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FULL PAPER

Microbiological quality and antimicrobial potential of extracts of different spices

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Abstract

The present study was carried out to determine the antimicrobial traits of spices for validating its potential as food preservatives and therapeutic alternatives along with their microbiological quality. In this regard, a total of 4 locally available spices including Saffron (*Crocus sativus*), Nutmeg (*Myristica fragrans*), Mace (*Myristica fragrans*) and Shahi Jeera (*Bunium bulbocastanum*) were collected from different areas of Dhaka, Bangladesh. The samples were found to harbor total viable bacteria and fungi up to 10^5 CFU/g and 10^4 CFU/g, respectively. Presence of specific bacterial species was also documented. Among them, *Staphylococcus* spp. and *Pseudomonas* spp. were prevalent as found in all samples. Presence of *Bacillus* spp. and *Escherichia coli* were also evident in Nutmeg and Shahi Jeera. Whereas, *Klebsiella* spp. was absent in all samples. Antibacterial properties of the samples were determined by the agar well diffusion method. The ethanolic and methanolic extracts of all the spice samples showed remarkable anti-bacterial activity against most of the tested bacterial isolates, although crude extract could merely affect the bacterial growth. The presence of antibacterial effects revealed that the spices could be used in food conservation and as natural antimicrobials.

1. Introduction

Presence of pathogenic microorganisms in food may cause spoilage and contribute to foodborne diseases. Recently, the occurrences of multidrug- and disinfectant-resistant bacteria in different food commodities have been increased drastically which resulted in the increase in morbidity and mortality (Miladi *et al*., 2016). Chemical preservatives could be toxic for human and can lead to the development of microbiological resistance which consequently enhances the consumers' risk (De Souza *et al*., 2005). In addition, chemical preservatives merely have any effect on several pathogenic bacteria like *Listeria monocytogenes* in food products or fail to retard the growth of spoilage microorganisms (Silva and Domingues, 2017). On the other hand, therapeutic options for common infectious diseases are declining day by day due to antibiotic resistant bacteria which limit the effectiveness of antibiotics (Högberg *et al*., 2010; Paphitou, 2013). Therefore, natural antimicrobials are receiving particular attention as suitable substitutes of synthetic antimicrobial therapeutics as well as of chemical preservatives because they have negligible side effects and have efficacy against human pathogens (Silva and Domingues, 2017).

Spices have been defined as plant substances derived from indigenous or exotic origin used to enhance the flavor and aroma of foods from ancient times (Newberne *et al*., 2000). Spices are inclusive of leaves (bay, mint, rosemary, coriander, laurel, oregano), flowers (clove), bulbs (garlic, onion), fruits (cumin, red chilli, black pepper), stems (coriander, cinnamon), rhizomes (ginger) and other plant parts (Shelef, 1983). Spices are known to stabilize the foods from the microbial deterioration and some spices are evident to have a particularly inhibitory effect on some food-spoilage microorganisms. Also, many spices have been applied to treat infectious diseases (Kizil and Sogut, 2003).

Spices have been well known for their medicinal, preservative and antioxidant properties as revealed by many investigations (Joe *et al*., 2009; Anjeza and Mandal, 2012). Therefore, spices have gained a large interest in recent days. Some experiments demonstrated that the employment of spices in place of chemicals and synthetics have become indispensable because they impart secondary metabolites which have antimicrobial

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effects (Nabavi *et al*., 2015). Spices active compounds such as carvacrol, carvone, thymol, trans-cinnamaldehyde etc. have been included in class of naturally occurring food preservatives (Brul and Coote, 1999). Considering the facts, the present study was undertaken to examine the inhibitory potential of spices commonly used in Bangladesh using their crude, ethanolic and methanolic extracts. The microbiological quality of the spice samples was also checked.

2. Materials and methods

2.1 Study area, sampling and sample processing

A total of 4 types of locally used spice samples including Saffron (*Crocus sativus*), Nutmeg (*Myristica fragrans*), Mace (*Myristica fragrans*), and Shahi jeera (*Bunium bulbocastanum*) were collected from local market of Dhaka, Bangladesh during January 2018 to March 2018. The samples were homogenized and serially diluted up to 10^{-4} for microbiological assay using standard methods (Sharmin *et al*., 2015; Jahan *et al*., 2018).

2.2 Microbiological analysis

2.2.1 Estimation of total viable bacteria and fungi

For the enumeration of total viable bacteria (TVB) and the total fungal load, 0.1 mL of each sample from the dilutions 10^{-2} and 10^{-4} was introduced onto the nutrient agar (NA) and Sabouraud's dextrose agar (SDA) plates, respectively, by means of spread plate technique. Plates were incubated at 37°C for 24 hrs and at 25°C for 48 hrs for total viable bacteria and fungi, respectively (Sharmin *et al*., 2015; Jahan *et al*., 2018; Munshi *et al*., 2018).

2.2.2 Estimation of E. coli, Klebsiella spp., Bacillus spp., Staphylococcus spp. and Pseudomonas spp.

From the dilutions 10^{-2} and 10^{-3}, 0.1 mL of each sample was spread onto MacConkey agar for the enumeration of coliforms (especially, *E. coli* and *Klebsiella* spp.), respectively. Plates were incubated for 24 hrs at 37°C. Likewise, *Staphylococcus* spp., *Pseudomonas* spp. and *Bacillus* spp. were isolated onto Mannitol Salt Agar (MSA), *Pseudomonas* agar and Starch agar (Sharmin *et al*., 2015; Jahan *et al*., 2018; Munshi *et al*., 2018).

2.3 Extraction of the samples and conduction of antimicrobial assay

The powder form of samples was prepared through grinding and 15 g of powder was added in 85 mL of ethanol and methanol in Durham's bottle to prepare ethanolic and methanolic extracts, respectively which were kept in shaking water bath at 130 rpm for 24 hrs at 24°C. Afterward, the extract solution was filtered followed by the collection of pellet of the sample to observe their anti-bacterial properties against different previously isolated pathogenic strains such as *E. coli*, *Pseudomonas* spp., *Vibrio* spp., *Klebsiella* spp., *Staphylococcus aureus* and *Bacillus* spp. preserved in the Microbiology Laboratory, Stamford University Bangladesh (Angiolella *et al*., 2018; Jahan *et al*., 2018). Agar well diffusion method was employed. At first the lawns of bacterial suspensions (10^5 CFU/mL or 0.5 OD measured by spectrophotometer) were prepared and 100 µL of the crude extract, ethanolic and methanolic extracts at a concentration of ~11.1mg/mL each were introduced into the wells. Buffer peptone water, absolute ethanol and methanol were used as negative controls while the antibiotic discs of gentamicin (10 µL) were used as positive control (Jahan *et al*., 2018). Plates were incubated at 37°C for 12-18 hrs and examined for formation of the zone of inhibitions (mm).

3. Results and discussion

3.1 Presence of microorganisms in the spice samples

Like other agricultural commodities, spices are prone to environmental microbial contamination during harvest, processing, and in retail markets by dust, wastewater as well as by the animal and human excreta (Freire and Offord, 2002). Previous studies on spices supported the fact and found different microorganisms including total heterotrophs, *Bacillus cereus*, *Clostridium perfringens*, *E. coli*, *Salmonella* spp. and toxigenic molds in the spice samples (Garcia *et al*., 2001; Banerjee and Sarkar, 2003; Sospedra *et al*., 2010). In the present study microbial contaminations were well documented in cohort with those previous findings. All the samples were found to harbor viable bacteria in a range of 3.7×10^5 CFU/g to 1.2×10^4 CFU/g, while fungi were present in an average of 10^3 CFU/g (Table 1). All the samples conferred the presence of specific bacterial isolates in a large extent. *Pseudomonas* spp. and *Staphylococcus* spp. was encountered predominantly in all the samples in an average of 10^4 CFU/g. Only Nutmeg and Shahi Jeera showed the presence of *E. coli* and *Bacillus* spp. All the samples were devoid of the growth of *Klebsiella* spp. (Table 1). Spices are grown and harvested in warm and humid areas which may support the growth of wide variety of microorganisms. Presence of microorganisms in spices could be source of food contamination (Banerjee and Sarkar, 2003).

3.2 In vitro anti-bacterial activity of the extracts of spice samples

Antibacterial activity of spices has been evident by several researchers previously (Outara *et al*., 1997; Al-

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Table 1. Microbiological load in the tested spice samples.

Samples	Total Viable Bacteria (CFU/g)	Fungi (CFU/g)	*E. coli* (CFU/g)	*Staphylococcus* spp. (CFU/g)	*Pseudomonas* spp. (CFU/g)	*Bacillus* spp. (CFU/g)
Saffron	3.7×10^4	1.8×10^3	0	1.1×10^4	5.2×10^4	0
Nutmeg	6.0×10^4	2.3×10^4	1.0×10^3	1.6×10^4	8.0×10^4	2.1×10^4
Mace	1.2×10^5	1.8×10^4	0	5.0×10^3	4.0×10^4	0
Shahi jeera	1.0×10^5	2.8×10^4	4.0×10^3	2.0×10^4	3.2×10^4	4.6×10^4

Table 2. Antimicrobial activity of saffron (*Crocus sativus*).

Test bacteria	Zone of Inhibition in diameter (mm)						
	Crude fraction	Negative control (BPW)	Negative control (Ethanol)	Ethanolic extract	Negative control (Methanol)	Methanolic extract	Positive control (Gentamicin, 10 µg)
E. coli	0	0	0	12 mm	0	13 mm	21 mm
Pseudomonas spp.	9 mm	0	0	13 mm	0	11 mm	23 mm
Vibrio spp.	0	0	0	0	0	0	21 mm
Bacillus spp.	0	0	0	0	0	0	20 mm
Klebsiella spp.	0	0	0	13 mm	0	13 mm	18 mm
Staphylococcus spp.	0	0	0	11 mm	0	10 mm	23 mm

Table 3. Antimicrobial activity of nutmeg (*Myristica fragrans*).

Test bacteria	Zone of Inhibition in diameter (mm)						
	Crude fraction	Negative control (BPW)	Negative control (Ethanol)	Ethanolic extract	Negative control (Methanol)	Methanolic extract	Positive control (Gentamicin, 10 µg)
E. coli	9 mm	0	0	13 mm	0	12 mm	21 mm
Pseudomonas spp.	8 mm	0	0	11 mm	0	13 mm	23 mm
Vibrio spp.	0	0	0	0	0	0	21 mm
Bacillus spp.	0	0	0	0	0	0	20 mm
Klebsiella spp.	0	0	0	14 mm	0	13 mm	18 mm
Staphylococcus spp.	0	0	0	0	0	0	23 mm

Table 4. Antimicrobial activity of mace (*Myristica fragrans*).

Test bacteria	Zone of Inhibition in diameter (mm)						
	Crude fraction	Negative control (BPW)	Negative control (Ethanol)	Ethanolic extract	Negative control (Methanol)	Methanolic extract	Positive control (Gentamicin, 10 µg)
E. coli	0	0	0	8 mm	0	10 mm	18 mm
Pseudomonas spp.	9 mm	0	0	16 mm	0	22 mm	21 mm
Vibrio spp.	0	0	0	0	0	0	19 mm
Bacillus spp.	0	0	0	0	0	0	23 mm
Klebsiella spp.	0	0	0	17 mm	0	19 mm	23 mm
Staphylococcus spp.	0	0	0	14 mm	0	13 mm	21 mm

Table 5. Antimicrobial activity of shahi jeera (*Bunium bulbocastanum*).

Test bacteria	Zone of Inhibition in diameter (mm)						
	Crude fraction	Negative control (BPW)	Negative control (Ethanol)	Ethanolic extract	Negative control (Methanol)	Methanolic extract	Positive control (Gentamicin, 10 µg)
E. coli	0	0	0	0	0	0	19 mm
Pseudomonas spp.	0	0	0	16 mm	0	12 mm	24 mm
Vibrio spp.	0	0	0	0	0	0	20 mm
Bacillus spp.	0	0	0	11 mm	0	13 mm	21 mm
Klebsiella spp.	0	0	0	0	0	0	22 mm
Staphylococcus spp.	8 mm	0	0	12 mm	0	9 mm	23 mm

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Jedah *et al*., 2000; Grohs and Kunz, 2000; De Souza *et al*., 2005; Silva and Domingues, 2017). The present investigation revealed that all the spice samples exhibited antibacterial activity (Tables 2-5). In most cases, the crude extract of the samples had almost no antibacterial activity. However, ethanolic and methanolic extracts of the spices showed their efficacy in eliminating pathogens. *Pseudomonas* spp. was found to be inhibited by all the samples, even by the crude extracts. Whereas, *Vibrio* spp. showed resistance against all the spice extracts. *E. coli* were eliminated by the ethanolic and methanolic extracts of Saffron, Nutmeg and Mace (Tables 2-5). Except for Nutmeg, all the other spice samples were successful in affecting the growth of *Staphylococcus* spp. Growth of *Bacillus* spp. was only reduced by the ethanolic and methanolic extracts of Shahi Jeera (Tables 2-5). In a similar study, Pandit and Shelef (1994) tested the antibacterial effect of 18 spices and found significant inhibitory effect. A study carried out by Al-Jedah *et al*. (2000) evident notifiable effects against bacteria with combined spices. Finding of antibacterial traits of spices claims their efficacy as alternative to chemical food preservatives and therapeutics (Al-Jedah *et al*., 2000; Grohs and Kunz, 2000; De Souza *et al*., 2005).

4. Conclusion

The presence of viable bacteria and fungi was evident in this study along with the specific bacterial isolates from the spice samples which was of public health concern. The present study also demonstrated that all the spice samples, especially their ethanolic and methanolic extracts possessed significant antibacterial activity. Such evidence suggests that they could be a potential source for antimicrobial agents against some foodborne pathogens and could be suitable candidates to be used as food preservatives. The spices claim their candidature for future studies of synergism, compatibility, and efficacy in foods or food-processing systems and mechanisms of activity against specific pathogens.

Conflict of Interest

Authors declare no conflict of interest.

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