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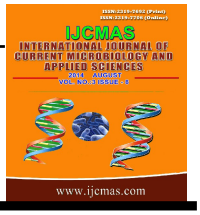

Original Research Article

Antimicrobial activity of some commonly used Indian Spices

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Keywords

Spices, Antimicrobial Activity, Activity Index

A B S T R A C T

Now-a-days majority of world population rely on the plant preparations as medicines to cure diseases, as they are considered safe and as effective as allopathic preparations without any side effects. Spices are plant products having aroma. They are mainly used during cooking to impart flavor and taste to the dish. They also possess medicinal values. The present study was designed to evaluate the antimicrobial activity of eight Indian spices against Gram positive and Gram negative pathogenic bacteria viz., *S. aureus, B. subtilis, B. cereus, E.coli, S. typhi, P. aeruginosa* using aqueous, ethanolic and methanolic extracts. Among all extracts tested alcoholic extract of Star anise (*Ilicium verum),* and black pepper (*Piper nigrum*) showed maximum antimicrobial activity against gram negative bacteria while alcoholic extract of clove (*Syzygium arromaticum*) and black pepper showed maximum activity against gram positive bacteria. By and large, all spices used during the studies proved as antibacterial compounds with maximum activity index (AI) 1.42 exhibited by alcoholic extract of black pepper against *E.coli.*

Introduction

India is one of the nations blessed with rich heritage of traditional medical system and rich biodiversity to complement the herbal needs of the treatment administrated by these traditional medical systems (Pei S.J., 2001).

Medicinal plants constitute major sources of number of primary and secondary metabolites which are the bioactive compounds of great therapeutic value. (Evans et al, 2002). Medicinal plants are important resources to traditional society's health care system and it is estimated that 70% to 80% of rural population in developing Asian nations depends on home care and traditional medicines for therapies. Major part of these therapies involves the use of plant extract or their active principles which can be used for treating diseases like asthma, intestinal disorder, and recurrent fever. (Tenover et al, 2004, Chang, H.W., 1995).

Also, herbal medicines are gaining more interest for their cost effective and ecofriendly attributes, even though pharmacological industries have produced a

number of new antibiotics in the last three decades (Arora and Kaur, 1999). The emergence of resistance to conventional antimicrobials is a serious problem. This necessitates the constant development of newer agents which can inhibit the growth of the pathogen or kill it (Gold and Millering, 1995). Hence more studies pertaining to the use of plants as therapeutic agents for the control of antibiotic resistant microbes should be emphasized. (Mishra and Behal, 2010).

Spices are plant substances with strong taste, used to enhance the taste of foods. Although the primary purpose of spices is to impart flavor and piquancy to food, they are important due to its medicinal, antimicrobial and antioxidant properties (Joe et al, 2009; Aneja et al, 2012,). The spices have unique aroma and flavor which are derived from compounds known as phytochemicals or secondary metabolites. (Avato et al, 2006, Panpatil et al, 2013). Numerous classes of phytochemicals including isoflavonoids, anthocyanins, and flavonoids are found associated with the spices. (Shan et al, 2007)

Keeping this view in mind, in the present work the study was carried out on the antimicrobial effect of aqueous, methanolic and ethanolic extract of common spices used in Indian dishes namely cumin, star anise, cardamom, lichens, black pepper, cinnamon, malabathrum and cloves against selected gram negative and gram positive bacteria.

Materials and Methods

Spices selected

Spices selected for the present study were cumin seeds (*Cuminum cyminum*), star anise (*Iilicium verum*), Cardamom (*Elettaria cardamomum*), malabatathrum (*Cinnamomum tamala*), Cloves (*Syzygium arromaticum*), Black pepper (*Piper nigrum*), Cinnamon (*Cinnamomum zeylanicum*) and Dagadphool (Foliose lichen).

Extract Preparation

Air-dried and coarsely powdered material of the spices was extracted for 8 hours with distilled water in Soxhlet apparatus and then the extract was filtered and used for the study as aqueous extract. Similarly ethanol and methanol extracts were prepared.

Test organisms used in the study

Three gram positive and three gram negative organisms were used as test organisms in the study. Gram positive bacteria were *Bacillus subtilis, Bacillus cereus* and *Staphylococcus aureus* while gram negative bacteria were *Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa*. The cultures were procured from NCCL, Pune and all the cultures were maintained on nutrient agar slants.

Antimicrobial activity

The antimicrobial activity of the extracts was carried out by well diffusion method. (Parez et al, 1990) using 100 µl of suspension containing 10^8 CFU/ml of bacteria spread on nutrient agar (NA) medium. Sterile 8 mm diameter cork borer was used to prepare well in the nutrient agar. Each well was filled with 0.1 ml of extract of plant material. Negative control was prepared using the same solvents employed to prepare the plant extracts. Penicillin G (10U /disc), was used as a positive reference to determine the sensitivity of bacterial species tested. The inoculated plates were incubated at 37°C for 24 h. The antibacterial activity was measured as the diameter in mm of clear zone of growth inhibition around the well.

Results and Discussion

In India, spices are ethnically used as active ingredients in ayurvedic medicines and reported to possess a number of pharmacological effects to treat different human ailments (Bonjar *et al*., 2004). Several investigations have been directed towards their anti-microbial properties (Voravuthikunchai *et al*., 2005 Vaishnavi et al 2007). In the present work, among the eight spices tested against six bacterial pathogens, all the spices showed antibacterial activity. The results of antibacterial activity of these spices against six pathogens tested are given in Table 1 and Table 2. Out of three types of extracts tested, ethanolic and methanolic extracts of spices had demonstrated better activity against pathogens than aqueous extracts of the spices. This may be due to the better solubility of active ingredients of spices in alcoholic solvents than water. Ahmad et al (1998) also reported that alcoholic extracts of medicinal plants had greater activity than their aqueous extracts.

Table.1 Antimicrobial activity of spices against gram negative bacteria

Sr. No.	Spices		Nature of Extract	Test bacteria Zone of inhibition in mm		
	Botanical Name	Common Name		*E.coli*	*S.typhi*	*P.aeruginosa*
1.	*Cuminum cyminum*	cumin	Aq. M. E.	- 14 11	10 16 14	- 11 15
2.	*Iilicium verum*	Star anise	Aq. M. E.	12 19 16	12 16 17	13 19 16
3	*Elettaria cardamomum*	Cardamom	Aq. M. E.	- 11 15	12 12 20	-- 12 13
4.	*Cinnamomum tamala*	Malabatathrum	Aq. M. E.	- 14 16	12 14 16	- 15 13
5.	*Syzygium arromaticum*	Cloves	Aq. M. E.	-- 17 16	16 18 17	-- 19 19
6.	*Piper nigrum*	Black pepper	Aq. M. E.	-- 18 20	-- 12 22	- 12 16
7.	*Cinnamomum zeylanicum*	Cinnamon	Aq. M. E.	-- 15 13	11 16 14	- 12 12
8.	Foliose lichen	Lichen	Aq. M. E.	-- 15 12	-- 15 15	-- 18 16

Table.2 Antimicrobial activity of spices against gram positive bacteria

Sr. No.	Spices		Nature of Extract	Test bacteria Zone of inhibition in mm		
	Botanical Name	Common Name		*B.cereus*	*B.subtilis*	*S.aureus*
1.	*Cuminum cyminum*	cumin	Aq. M. E.	11 12 16	-- 13 14	11 -- 13
2.	*Iilicium verum*	Star anise	Aq. M. E.	13 17 17	13 17 16	21 15 16
3	*Elettaria cardamomum*	Cardamom	Aq. M. E.	14 15 13	11 18 15	11 13 14
4.	*Cinnamomum tamala*	Malabatathrum	Aq. M. E.	14 13 12	11 12 12	12 12 11
5.	*Syzygium arromaticum*	Cloves	Aq. M. E.	-- 21 18	-- 18 21	18 26 19
6.	*Piper nigrum*	Black pepper	Aq. M. E.	15 16 19	12 20	17 11 16
7.	*Cinnamomum zeylanicum*	Cinnamon	Aq. M. E.	10 14 14	- 13 17	15 12 21
8.	Foliose lichen	Lichen	Aq. M. E.	11 17 17	 15 16	-- 14 17

Figure.1 Activity Index of spices against Gram Negative bacteria [*E. coli*]

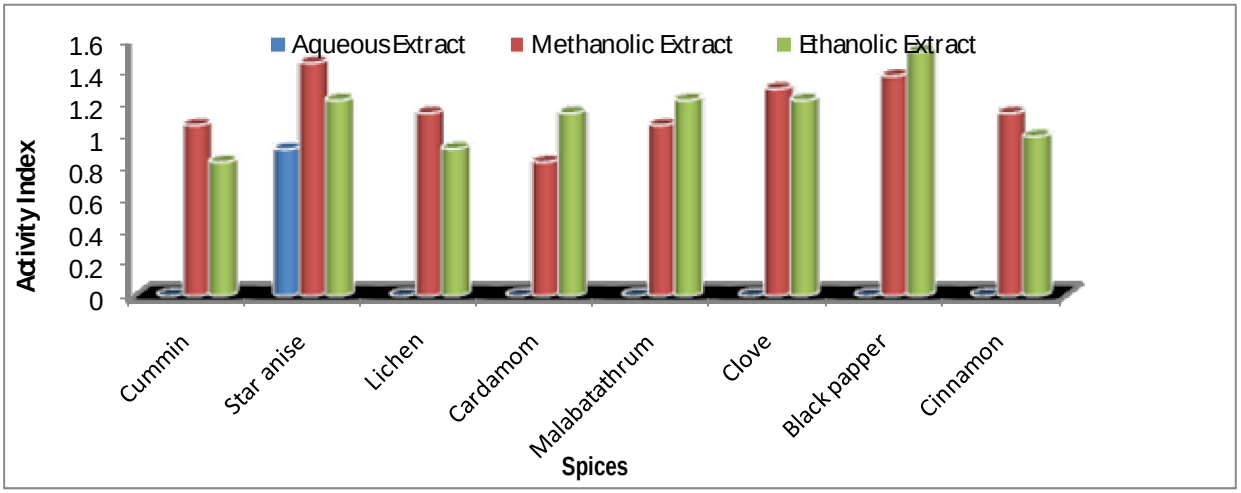
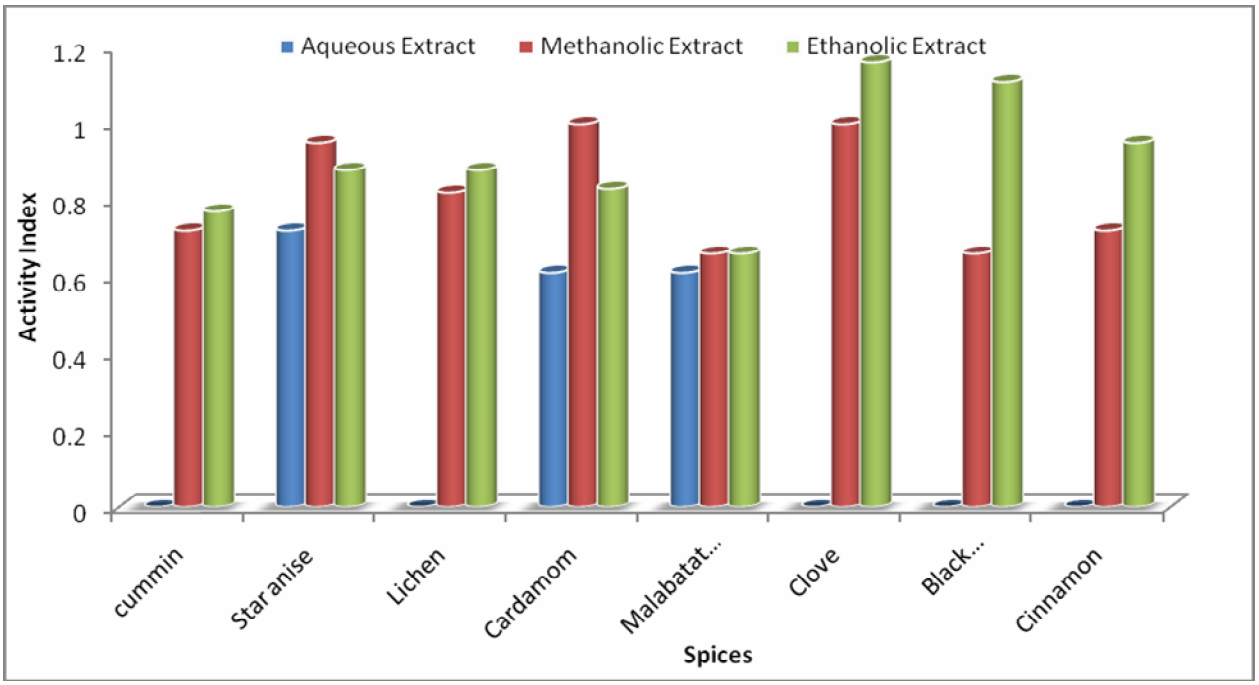


Figure.2 Activity Index of spices against Gram Positive bacteria [*B. subtilis*]



Among the spices tested, the extracts of star anise, clove and black pepper had shown maximum activity against pathogens than other spices. Cummin, malabatathrum and cardamom had shown less activity, while lichen and cinnamon had exhibited moderate activity.

The efficacy of spices was compared between gram negative and gram positive bacteria. It was observed that spices like cloves, and star anise had shown highest antimicrobial zones against gram negative bacteria while black pepper exhibited maximum zones against gram positive bacteria. Shihabudeen et al (2010) and Ceylan and Fung (2004) demonstrated that the gram positive bacteria were more sensitive to spices than gram negative bacteria because of their cell wall structure. But in the present work the spices were found to be effective against both gram positive as well as gram negative bacteria.

The antibacterial activity of spices was compared with the standard antibiotic Penicillin G (10 U/disc) and activity index of spices was determined against gram positive and gram negative bacteria. (Fig.1 and Fig 2). The activity index of clove, star anise and black pepper against *E.coli* was found to be more than one, while activity index of clove and black pepper was more than one against *Bacillus subtilis*. This indicated that these spices are exhibiting more antimicrobial activity than the antibiotic penicillin which is generally used against bacterial pathogens.

It is established in this study that the spices reduce and inhibit the growth of pathogens. This is also applicable to food pathogens and food poisoning organisms as spices are used in various food preparations. Thus the results of present study provided the justification for therapeutic potential of the spices and imply the need for the further study on the

use of spices as supplementary or alternative medicines**.**

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