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Antimicrobial properties of Indian spices

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ABSTRACT

The present study was designed to evaluate the antimicrobial activity of both aqueous and methanol extracts of five Indian spices, namely *Allium sativum*, *Curcuma longa*, *Zingiber officinale*, *Caryophyllus aromaticus* and *Cinnamomum tamala*. All of these have been traditionally used in folk medicine and are still used in the alternative system of health care. Antimicrobial activity of these commonly used Indian spices was tested against six strains of both Gram positive and Gram negative bacteria, namely *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Vibrio cholerae*, *Klebsiella pneumoniae and Pseudomonas aeruginosa*. Susceptibility of the microorganisms to the extracts of these plants was compared. The result showed that, the methanol extracts of spices exhibited higher activity against the tested organisms rather than aqueous extract of those spices. Minimum inhibitory concentration (MIC) of the methanol extract of selected spices was studied. The results showed that the extracts of cloves and garlic had good inhibitory action than the extract of turmeric and ginger.

KEYWORDS

Antimicrobial activity;
Indian spices;
MIC;
Pathogen;
Plant extracts.

INTRODUCTION

In contemporary Indian spices are used to rustle up scrumptious delicacies. However the Indian spices are more than just ingredients to add flavour and aroma to food, also it has an important role in economy. The Indian climate supports the growth of an array of spices and as a result the nation produces 75 types of spices out of 109 listed with the International Organization for Standardization (ISO). These spices are being used not only for flavouring foods but also in medicines, pharmaceutical, perfumery, cosmetic and several other industries.

For centuries, Indian spices have made a significant contribution both in the health care system and the food industry. Ancient Asian literature is a treasure of information related to the problems of health care and other environmental aspects. Indian spices have been used for years in different traditional forms of medicine like Ayurveda, Unani and Sino-Tibetian systems. The Vedic literature (2500 B.C.) is the main source of information that contributes to the development of Ayurveda. Particularly in Ayurveda, spices contributed a major amount for the treatment of key disorders of the body. Spices are used as chief ingredients in the preparation of Homeopathic medicine. In ancient In-

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dia, natural herbs and spices were consumed either in food, or used as medicine in order to maintain proper sanitation, health and hygiene, and to increase longevity of life[1].

In this respect, spices, such as clove (toothache, fever and pain), cinnamon (nervous problems, stomach/intestine infections), mustard, garlic (antiseptic, diuretic), ginger (digestive aid, cold), mint etc. have been reported to possess very good medicinal properties. Apart from being a major part of the Indian culinary, spices also contribute to the modern allopathic system of healthcare by providing large number of medicines or parent compounds. Reports indicate that spices have dual type of action. Short-term effects include inflammation, pain, heat, redness and swelling. Long-term effects include anti-inflammation, analgesic, antimicrobial, antioxidant and antimutagenic actions[2-6].

A large number of plants are used to combat different types of infectious diseases and possess antimicrobial activity. In modern era characterised by increasing consumer choice, self-medication and quest for natural therapy, herbal products are used increasingly as an alternative to drugs and supplements[7]. In particular, extracts from many kinds of oriental spice plants are known to possess antimicrobial effect besides being used for the purpose of food preservation, appetiser promotion and medicinal purposes[8-10]. The essential oil of several plants shows activity against several bacteria, like *Staphylococcus*, *Bacillus*, *Listeria* and *Kleisbella*[11,12]. Conner & Beuchat[13] studied essential oils of thirty-two spices for inhibitory effect on thirteen food spoilage and industrial yeasts. They also found varying degree of inhibitory action amongst various spices; some being strongly antimicrobial, while others showing no antimicrobial activity at all.

In this communication we have reported the antimicrobial activity of the extracts of five widely used Indian spices against some common gram positive and gram negative pathogenic microorganisms.

MATERIALS AND METHODS

Spices used for antimicrobial activity

The antimicrobial activity of five well-known and commonly used Indian spices, namely garlic, turmeric, ginger, clove and cinnamon leaf was verified. All the selected spices were procured from the local market. They were categorised into dry and wet spices. Different parts of the plants were used enumerated as follows:

Name of Spices	Botanical name	Part of plant
Garlic	*Allium sativum*	Bulb
Turmeric	*Curcuma longa*	Rhizome
Ginger	*Zingiber officinale*	Rhizome
Clove	*Caryophyllus aromaticus*	Buds
Cinnamon leaf	*Cinnamomum tamala*	Leaf

Preparation of plant / spice extracts

Clean dry spice samples were collected in a cotton bags. The materials were grinded with the help of mixer grinder. Then these materials were used for the preparation of aqueous and methanol extracts.

a) Preparation of aqueous extract

2 gm of grinded materials were mixed with 20 ml of sterile distilled water and kept on a rotary shaker for 12 hours at 30°C. Thereafter, it was filtered with the help of Whatman No. 1 filter paper. The filtrate was then centrifuged at 2000 rpm for 10 min. Then the supernatant was collected and stored at 4°C for further use.

b) Preparation of methanol extract

10 gm of grinded materials were soaked in 30 ml of 70% methanol and were kept at 30°C for 12 hours on a rotary shaker. After 12 hours the previous portion of added methanol was evaporated so to make the same volume methanol was added and then it was placed on a rotary shaker for another 12 hours at 30°C. After that it was filtered through Whatman No. 1 filter paper. The filtrate was centrifuged at 2000 rpm for 10 min. Then the supernatant was collected and stored at 4°C for further use.

For MIC determination the supernatant was collected and allowed to evaporate until completely dry. Then 30 mg of dry extract was re-suspended in 1 ml of 70% methanol. The final concentration of the extract was 30 mg/ml.

Microorganisms used

Two gram positive (*Staphylococcus aureus and*

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Bacillus cereus) and four gram negative (*Escherichia coli, Vibrio cholerae, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) pathogenic bacterial samples were collected from the Department of Microbiology, Vidyasagar University, Midnapore. The organisms were sub-cultured in nutrient broth and nutrient agar for use in experiment.

In vitro Antibacterial Study

Following methods were performed to determine the antimicrobial activity of spice extracts –

a) The modified agar-well diffusion method of Cappuccino and Sherman[14] was employed to study the antimicrobial activity of the plant extracts. 3.7% of Muller Hinton Agar was mixed with hot distilled water and autoclaved at 15 lb pressure for 15 minutes. After autoclaving, it was allowed to cool to 45°C-50°C. Then the medium was poured into sterilized Petri dishes with a uniform depth of approximately 4 mm. The agar medium was allowed to cool to room temperature. To standardize the inoculums density for sensitivity test, a $BaSO_4$ turbidity standard, equivalent to 0.5 Mac Farland standards were used. For the transformation of bacteria to Petridish a swab dipped in standard inoculums was used. After dipping, the swab was used to spread the bacteria on the media in a confluent lawn. Then the Petri dishes were left for 3 to 5 minutes. Using cork borer, 6 mm diameter wells were made in all the plates. Different extracts were added to the groove with one blank of each. Plates were incubated for 24 hours at 37°C. After 24 hours the plates were examined. Results were recorded as the presence or absence of inhibition zone. The inhibitory zone around the well indicated absence of bacterial growth and it was reported as positive and absence of zone is negative. The diameters of the zones were measured using diameter measurement scale. The effect of plant extract was compared with that of standard antibiotic tetracycline and levofloxacin.

b) The minimum inhibitory concentration (MIC) was evaluated by dilution method[15] on plant extracts to observe the antimicrobial activity. Antibacterial agents were incorporate in different concentration with liquid media. These media were inoculated with the test bacteria and incubated. The lowest dilution at which there is no growth of organisms is considered significant. The turbidity of the test sample is measured by spectrophotometer with respect to blank (media without microorganism).

Statistical Analysis

Since the readings of control (distilled water) in the *in vitro* antibacterial studies of medicinal spice were zero, the data was analyzed by simple arithmetic means of the different extracts and standard error was compared to the control. No other statistical test was applied to show significance since the extracts were either positive or negative for the antibacterial studies.

RESULTS

Aqueous extract of *Allium sativum, Caryophyllus aromaticus* and *Zingiber officinale* showed low activity against most of the tested organisms, except that *Zingiber officinale* is not effective against *Staphylococcus aureus* and *Bacillus cereus* (Figure 1, 4 & 3). But no antimicrobial activity was noticed with the aqueous extract of *Curcuma longa* and *Cinnamomum tamala* against test organisms (Figure 2 & 5).

The methanol extract of the spices showed different levels of antimicrobial activity toward test organ-

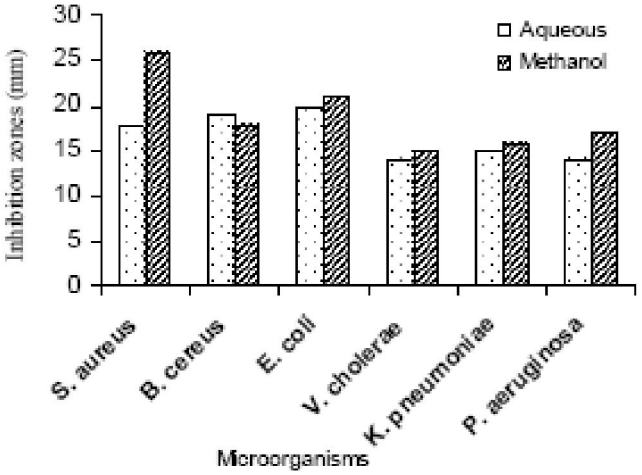

Figure 1 : Antibacterial activity of *Allium sativum* in aqueous and methanol extracts against both gram (+ve) and gram (-ve) microorganisms.

isms. Extract of *Allium sativum and Caryophyllus aromaticus* showed highest antimicrobial activity against all the tested organisms (Figure 1 & 4). The methanol extract of *Curcuma longa* and Zingiber *officinale* exhibited low antimicrobial activity against the test organisms (Figure 2 & 3). *Cinnamomum tamala* showed little antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* (Figure 5)

To screen the antibacterial activity against tested organisms, tetracycline and levofloxacin were used as a standard. It was found that levafloxacin (5µg/ml) showed higher activity than tetracyclin (30µg/ml) against tested microorganisms (Figure 6).

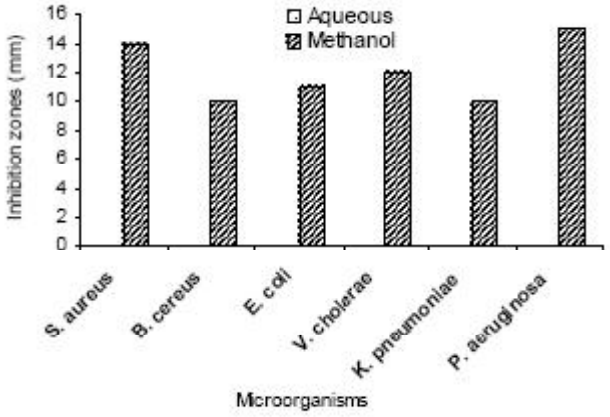

Figure 2 : Antibacterial activity of *Curcuma longa* in aqueous and methanol extracts against both gram (+ve) and gram (-ve) microorganisms.

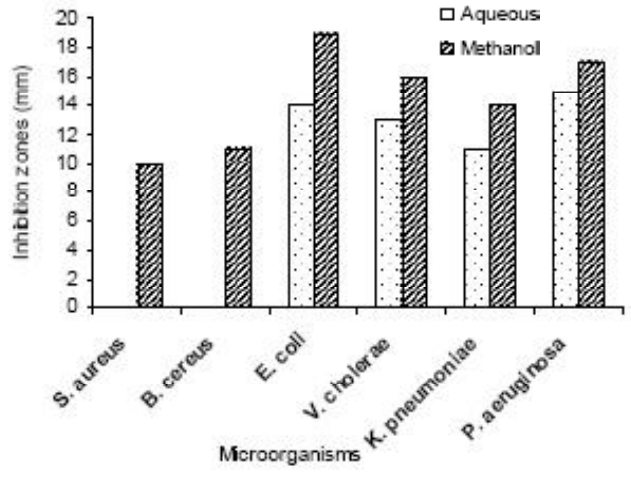

Figure 3 : Antibacterial activity of *Zingiber officinale* in aqueous and methanol extracts against both gram (+ve) and gram (-ve) microorganisms.

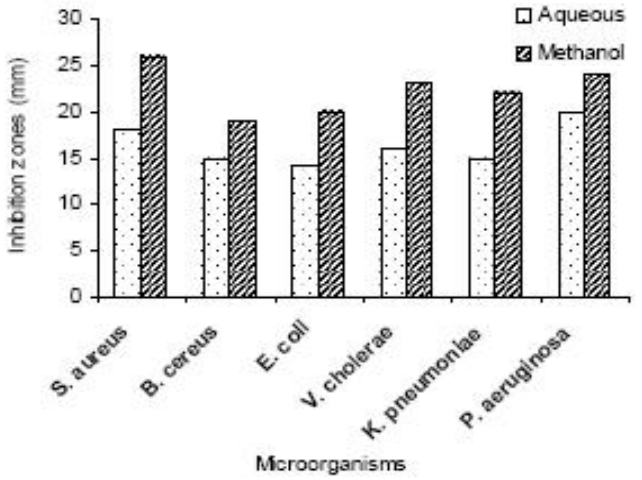

Figure 4 : Antibacterial activity of *Caryophyllus aromaticus* in aqueous and methanol extracts against both gram (+ve) and gram (-ve) microorganisms.

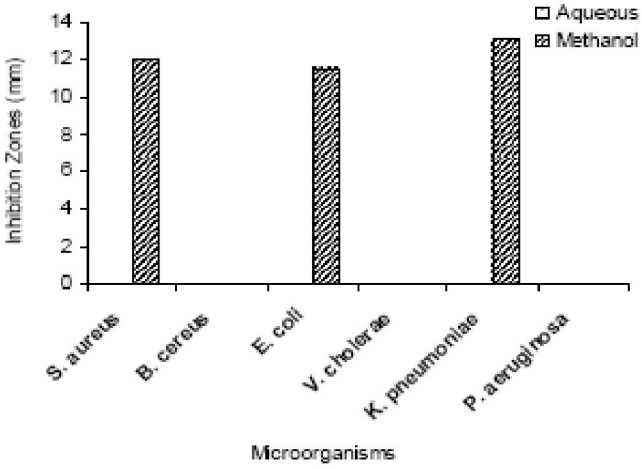

Figure 5 : Antibacterial activity of *Cinnamomum tamala* in aqueous and methanol extracts against both gram (+ve) and gram (-ve) microorganisms.

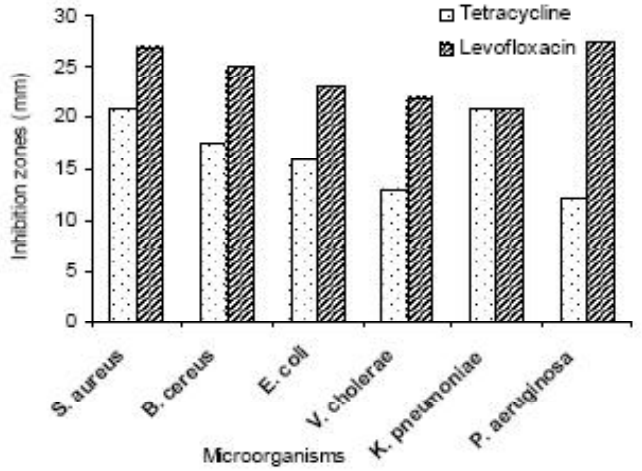

Figure 6 : Antibacterial activity of Tetracyclin (30µg/ml) and Levafloxacin (5µg/ml) standards against both gram (+ve) and gram (-ve) microorganisms.

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TABLE 1 showed the MIC values of methanol extracts of selected spices and the standard levafloxacin. The result indicates that standard antibiotic levafloxacin has much higher antimicrobial activity than the four selected methanol spice extracts. Among the spice samples, the effectiveness of inhibitors can be sequenced as follows in descending order against different pathogens: *Staphylococcus aureus*: Clove > Turmeric > Garlic > Ginger; *Bacillus cereus:* Clove > Garlic > Turmeric > Ginger; *Escherichia coli*: Garlic > Clove > Turmeric > Ginger; *Vibrio cholerae*: Clove > Turmeric > Ginger > Garlic; *Klebsiella pneumoniae*: Clove > Garlic > Turmeric > Ginger; *Pseudomonas aeruginosa*: Clove > Turmeric > Garlic > Ginger.

TABLE 1 : The MIC of the *Allium sativum, Curcuma longa, Zingiber officinale* and *Caryophyllus aromaticus* methanol extracts and levofloxacin against the microorganisms.

Data represents Mean ± Standard Error of Mean.

Microorganisms	MIC (mg/ml)				
	Allium sativum	*Curcuma longa*	*Zingiber officinale*	*Caryophyllus aromaticus*	*Levofloxacin*
Staphylococcus aureus	2.73 ± 0.16	2.08 ± 0.30	4.78 ± 0.34	0.83 ± 0.08	0.041 ± 0.006
Bacillus cereus	2.92 ± 0.21	3.25 ± 0.26	3.84 ± 0.51	2.18 ± 0.19	0.070 ± 0.008
Escherichia coli	1.49 ± 0.11	2.92 ± 0.31	3.15 ± 036	1.72 ± 0.24	0.061 ± 0.005
Vibrio cholerae	3.05 ± 0.29	2.48 ± 0.27	3.01 ± 0.28	1.53 ± 0.11	0.034 ± 0.004
Klebsiella pneumoniae	2.09 ± 0.18	2.53 ± 0.12	2.93 ± 0.15	1.61 ± 0.17	0.092 ± 0.009
Pseudomonas aeruginosa	2.24 ± 0.24	1.98 ± 0.09	2.76 ± 0.24	1.39 ± 0.13	0.065 ± 0.01

DISCUSSION

Successful prediction of Indian spices is largely dependent on the type of solvent used in the extraction procedure. Traditionally medicinal Indian spices are used primarily with water but in our studies we found that tested spices extract in organic solvent (methanol) provided more consistent antimicrobial activity compared to those extracted in water, as also reported earlier[16,17]. These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and in addition to their intrinsic bioactivity. In the present study, the antimicrobial activity of aqueous and methanol extracts of the five spices, namely *Allium sativum, Curcuma longa, Zingiber officinale, Caryophyllus aromaticus* and *Cinnamomum tamala* were examined to a preliminary screening for antimicrobial activity against six standard bacteria: two gram positive (*Staphylococcus aureus and Bacillus cereus)* and four gram negative (*Escherichia coli, Vibrio cholerae, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). The results of screening are presented in Figure 1-5. It is clear that the methanol extract of selected Indian spices exhibited higher activity against the tested organisms rather than aqueous extract of those spices. Methanol extract of *Allium sativum, Curcuma longa, Zingiber officinale* and *Caryophyllus aromaticus* showed pronounced activity against all the tested gram positive and gram negative microorganisms except *Cinnamomum tamala*. It was surprising that there is difference in the antibacterial activities of the extracts of the different spices. This could be due to the phytochemical differences between them. In our study it was found that clove, garlic and turmeric showed higher antimicrobial activity than ginger. Among them clove extract showed excellent antibacterial activity in the present investigation. According to a review by Snyder[18], similar observations were made where cloves, cinnamon and mustard were recognised as strong antimicrobial agents, while ginger and mint as weak ones. Arora & Kaur[19] tested various spices for antimicrobial activity of the different spices tested; only garlic and clove were found to possess antimicrobial activity. Rhee et al.[20] reported that clove showed strong activity towards *E. coli* and *B. cereus*, but relatively less towards *S. aureus* at 0.5% and 1% concentrations. The potent antimicrobial activity of clove can be predominantly attributed to eugenol. These are the phenolic components of clove, which render them effective against the tested micro-organisms. This was confirmed by Farag

et al.[21], where eugenol, a major component of clove was found to limit the growth of *B. cereus* by inhibiting the production of certain enzymes needed for its growth. Bhak et al.[22] showed clove to have strong inhibitory actions, while mustard and garlic had only slight antimicrobial activity. Figure 1-5 clearly indicates that clove was the most active against all the tested organisms except *E. coli*. Where as garlic is the most active against *E. coli*. These results are corroborated by some researchers who believe that Allicin is the principal antimicrobial compound of freshly crushed garlic[23,24]. In a similar study carried out by Adler & Beuchat[25], the addition of garlic to a food substrate enhanced the inactivation of *E. coli* at varying temperatures.

As we have found better result with methalonic extract of *Allium sativum, Curcuma longa, Zingiber officinale* and *Caryophyllus aromaticus* against most of the tested pathogens. So, the MIC values of methanol extracts of those spices were performed (TABLE 1). The results showed that the MIC of *Allium sativum* extract against all the tested organisms varied between 1.49 ± 0.11 and 3.05 ± 0.29, MIC of *Curcuma longa* extract against all the tested microorganisms ranged between 1.98 ± 0.09 and 3.25 ± 0.26, MIC of *Zingiber officinale* extract against all the studied microorganisms varied between 2.76 ± 0.24 and 4.78 ± 0.34, and MIC of *Caryophyllus aromaticus* extract against all the tested microorganisms ranged between 0.83 ± 0.08 and 2.18 ± 0.19. The standard antibiotic levafloxacin had MIC values varying between 0.034 ± 0.004 and 0.092 ± 0.009. The results indicate that standard antibiotic has stronger activity than the plants extracts used in this study (TABLE 1). This higher amount of inhibitory activity of antibiotic may be due to its pure nature whereas in crude extracts of spices, different non-antimicrobial compounds were present. Arora & Kaur[19] reported that the antimicrobial effect of garlic extract was apparent within 1h of incubation. The extract killed 93% of *Staphylococcus epidermis* and *Salmonella typhi* within 3h. Ginger showed very mild inhibitory action against the three pathogenic bacteria *E. coli, B. cerus, S. aereus* and was unable to showed little or no inhibition on different test bacteria. Mint was found to show better antimicrobial properties. It strongly inhibited (2% and 3%) the three food borne pathogens. Among the dry spices, clove was the only spice to show complete bactericidal effect against all three food borne pathogens at 3% concentration. Fabian *et al.,*[26] tested 10% extracts of cinnamon and clove against *B. subtilis* and *S. aureus*. They found cinnamon to be a slight inhibitor, while clove a strong inhibitor at 1:100 and 1:800, respectively.

Recently, Samy[27] used methanolic extracts of ginger which did not present antimicrobial effect against *S. aureus* and *E. coli*. However, Indu et al.[28], using a different method of ginger extract preparation, verified an inhibitory action against *E. coli* as well as high antimicrobial activity of garlic extracts against *E. coli* and *Salmonella*. Ahmad and Aqil[29] concluded that ethanolic extracts of garlic did not have anti-*E. coli* or anti-*Shigella* action. Using another methodology, Vuddhakul et al.[30] observed that garlic extracts inhibited the growth of *V. parahaemolyticus, E. coli* and *S. aureus*; however, lemongrass and ginger extracts did not show any antimicrobial activity. Such behavior of the antibacterial action was also verified by Adonizio et al.[31], who used lemongrass extracts and did not observe antibacterial effects.

Comparisons with pertinent data from literature indicate that, the methodology adopted in studies on antimicrobial activity showed the most diverse results. Plant extracts have shown different inhibitory effect on the growth of the bacteria studied. It is therefore recommended that the nature and number of the active antibacterial principles should be considered during examining each plant extract.

It can be concluded that the results of the present study gives an idea about the antimicrobial activity of clove, garlic, turmeric and ginger against the test strains. These spices act through their natural inhibitory mechanisms either by inhibiting or killing the pathogens completely. With the increasing awareness of people towards natural food and natural therapies, spices might act as the most obvious alternative. In developing countries like India, where spices are produced and used as food additives, their use as antimicrobial agents and potential preservatives can be extremely useful.

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