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Screening the efficacy of extracts of different spices in inhibiting the growth of foodborne bacterial isolates

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Abstract

Owing to the presence of a diverse group of phytochemicals, spices could be potential sources of antibacterial and antioxidant agents. The present investigation was aimed to determine and compare the antimicrobial activities of different spices. A total of 5 spices including cardamom (Seeds, *Elettaria cardamomum*), cinnamon (Barks, *Cinnamomum verum*), clove (Flower buds, *Syzygium aromaticum*), Indian bay leaf or Tejpat (Dried leaves, *Cinnamomum tamala*), and cumin (Seeds, *Cuminum cyminum*) were collected. Different extracts (crude, aqueous, ethanolic, and methanolic) of spices were prepared and examined for antimicrobial activity against previously isolated foodborne bacterial isolates. Extracts from the tested spices showed significant inhibitory effects (mostly with >10 mm mean zone of inhibition) as revealed by the agar well diffusion technique. Clove among the tested spices was found to be the prominent one in eliminating foodborne pathogens. Methanolic extracts followed by ethanolic extracts were determined to be most effective against the bacterial isolates when the relative effectivity of different extracts was compared. The frequently encountered minimal inhibitory and bactericidal concentrations of the spices were 12 and 24 mg/mL, respectively. All the spice extracts showed considerable antimicrobial traits which validate their potential and applicability as natural food preservatives and decontaminants.

1. Introduction

Despite the food safety concern and recent technological advancements, foodborne diseases remain to be a big concern worldwide as well as in Bangladesh (Gottardi *et al*., 2016; Noor and Feroz, 2016). Several microorganisms responsible for food spoilage and foodborne illnesses can frequently contaminate foods from various sources such as water, air, dust, equipment, sewage, insects, rodents, and personnel (Chakraborty *et al*., 2020). To ensure food safety and quality, controlling the growth of spoiling and pathogenic foodborne microorganisms is crucial. Suppression of one or more essential factors associated with microbial survival could ensure food conservation (de Souza *et al*., 2005). However, synthetic preservatives that have been used in foods for decades could be toxic to humans and may have adverse health consequences (de Souza *et al*., 2005; Chakraborty *et al*., 2020). Therefore, the concern for replacing synthetic preservatives with renewable, effective, and non-toxic compounds is raising (de Souza *et al*., 2005; Purkait *et al*., 2018). Especially plant products with antimicrobial properties attract special focus for potential use in food processing to control bacterial and fungal growth (Jahan *et al*., 2018; Hossaini *et al*., 2020; Hossaini *et al*., 2021). Plant-derived antimicrobial compounds have been used for food preservation for centuries (Dhiman *et al*., 2016).

Since ancient times, spices have been used to heighten the flavour and taste of cooked food (D'Souza *et al*., 2017). Spices are usually dry, colourful, aromatic, pungent, and used as seasoning agents applied in minimal amounts (de Souza *et al*., 2005; D'Souza *et al*., 2017). Spices comprise different plant materials including leaves, flowers, fruits, roots, rhizomes, bulbs and more (de Souza *et al*., 2005; Chakraborty *et al*., 2020). Spices and their combination constitute naturally occurring food additives and are an integral part of the daily diets of large numbers of the world's population

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(Škrinjar and Nemet, 2009). Some spices are known to have antibacterial and antioxidant effects. They aid in the prevention and treatment of a range of diseases such as cancer, ageing, metabolic, neurological, cardiovascular, and inflammatory disorders (Teodoro, 2019). Their role in food safety and preservation has also been established (Gottardi *et al*., 2016).

Spices can assist in food preservation and food safety maintenance by imparting antimicrobial and antioxidant properties against food spoilage and foodborne pathogenic microorganisms (Tajkarimi *et al*., 2010; Gottardi *et al*., 2016). Thereby, they can enhance the shelf-life of foods (Shan *et al*., 2007; Diman *et al*., 2016). Several active compounds such as phenolic compounds, carvacrol, eugenol, carvone, thymol, trans-cinnamaldehyde, and gingerol have been identified to have antimicrobial and antioxidant potential (Chakraborty *et al*., 2020). However, frequent evaluation of the antimicrobial activity of different spices against foodborne microorganisms is crucial to claim their effectiveness as food preservatives (Chakraborty *et al*., 2020). Therefore, the present study was undertaken to examine the inhibitory potential of spices commonly used in Bangladesh against foodborne bacteria through agar well diffusion and microdilution methods.

2. Materials and methods

2.1 Samples

Five categories of commonly used spices, Cardamom (Seeds, *Elettaria cardamomum*), Cinnamon (Barks, *Cinnamomum verum*), Clove (Flower buds, *Syzygium aromaticum*), Indian Bay Leaf or Tejpat (Dried leaves, *Cinnamomum tamala*), and Cumin (Seeds, *Cuminum cyminum*) were collected from the local market of Dhaka, Bangladesh during October 2020 to December 2020. Following collection, samples were quickly transported to the laboratory of the Department of Microbiology, Stamford University Bangladesh. They were allowed to dry then processed for extraction.

2.2 Preparation of spice extracts

Each spice sample (dried) was blended to get the powdered form which was directly employed as a crude fraction. The aqueous extract was prepared by adding 10 g of each sample powder to the 90 mL of buffer peptone water followed by homogenization. The solvent extracts (ethanolic and methanolic) were prepared by mixing 15 g of each powdered spice sample to 85 mL of ethanol and methanol in separate Durham's bottles that were kept in a shaking water bath at 130 rpm for 24-48 hrs at 24°C. Afterwards, the solvent extracts were filtered using Whatman filter papers. The filtered extracts were then kept in a rotary evaporator for evaporation of methanol and ethanol followed by dissolving the extracts in 10% dimethyl sulfoxide (DMSO) to make a final concentration of 10 mg/mL (Jahan *et al*., 2018; Chakraborty *et al*., 2020; Hossaini *et al*., 2020; Hossaini *et al*., 2021). The extracts were stored at 4°C until use.

2.3 Test microorganisms

The bacterial isolates used in this study for the antimicrobial assay were previously isolated and biochemically identified from different food samples including fried chicken, chicken sausage, ice creams and mango juices in the laboratory of the Department of Microbiology, Stamford University Bangladesh employing standard protocol for microbiological analysis (Akter *et al*., 2019; Hossain *et al*., 2020; Hossaini *et al*., 2020). The selected foodborne bacterial isolates include *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Vibrio* spp., *Listeria* spp., *Salmonella* spp. and *Staphylococcus* spp. bacteria were preserved at -20°C and subcultures were made on Nutrient agar (HiMedia Laboratories, Mumbai, India) before tested in the current study.

2.4 Determination of the antimicrobial activity of spice extracts by agar well diffusion method

Modified agar well diffusion method (Sharmin *et al*., 2014; Munshi *et al*., 2018; Chakraborty *et al*., 2020; Hossaini *et al*., 2020; Hossaini *et al*., 2021; Kabir *et al*., 2021) was applied to determine the anti-bacterial potential of crude fraction, aqueous and solvent extracts of the spice samples against different previously isolated foodborne bacterial strains. First, the lawn was prepared on Mueller-Hinton agar (MHA, Oxoid Ltd., England) by spreading each bacterial suspension (turbidity adjusted to the 0.5 McFarland standard which is equal to 10^5 CFU/mL) and wells (8 mm) were made after the media dried. Crude fractions, aqueous, ethanolic, and methanolic extracts at a residual concentration of 10 mg/mL were introduced into the wells generated on MHA. Buffer peptone water, absolute ethanol, and methanol were used as negative controls, whereas Gentamicin antibiotic disc (10 µg) was used as a positive control. After incubation at 37°C for 12-18 hrs, the plates were examined for the formation of a zone of inhibition (mm) around the wells.

2.5 Assessment of the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of spices

For the determination of the minimal inhibitory and bactericidal concentrations of the spice samples, the microdilution method was employed (Sharmin *et al*., 2014; Hossaini *et al*., 2020; Hossaini *et al*., 2021; Kabir *et al*., 2021). After overnight (~12 hrs) incubation, 100 µL of each bacterial suspension (turbidity adjusted to 0.5

McFarland standard) was inoculated into properly labelled tubes containing 3 mL Mueller-Hinton (MH) broth (Oxoid Ltd., England). Spice samples were further added into the suspension at residual concentrations of 1.5, 3, 6, 12, 24, and 48 mg/mL, and the tubes were incubated at 37°C for 18-24 hrs. The lowest concentration (mg/mL) of each sample that could visibly inhibit the growth of the tested bacteria was considered as the MIC value and the lowest concentration (mg/mL) that could clear the growth i.e to kill the tested bacteria was determined as the MBC value (Sharmin *et al*., 2014; Hossaini *et al*., 2020; Hossaini *et al*., 2021).

2.6 Statistical analysis

All the experiments carried out in this study were in triplicate. Statistical analysis was conducted using SPSS statistics version 20.0 (IBM, Georgia, USA) and Microsoft Office Excel Professional Plus 2016 (Microsoft Corporation, Redmond, Washington, USA) program packages, and the mean values and standard deviations (SD) were determined. Data were analyzed by one-way ANOVA, and mean values were separated by the posthoc statistic of Tukey's HSD (honest significant difference). The mean differences in the results of different extracts were considered to be significant at $P < 0.05$.

3. Results and discussion

3.1 Antimicrobial potential of the tested spices

All the spice samples showed their efficiency in reducing the growth of foodborne bacterial isolates and significant differences ($P < 0.05$) were observed in the mean zone of inhibition (mm) by different extracts (Table 1). In most cases, ethanolic and methanolic extracts were successful in exhibiting their antimicrobial potential to a significant extent. Whereas, crude fractions and aqueous extracts except for Cloves were found to impart no activity against a majority of the tested bacterial isolates (Table 1). Mean±SD zone of inhibition found for different extracts of Cardamoms was minimal with the highest measurement of 12.7±1.53 mm and no effect was found against *Vibrio cholerae* (Table 1). However, Tajkarimi *et al*. (2010) and Savan and Kucukbay (2013) reported antimicrobial potential in Cardamom against a range of bacterial isolates from food samples. The extracts of Cinnamons, especially the ethanolic and methanolic extracts, had antimicrobial activity against the foodborne bacterial isolates with mean±SD zone of inhibition ranging from 9.3±0.58 mm to 18.0±1.00 mm. Tajkarimi *et al.* (2010) and Purkait *et al.* (2018) found Cinnamon to be effective against both Gram-positive and Gram-negative bacteria isolated from spoiled and contaminated foods. With the inhibitory zones ranging from 7.3±0.58 mm to 15.3±1.15 mm, ethanolic and methanolic extracts of Indian Bay Leaf samples had noticeable antimicrobial activity though crude fraction and aqueous extract merely had any effect. Nearly similar findings were found for the Cumin samples with mean±SD zone of inhibition ranging from 7.7±0.58 mm to 15.7±1.53 mm (Table 1). Tajkarimi *et al.* (2010) and Xu *et al.* (2014) evident antimicrobial efficacy in Indian bay leaf against food spoilage bacterial isolates. Al-Jedah *et al.* (2000) found a static effect in Cumin against different bacteria of fish sauce. A study by Wakoli *et al.* (2014) had evidence of the control of food spoilage by Cumin. However, Purkait *et al.* (2018) found Cumin to be ineffective against Gram-negative bacteria.

Clove was proved to be the most effective spice among the tested samples in retarding the growth of bacteria as all the extracts including crude fraction and aqueous extract imparted remarkable inhibitory effects with the highest 25.7±0.58 mm inhibition zone (Table 1). Zhang *et al.* (2009) found a strong inhibitory effect in Clove against meat spoilage bacteria. Several other researchers also reported significant antimicrobial activity in Clove against foodborne bacterial isolates (Leuchner and Zamparini, 2009; Shan *et al*., 2007). Similar to the findings of the present study, Chouhan *et al.* (2017) and Purkait *et al.* (2018) also found higher antimicrobial potential in Clove than other tested spices against foodborne bacteria. The methanolic extract was found to be most effective in eliminating the growth of foodborne bacteria. in cohort with the findings of previous studies (Shan *et al*., 2007; Negi, 2012; Dhiman *et al*., 2016).

3.2 The MIC and MBC of the spices

The MIC of the spice samples against the foodborne bacterial isolates was ranged between 6 and 48 mg/mL (Figure 1). In the majority of the cases (21 instances), the MIC was found to be 12 mg/mL. The MIC of 24 mg/mL

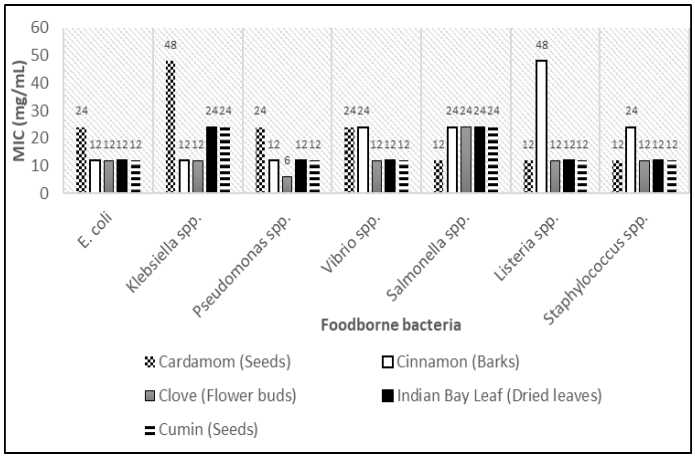

Figure 1. Minimum inhibitory concentration (MIC) of the spice samples. Mean values have been shown here.

Table 1. Antimicrobial potential of different extracts of the tested spices.

Spices	Foodborne bacteria	Zone of inhibition in diameter (mm)				Positive control
		Crude fraction	Aqueous extract	Ethanolic extract	Methanolic extract	(Gentamicin 10 µg)
Cardamom (Seeds, *Elettaria cardamomum*)	*E. coli*	0±0	0±0	6.7±0.58^{dx}	6.7±1.15^{ex}	18.7±0.58^{ax}
	Klebsiella spp.	0±0	0±0	8.3±0.58^{dy}	8.7±0.58^{ey}	21.0±1.00^{ay}
	Pseudomonas spp.	0±0	0±0	11.7±0.58^{dz}	0±0	21.7±0.58^{az}
	Vibrio spp.	0±0	0±0	0±0	0±0	19.7±0.58^{au}
	Salmonella spp.	0±0	0±0	6.7±1.53^{dv}	7.0±1.00^{ev}	20.0±1.00^{av}
	Listeria spp.	0±0	12.7±0.58^{cw}	8.7±0.58^{dw}	12.7±1.53^{ew}	23.3±0.58^{aw}
	Staphylococcus spp.	0±0	0±0	10.3±0.58^{dt}	6.7±0.58^{et}	22.7±0.58^{at}
Cinnamon (Barks, *Cinnamomum verum*)	*E. coli*	0±0	0±0	9.7±0.58^{dx}	13.7±0.58^{ex}	19.7±0.58^{ax}
	Klebsiella spp.	0±0	0±0	9.7±1.15^{dy}	15.7±0.58^{ey}	20.3±1.15^{ay}
	Pseudomonas spp.	0±0	0±0	11.3±1.15^{dz}	13.7±0.58^{ez}	20.7±0.58^{az}
	Vibrio spp.	0±0	9.3±0.58^{au}	9.7±0.58^{du}	18.0±1.00^{eu}	18.7±0.58^{au}
	Salmonella spp.	0±0	0±0	9.3±1.15^{dv}	13.3±0.58^{ev}	19.3±0.58^{av}
	Listeria spp.	18.3±0.58^{bw}	11.0±1.00^{cw}	15.0±1.00^{dw}	16.7±1.15^{ew}	22.0±1.00^{aw}
	Staphylococcus spp.	19.3±1.15^{bt}	0±0	10.3±1.53^{dt}	10.7±0.58^{et}	21.3±0.58^{at}
Clove (Flower buds, *Syzygium aromaticum*)	*E. coli*	20.3±1.53^{bx}	9.7±0.58^{cx}	20.7±1.15^{dx}	19.7±0.58^{ex}	19.3±1.15^{ax}
	Klebsiella spp.	25.7±0.58^{by}	11.7±0.58^{cy}	15.7±0.58^{dy}	22.3±1.15^{ey}	21.7±0.58^{ay}
	Pseudomonas spp.	18.7±0.58^{bz}	11.3±1.15^{cz}	20.0±1.00^{dz}	16.7±0.58^{ez}	21.7±0.58^{az}
	Vibrio spp.	21.0±1.00^{bu}	11.7±0.58^{cu}	18.7±0.58^{du}	21.7±0.58^{eu}	20.0±1.00^{au}
	Salmonella spp.	19.7±0.58^{bv}	15.3±1.15^{av}	17.7±0.58^{dv}	19.7±0.58^{ev}	19.7±0.58^{av}
	Listeria spp.	25.3±1.15^{bw}	10.7±0.58^{cw}	16.3±1.15^{dw}	23.7±0.58^{ew}	21.0±1.00^{aw}
	Staphylococcus spp.	24.7±0.58^{bt}	14.7±0.58^{ct}	23.7±0.58^{dt}	24.3±1.53^{et}	22.7±0.58^{at}
Indian Bay Leaf (Dried leaves, *Cinnamomum tamala*)	*E. coli*	0±0	0±0	10.3±0.58^{dx}	9.7±0.58^{ex}	19.0±1.00^{ax}
	Klebsiella spp.	0±0	0±0	0±0	10.3±1.15^{ey}	21.3±0.58^{ay}
	Pseudomonas spp.	0±0	0±0	11.3±1.15^{dz}	10.7±0.58^{ez}	20.7±0.58^{az}
	Vibrio spp.	0±0	0±0	7.7±0.58^{du}	10.7±0.58^{eu}	20.7±0.58^{au}
	Salmonella spp.	0±0	0±0	7.3±1.15^{dv}	14.7±0.58^{ev}	20.3±1.15^{av}
	Listeria spp.	0±0	14.7±0.58^{cw}	14.7±0.58^{dw}	15.3±1.15^{ew}	21.3±0.58^{aw}
	Staphylococcus spp.	0±0	0±0	7.3±0.58^{dt}	8.7±0.58^{et}	22.3±0.58^{at}
Cumin (Seeds, *Cuminum cyminum*)	*E. coli*	0±0	0±0	11.7±0.58^{dx}	12.7±0.58^{ex}	19.3±1.15^{ax}
	Klebsiella spp.	0±0	0±0	8.7±1.15^{dy}	9.3±0.58^{ey}	21.0±1.00^{ay}
	Pseudomonas spp.	12.3±0.58^{bz}	14.3±1.15^{cz}	14.3±0.58^{dz}	15.7±1.53^{ez}	19.7±0.58^{az}
	Vibrio spp.	0±0	0±0	0±0	8.7±0.58^{eu}	18.7±0.58^{au}
	Salmonella spp.	0±0	0±0	8.3±1.15^{dv}	10.7±0.58^{ev}	20.7±1.53^{av}
	Listeria spp.	0±0	8.7±0.58^{cw}	12.7±0.58^{dw}	13.3±0.58^{ew}	20.3±0.58^{aw}
	Staphylococcus spp.	0±0	0±0	7.7±0.58^{dt}	10.7±1.15^{et}	22.7±0.58^{at}

Values are presened as mean±SD. Values with different superscripts within each row are significantly different ($P < 0.05$). Buffered peptone water, methanol, and ethanol were used as negative controls and found to not affect bacterial growth.

was encountered in 11 instances (Figure 1). On the other hand, the MBC of the tested spices against the majority of bacterial isolates was 24 mg/mL (Figure 2). The lowest MBC was found to be 12 mg/mL (in 4 instances), whereas the highest MBC was 48mg/mL (in 5 instances). Different studies found varied results for MIC and MBC in spices against microorganisms from different foods (Shan *et al*., 2007; Dhiman *et al*., 2016; D'Souza *et al*., 2017). Chlipala *et al.* (2010) and Purkait *et al.* (2018) in their studies found MIC of Clove, Cinnamon, and other spice samples as <100 µg/mL. Bassolé and Juliani (2012) reported MIC of various spices as 0.625 - 1.25 µL/mL

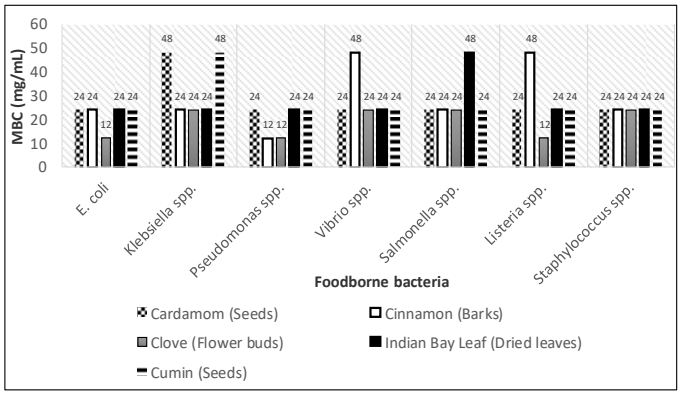

Figure 2. Minimum bactericidal concentration (MBC) of the spice samples. Mean values have been shown here.

against different bacterial isolates. Dhiman *et al.* (2016) evident the MIC of spice samples in a range of 25 to 50 mg/mL.

4. Conclusion

The present study confers the presence of considerable antibacterial potential in the tested spices against selected foodborne bacteria. The solvent extracts exhibited strong antimicrobial activity compared to crude and aqueous extracts. Such pieces of evidence indicate that spices could be potent sources of inhibitory substances against food spoilage- and disease-causing microorganisms. The findings inspire us to suggest the uses of spices in the development of effective as well as safe natural food preservatives.

Conflict of interest

The authors declare no conflict of interest.

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