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Anti-enteric Potentials of Six Common Food Spices

M. Reenaa[*] and Gurpreet Kaur

Department of Microbiology, Ethiraj College for Women, Chennai, Tamil Nadu, India

Corresponding author

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A B S T R A C T

Spices have been used for food flavoring since ancient times. They are also used as home remedies due to their medicinal properties. Food borne pathogens cause considerable acute and chronic illnesses in the human population. Indiscriminate and sometimes unwanted use of antibiotics to treat infections caused by these pathogens has resulted in a tremendous increase in bacterial drug resistance. Herbs and spices are safe as they have been in human consumption for centuries. In this study an attempt has been made to investigate and compare the antimicrobial properties of aqueous extract and oil extract of six commonly used Indian spices, namely garlic, cinnamon, clove, ginger, cardamom and cumin. Antibacterial activity of both the aqueous extract and oil extract of the six chosen spices were tested against three food borne pathogens, *Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa*. Except cumin all the other five spices exhibited antibacterial activity against the test bacterial pathogens. Among these, garlic exhibited activity against all 3 test bacterial isolates in all test concentrations (9/9; 100%; $p<0.05$) followed by clove and ginger. Aqueous extracts are relatively less effective against the test bacterium ($p<0.05$) compared to oil extracts of spices especially at the lower test concentration (50□ l/disc). Additional phytochemicals and pharmacology studies are recommended for the furtherance of the medicinal applications of these spices.

Introduction

Spices are the heart and soul of the great variety of hot and spicy Asian cuisines. India is blessed with rich heritage of traditional medical system and complemented with remarkable natural biodiversity (Kapilan, 2015). The natural products are found to be more effective as medicaments with least side effects compared to allopathic antibiotics.

A spice can be a seed, fruit, root, bark or other plant substances primarily used for flavoring, coloring or preserving the food. Spices are distinguished from herbs, which are leaves, flowers, or stems from plants that are often used primarily as home remedies instead of being a component of a cuisine recipe. The terminology, spice and herb are often used interchangeably in scientific and colloquial communication. While, most spices can be used as herbs as home remedy not all herbs can be used as spices for culinary purposes. Spices have been used for many centuries by various cultures to enhance flavor and aroma of foods as recognized by our ancestors. Spices are also used in stabilizing several food items from deterioration. On the other hand, herbal medicines are gaining more interest as an alternative to allopathic antibiotics for their cost effective and eco-friendly attributes. The use of herbal medicines is limited to few developing countries or select global population owing to poor knowledge or evidence on their pharmacological attributes.

Food borne infections due to the consumption of food with microbial contamination or intoxication is a major public health concern (Hero, 2014). The current trend is to decrease the usage of synthetic additives and use spices instead in food to treat the etiology at the source itself. In the present study, the antibacterial effects of aqueous and oil extracts of cumin, ginger, garlic, cloves, cardamom and cinnamon were evaluated with three common enteric bacterial pathogens.

Materials and Methods

Spices

The spices (Table 1; Fig 1), garlic bulbs, ginger rhizomes, cinnamon bark, clove, cumin seeds and cardamom used in this study were collected from local farmers market. These spices were identified and confirmed in the Department of Plant Biology and Plant Biotechnology, Ethiraj College for Women, Chennai.

Bacterial Strains

To assess the antibacterial activity of the spices, three food related (enteric) pathogenic bacteria *Salmonella typhimurium* (MTCC No. 3224) *Escherichia coli* (MTCC No. 10312) and *Pseudomonas aeruginosa* (MTCC No. 6204) were procured from the MTCC, Chandigarh, and used in this study.

Preparation of Aqueous Spice Extract

Two grams of the chosen spice was weighed in a monopan balance and placed in 250 ml beaker with sterile distilled water in an orbital shaker for 5 minutes to wash off surface contaminants. The moist spice was immediately transferred to surface sterilized mortar and crushed with a pestle inside the laminar air flow hood (BSC Class II). The crushed spice was transferred in entirety into a conical flask containing 20 ml sterile distilled water and was heated to boil at medium flame for 15 minutes to extract the water soluble compounds into solution. The flask was sealed with sterile cotton plug and allowed to cool at room temperature. The aqueous spice extract was filtered using pre-sterilized filter paper (Whatmann No.1) inside the laminar air flow hood, filtrate aliquoted in cryotubes and stored at 4^oC till use.

Preparation of Oil Spice Extract

Two grams of the chosen spice was weighed in a monopan balance and placed in 250 ml beaker with sterile distilled water in an orbital shaker for 5 minutes to wash off the surface contaminants. The moist spice was immediately transferred to surface sterilized mortar and crushed with a pestle inside the laminar air flow hood

(BSC Class II). The crushed spice was transferred in entirety into a conical flask containing 20 ml pre-heated cooking oil and cooked for 5 minutes to extract the fat soluble compounds into solution. The flask was sealed with sterile cotton plug and allowed to cool at room temperature. The oil spice extract was filtered using pre-sterilized filter paper (Whatmann No.1) inside the laminar air flow hood, filtrate aliquoted in cryotubes and stored at 4^oC till use.

Test of Spice Extracts for Anti-enteric Bacterial Activity

Aqueous and oil spice extracts were incorporated into pre-sterile filter discs (Hi-Media, India) at different volume (50 µl, 75µl and 100 µl/disc) allowed to air dry inside the laminar air flow chamber. The three test bacterial cultures *viz*., *E. coli, P. aeruginosa* and *S. typhimurium* were grown overnight in nutrient broth and adjusted to 0.5 McFarland with sterile saline.

Air dried Mueller-Hinton (MH) agar was seeded with the bacterial culture using a sterile swab and allowed to dry. Aqueous or oil spice extract impregnated filter discs were placed on the bacteria seeded MH agar and incubated at 37^oC for 24 hrs.

Zone of inhibition (mm) was measured around the filter discs against respective bacterial pathogens and data discussed.

Data Analysis and Statistics

Antibacterial efficacy of two different extracts of six spices against 3 different test bacterial isolates was tested for within group and within extract significance using Analysis of variance (ANOVA; MS EXCEL2016) single factor with the alpha set at 0.05.

Results and Discussion

Six most commonly used spices (Table 1; Figure 1) in day to day Indian cooking were screened for their anti-enteric bacterial activity. Recipes that use these spice process them either in aqueous medium resulting in a thick paste or soups or in oil rich curries that goes along with sautéed vegetables or meat. Mindful of these preparations, we hypothesized that boiling the spices in aqueous or oil medium will recover different bioactive compounds resulting in variation in their antibacterial outcome. To verify this hypothesis, six spices were heat processed in aqueous and oil matrix and the extracts were screened for their anti-enteric properties using three most common enteric pathogens, *E. coli*, *S. typhimurium* and *P. aeruginosa*.

Antibacterial profile of the aqueous spice extract was presented in Table 2. Among the tested spices, the aqueous extract of garlic was found to have superior antibacterial activity at different concentration (7/9; 78%), on all three test bacterial isolates. Even though, clove and ginger had modest antibacterial efficacy (4/9; 44%), they were effective either against *S. typhimurium* and *E.coli* (clove) or *S. typhimurium* and *P. aeruginosa* (ginger) (Table 2). Aqueous extract of cinnamon and cardamom was effective only against *P. aeruginosa* or *S. typhimurium* respectively while cumin aqueous extracts had no antibacterial activity even at 100 µl/disc.

Even among the spice extracts that exhibited antibacterial efficacy, only 1/6 (17%) were effective at lowest concentration (50 µl/disc) against *E. coli* and *P. aeruginosa* and none for *S. typhimurium*.

Data presented in Table 3 clearly indicate the superior antibacterial activity in the oil extracts of the chosen spices compared to aqueous extracts of the same. Among these, 4/6 (67%) spices have exhibited antibacterial activity against all the three test bacterium at all 3 test concentrations (9/9; 100%). While the oil extract of cardamom was effective only against *S. typhimurium* that too at concentrations >50µl/disc, cumin seed extract was ineffective.

Table.1 Spices evaluated in the present study

S. No.	SPICE	PARTS USED	BOTANICAL NAME	COMMON NAME
1.	Clove	Buds	*Sygizium aromaticum*	Laung (Hindi), lavangam (Tamil)
2.	Cardamom	Pods	*Elettaria cardamomum*	Elaichi (Hindi), Elakkai (Tamil)
3.	Ginger	Rhizomes	*Zingiber officinale*	Adrak (Hindi), Inji (Tamil)
4.	Garlic	Bulbs	*Allium sativum*	Lehsan (Hindi), Poondu (Tamil)
5.	Cumin	Seeds	*Cumimum cyminum*	Jeerah (Hindi) Jeeraka (Tamil)
6.	Cinnamon	Bark	*Cinnamomum zeylanicum*	Dalchini (Hindi) Pattai(Tamil)

Table.2 Antimicrobial activity of aqueous extract of spices as indicated by the inhibition zone against test bacterial isolates

Spice tested	Zone of Inhibition (mm)									Overall effect of spices +ve/tested (%)
	S. typhimurium			*E. coli*			*P. aeruginosa*			
	Extract test concentration (µl/disc)*									
	50	75	100	50	75	100	50	75	100	
Garlic	-	6	8	6	7	8	-	7	9	**7/9 (78%)**
Cinnamon	-	-	-	-	-	-	6	8	10	**3/9 (33%)**
Clove	-	6	8	-	7	9	-	-	-	**4/9 (44%)**
Ginger	-	7	7	-	-	-	-	7	8	**4/9 (44%)**
Cardamom	-	7	8	-	-	-	-	-	-	**2/9 (22%)**
Cumin	-	-	-	-	-	-	-	-	-	**0/9 (0%)**
Total number of effective spices (%)	**0/6 (0%)**	**4/6 (67%)**	**4/6 (67%)**	**1/6 (17%)**	**2/6 (33%)**	**2/6 (33%)**	**1/6 (17%)**	**3/6 (50%)**	**3/6 (50%)**	

***** Sterile water control had no zone of inhibition
- No zone

Table.3 Antimicrobial activity of oil extract of spices as indicated by the inhibition zone against test bacterial isolates

Spice tested	Zone of Inhibition (mm)									Overall effect of spices +ve/tested (%)
	S. typhimurium			*E. coli*			*P. aeruginosa*			
	Extract test concentration (µl/disc)*									
	50	75	100	50	75	100	50	75	100	
Garlic	9	10	10	9	9	10	8	9	9	**9/9 (100%)**
Cinnamon	9	9	10	8	8	9	10	11	11	**9/9 (100%)**
Clove	9	9	10	9	9	10	9	9	10	**9/9 (100%)**
Ginger	9	9	10	7	8	8	8	9	10	**9/9 (100%)**
Cardamom	-	7	8	-	-	-	-	-	-	**2/9 (22%)**
Cumin	-	-	-	-	-	-	-	-	-	**0/9 (0%)**
Total number of effective spices (%)	**4/6 (67%)**	**5/6 (83%)**	**5/6 (83%)**	**4/6 (67%)**	**4/6 (67%)**	**4/6 (67%)**	**4/6 (67%)**	**4/6 (67%)**	**4/6 (67%)**	

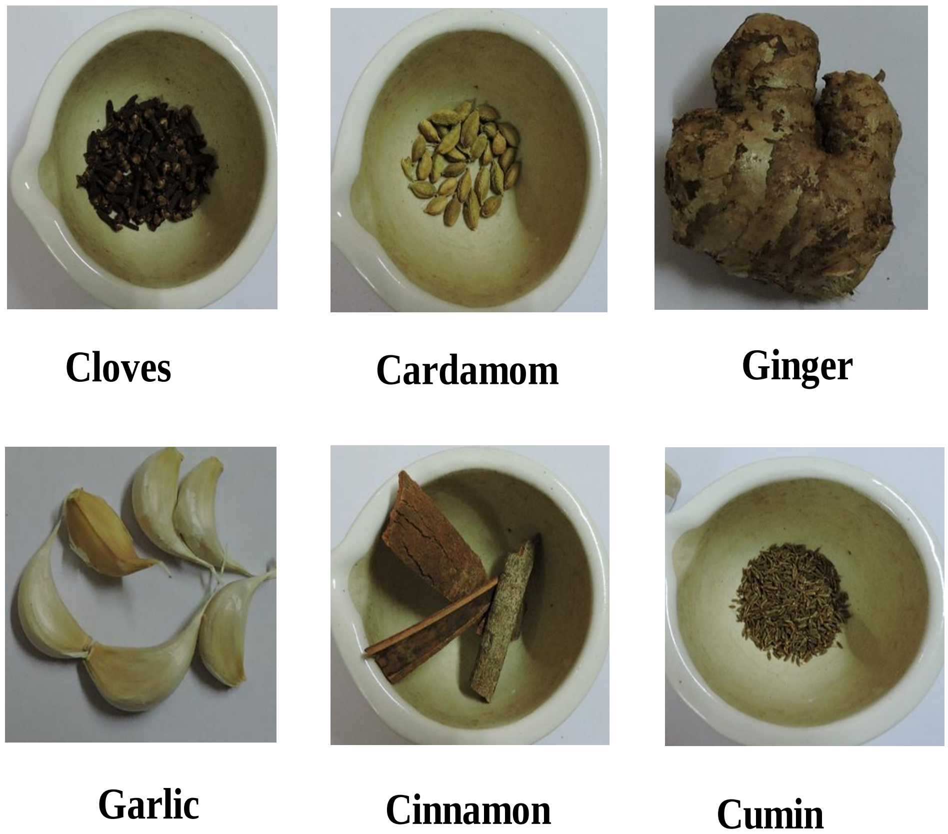
***** Cooking oil control had no zone of inhibition
- No zone

Table.4 Comparative analysis of antibacterial activity of spice extracts

Spice tested	Antibacterial activity					
	*S. typhimurium**		*E. coli**		*P. aeruginosa*	
	Aqueous	Oil	Aqueous	Oil	Aqueous	Oil
Garlic	++	+++	+++	+++	++	+++
Cinnamon	-	+++	-	+++	+++	+++
Clove	++	+++	++	+++	-	+++
Ginger	++	+++	-	+++	++	+++
Cardamom	++	++	-	-	-	-
Cumin	-	-	-	-	-	-
Total number of effective spices (%)	**4/6 (67%)**	**5/6 (83%)**	**2/6 (33%)**	**4/6 (67%)**	**3/6 (50%)**	**4/6 (67%)**

+++ = Effective at all 3 test concentrations; ++ = Effective at 2 test concentrations;
+= Effective at 1 test concentration; - = No antibacterial effect
*= $p<0.05$

Fig.1 Spices screened in this study



A head to head comparison of the aqueous and oil extracts (Table 4) clearly shows anti-enteric bacterial superiority of garlic over others with statistical significance over cardamom and cumin ($p<0.05$).

Both aqueous and oil garlic extract had demonstrated effect on all three test bacterial isolates. This was followed by clove and ginger that were effective against all three organisms except aqueous extract for *P. aeruginosa* and *E. coli* respectively. Further statistical analysis indicate a significant difference ($p<0.05$) among the aqueous and oil extract's effect against *S. typhimurium* and *E.coli* at 50µl/disc concentration.

Several researchers have previously attempted to assess and understand the antibacterial profile of various spices and herbs (Arora and Kaur, 1999; Minakshi *et al.*, 1999; Melvin Joe *et al.*, 2009; Bachir and Benali, 2012; Rajasekar *et al.*, 2012; Pandey *et al.*, 2014; Shete and Chitanand, 2014). Previous works have shown similar superior anti-bacterial activity in garlic extracts among the Indian spices that were tested (Indu *et al.*, 2006; Baljeet *et al.*, 2015). Boiling imparts thermal energy into organic compounds and complexes facilitating bond breakage and changes in structural conformation. As most of these bioactive compounds exert their bacteriostatic/bactericidal activities by either interfering the enzyme substrate complex leading to metabolic aberrations in the target bacterium or bind to a eukaryotic receptor binding protein impeding the binding of the virulence factor on to the host receptor.

Any changes in the bioactive compounds due to culinary processing can either result in enhancement or reduction in their reactivity with the target.

Based on the data generated in this study, it is possible the oil extraction process either conserve the structural conformation of the bioactive compounds and/or modify them in a manner resulting in a potentiated antibacterial activity.

This is a preliminary study and the data indicate the possibilities of the existence of interesting bioactive compounds in these spices that warrants well designed phytochemical and pharmacological studies using purified preparations.

It is obvious that these spices are included in traditional cooking not only for the taste and texture but also for their mysterious medicinal properties. With the advent of a variety of phytochemical tools, it is time open this 'magic box' and decipher the secret codes that can lead to the development of affordable, effective and ecofriendly medicaments.

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