. Our observation are in agreement with the reports of other workers (Arora and Kaur, 1999; Ahmad and Beg, 2001; Burt, 2004; Tajkarimi *et al*., 2010).

The DIZ and MIC values showed that gram positive bacteria *B. cereus* is more sensitive to spice extracts than gram negative and yeast. This was associated with the previous studies on other spices (Shan *et al*., 2007; Weerakkody *et al*., 2010). This is attributed to the differences in the outer layers of gram negative and gram positive bacteria. Gram negative bacteria possess an outer membrane and a unique periplasmic space not found in gram positive bacteria (Ceylan and Fung, 2004; Lopez *et al*., 2005; Shan *et al*., 2007).

The type of solvent used for the extraction of spices also has great impact on the antimicrobial activities of spices. The most commonly used solvents for investigations of antimicrobial activity in plants are methanol, ethanol and water (Ncube *et al*., 2008; Chang and Lin, 2012). In the present study, organic extracts of spices possess greater antimicrobial activities as comparison to aqueous extracts. The results were confirmed by previous study (Yano *et al*., 2006; Aneja *et al*., 2010, 2011; Sharma *et al*., 2012). Acetonic and methanolic extracts of spices exhibited greater DIZ against tested microbes. It may be due to the presence of more extraction of saponins which possess antimicrobial activities (Ncube *et al*., 2008). The antimicrobial activity of spices may be due to the presence of various secondary metabolites such as phenols, tannins, flavonoids, coumarins, thiosulfinates, glucosinolates and saponins (Cowan, 1999; Tajkarimi *et al*., 2010; Negi, 2012).

CONCLUSION

The results of present study confirm that all the tested spices possess antimicrobial activity against selected microbes associated with juices except *E. cardamom* and *M. fragrans* which might be due to the presence of phenolic compounds and is well supported by previously documented study. These spice extracts therefore have the potential to extend the shelf life or used as natural preservatives in fruit juices.

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