**Original Research Article****DOI - 10.26479/2018.0403.05****THERAPEUTIC EFFECT OF INDIAN SPICES IN THE TREATMENT OF GASTROINTESTINAL DISEASES CAUSED BY VIBRIO SPECIES****Pooja Singh¹, Shailendra Kumar Tripathi^{2*}**

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ABSTRACT: *Vibrio* causes cholera with other major gastrointestinal disease which is very fatal and in this study we have analyzed whether the medicinal effects of these spices, which are used on daily basis, can minimize the activity of *Vibrio* species. The present study was aimed to assess the therapeutic effect of Indian Spices in the treatment of gastrointestinal disease caused by *Vibrio* species. Total 5 types of spices i.e. cinnamon, black pepper, green cardamom, coriander, and cumin were selected based on the studies and research made on their medicinal values. In order to determine the antimicrobial activity/effect of spices bacteria *Vibrio* and its species were selected i.e. *cholera*, *Parahaemolyticus*, *alginolyticus*. The antibacterial activity of the extracts of all the spices were screened by analyzing the effect on the growth of *Vibrio* species through their zone of inhibition produced. Aqueous extracts of all five spices were obtained using three Solvents ethanol methanol and acetone extraction with the concentration of 70%, 85% and 100%. The liquid portion of extract was collected and rest was discarded. Antibacterial studies were investigated using agar well diffusion method to determine the effect of these spices against the *Vibrio* species. The result showed highest zone of *Vibrio* species inhibition in green cardamom, cumin seed and cinnamon and low zone of inhibition in black pepper and coriander. Hence the study suggests that these all three spices can be used for the treatment of cholera.

KEYWORDS: Antibacterial, Asafoetida, Cinnamon, Green Cardamom, Cumin seed, Turmeric***Corresponding Author: Dr. Shailendra Kumar Tripathi Ph.D.**

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1. INTRODUCTION

India has been recognized all over the world for spices and medicinal plants. Both exhibit a wide range of physiological and pharmacological properties. Our ancestors have recognized the usage of spices in food preservation and in treatment of clinical ailments. The first scientific evidence of the preservation potential of spices, describing antimicrobial activity of cinnamon oil against spores of anthrax bacilli were reported in 1830 [8], [9]. A variety of plant and spice based antimicrobials is used for reducing or eliminating pathogenic microorganisms and increase the shelf life of food. In India, natural herbs and spices are consumed either in food or used as medicine in order to maintain proper sanitation, health and hygiene and to increase longevity of life [17], [19]. Several spices such as ajowan, clove, ginger, black pepper, cumin and asafetida are commonly used in the Indian diet [1], [4], [11]. The spices have a unique aroma and flavor, which are derived from compounds known as phyto-chemicals or secondary metabolites [2], [21]. The phyto-chemicals are antimicrobial substances, which are capable of attracting benefits and repel harmful organisms. Diverse variety of phytochemicals namely isoflavones, anthocyanins and flavonoids are present in spices [3], [13]. In the present study, we have evaluated the antimicrobial effect of the extracts of five widely used spices in India such as Black pepper (*Piper nigrum*), Cumin seed (*Cuminum cyminum*), Coriander (*Coriandrum sativum*), Cinnamon (*Cinnamomum verum*) and Green cardamom (*Elettaria Cardamomum*). The effect of different solvents in the extraction of spices and their antimicrobial activity was also compared. This study will be useful to establish the traditional role of spices as preservatives, antiseptics and disinfectants.

2. MATERIALS AND METHODS

2.1. Experimental work

The antibacterial activity of five well-known and commonly used Indian spices, namely Black pepper (*Piper nigrum*), Cumin seed (*Cuminum cyminum*), Coriander (*Coriandrum sativum*), Cinnamon (*Cinnamomum verum*) and Green cardamom (*Elettaria cardamomum*) was verified against cholera causing *Vibrio cholera* bacterium. All the selected spices were procured from the local market of Lucknow in solid form and brought in laboratory for experiment.

2.2. Preparation of spice extracts

All the spices were first cleaned using tap water in order to remove any dirt or debris, and later using sterile distilled water. They were dried in laminar flow biological safety cabinet. They were crushed in a sterile mortar pestle until a fine powder was obtained. About 50 g of finely powdered spices was weighed separately and extracted with 500 ml solvents each in culture tubes. Ethanol, Methanol and Acetone was used as solvent in different concentrations 70%, 85% and 100% each separately for extraction of spices.

2.3. Isolation of *Vibrio* species

Bacteria isolated from water samples collected from Kurram Nagar (Fish market) of Lucknow. Isolation of *Vibrio* species was done by Spreading pond water over the TCBS media (Thio-citrate Bile Salt Agar) to get the colonies and subculture on TSA media (Traptone Soya Agar) by streaking plate for broth preparation.

2.4. Antibacterial test

The agar well diffusion plate method was used to evaluate their antimicrobial properties of extracted spice. Separate nutrient agar plates of all three extracts ethanol, methanol and acetone were prepared to compare the antimicrobial activity of all three extracts against *Vibrio* species. 200 μ l inoculum of each selected bacterium was uniformly spread on nutrient agar plates with the help of glass spreader. Well of 6mm diameter was bored in the plates after five minutes. 20 μ l of spices extract and standard antibiotic ampicillin were poured into the well separately with the help of sterile syringe in all three plates. The plates were allowed to diffuse in a refrigerator for about 30min, and then incubated for 24hr at 37 $^{\circ}$ C and thereafter plates were observed for the zone of inhibition (mm).

2.5. Statistical analysis

The basic statistics i.e. mean values with standard deviation of zone of inhibition (mm) of all five spices extract were first evaluated and summarized in table-1. One way ANOVA was performed to compare the mean zone of inhibition of all five spice extract. All the statistical analysis was performed on MS-Excel²⁰⁰⁷ and SPSS²⁰ software.

3. RESULTS AND DISCUSSION

In the present study, the antimicrobial activity of the five varieties of spice extracts was examined against *Vibrio cholerae* species in three solvents namely ethanol, methanol and acetone. *Vibrio* species was isolated from water samples collected from Kurram Nagar, fish market of Lucknow cultured on TCBS and TSA media (Figure-1). The mean diameters of the inhibition zones of all spice extracts against *Vibrio cholera* are given in Table-1. Methanol possessed less antimicrobial activities in comparison to ethanol and acetone. The results agree with observations of previous researchers [22], [18].

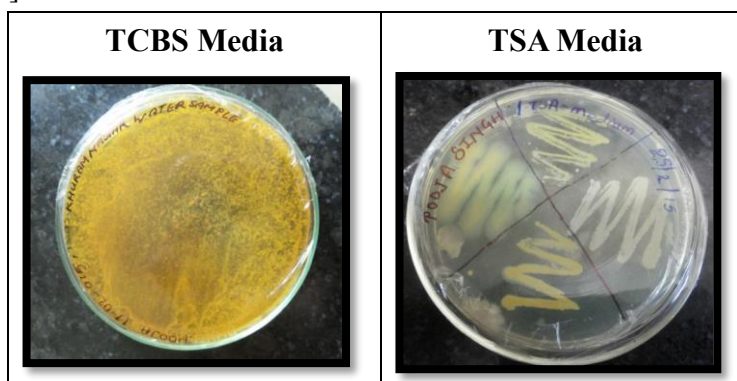


Figure-1: Colony of *Vibrio cholerae* cultured on TCBS & TSA media

The maximum zone of inhibition against *Vibrio cholera* species was exhibited by ethanolic extract (20.67 ± 8.14 mm) and acetonic extract (17.00 ± 6.08 mm) of green cardamom (*Elettaria cardamomum*) as compare to methanolic extract (3.33 ± 0.77 mm) (Figure-2). The antimicrobial activity of green cardamom against both gram-positive and gram-negative bacterial species is demonstrated by previous researcher up to some extent [14], [20].

Table 2: Antimicrobial activity of different spices

Name of Spices	Zone of inhibition in mm (Mean \pm SD)		
	Ethanol	Methanol	Acetone
Green cardamom	20.67 ± 8.14	3.33 ± 0.77	17.00 ± 6.08
Coriander	13.33 ± 5.77	10.00 ± 3.94	3.33 ± 0.67
Cumin seed	18.67 ± 3.21	8.33 ± 1.64	15.00 ± 3.23
Black pepper	7.33 ± 1.52	4.00 ± 0.89	10.00 ± 2.82
Cinemon	15.00 ± 3.66	18.33 ± 3.77	21.67 ± 4.64

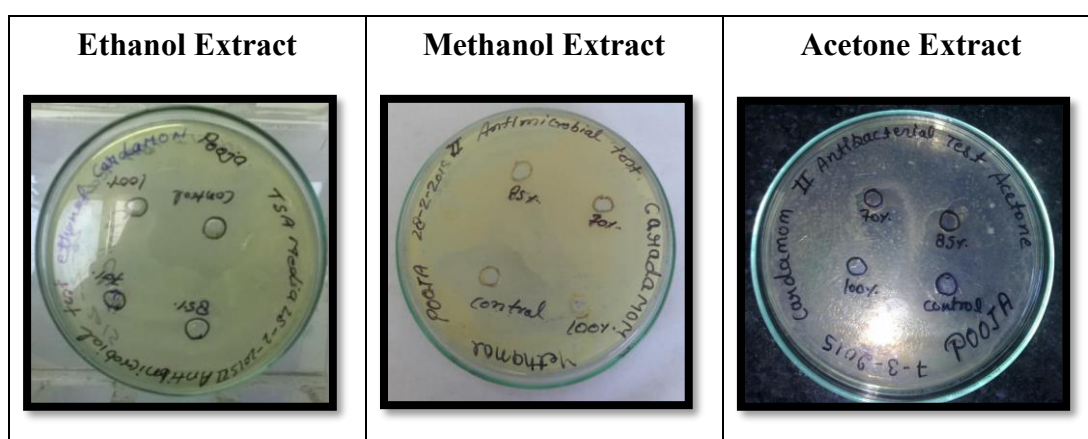


Figure-2: The antibacterial activity of green cardamom against *Vibrio cholera* species

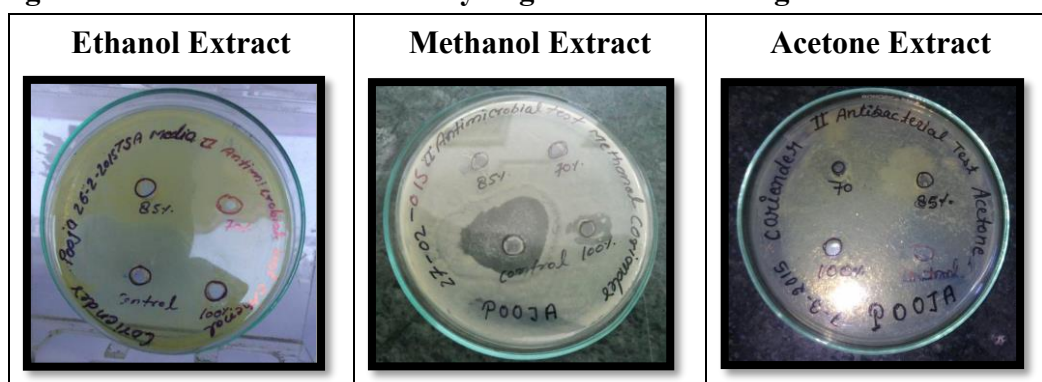


Figure-3: The antibacterial activity of coriander against *Vibrio cholera* species

Coriander (*Coriandrum sativum*) exhibited maximum zone of inhibition against *Vibrio cholera* species by ethanolic extract (13.33 ± 5.77 mm) and methanolic extract (10.00 ± 3.94 mm) in comparison to acetonic extract (3.33 ± 0.67 mm) (Figure-3). The inhibitory effect of *C. sativum* on potential spoilage bacteria, such as *Klebsiella pneumoniae* (*K. pneumoniae*), *Bacillus megaterium*,

Pseudomonas aeruginosa (*P. aeruginosa*), *S. aureus*, *Escherichia coli* (*E. coli*), *Escherichia cloaca*, *Enterococcus faecalis*, has been reported by many researchers [6], [12], [15], [16].

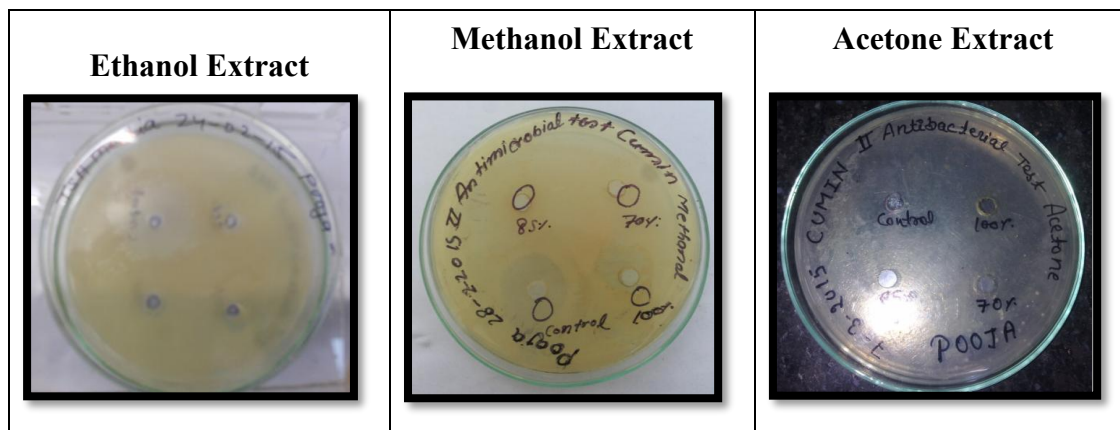


Figure-4: The antibacterial activity of cumin seed against *Vibrio cholera* species

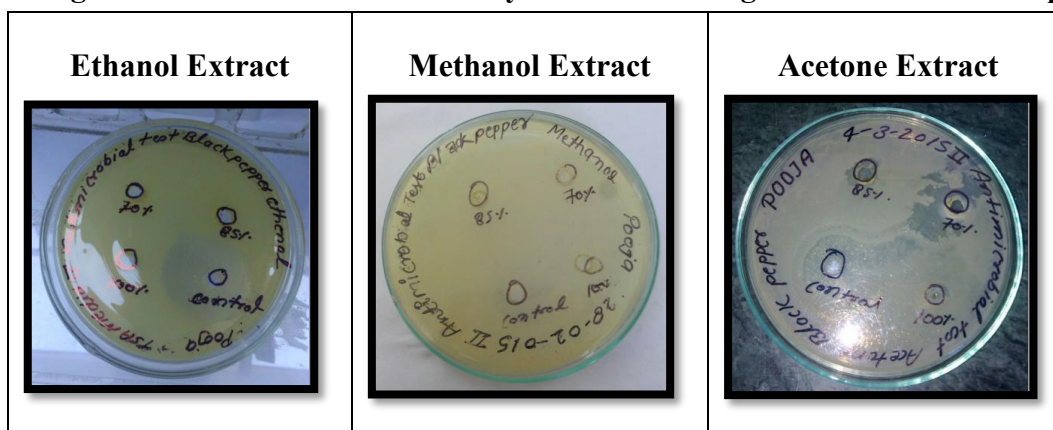


Figure-5: The antibacterial activity of black pepper against *Vibrio cholera* species

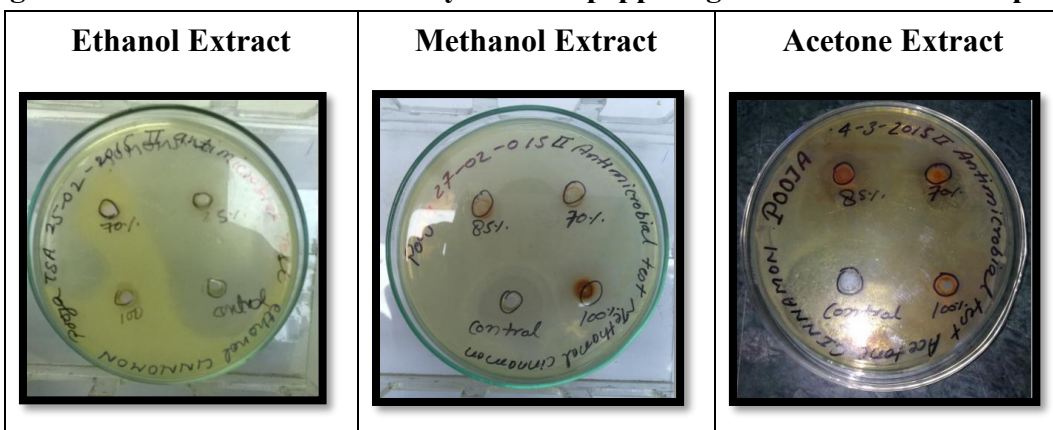


Figure-6: The antibacterial activity of cinemon against *Vibrio cholera* species

The acetonic extract ($10.00 \pm 2.82\text{mm}$) of black pepper (*Piper nigrum*) exhibited maximum zone of inhibition against *Vibrio cholera* as compare to other two solvents ethanolic extract ($7.33 \pm 1.52\text{mm}$) and methanolic extract ($4.00 \pm 0.89\text{mm}$) (Figure-5). Similar higher inhibition of growth against infection causing gram positive and gram negative bacteria was observed by other researchers also. The maximum zone of inhibition against *Vibrio cholera* species was exhibited by ethanolic extract.

(18.67 ± 3.21 mm) and acetic extract (15.00 ± 3.23 mm) of cumin seed (*Cuminum cyminum*) as compare to methanolic extract (8.33 ± 1.64 mm) (Figure-4). Other researchers have also reported the similar type of antibacterial activity of cumin seed against several microorganisms [7].

Acetic extract of cinamon (*Cinnamomum verum*) exhibited maximum zone of inhibition (21.67 ± 4.64 mm) against *Vibrio cholera* followed by methanolic extract (18.33 ± 3.77 mm) and ethanolic extract (15.00 ± 3.66 mm) (Figure-6). The Gram-positive bacteria were more sensitive to the antimicrobial compounds present in cinamon. Phenols, alcohols, aldehydes, ketones, ethers and hydrocarbons have been recognized as major antimicrobial components in Indian spices [5], [10]. The result of one way ANOVA performed using SPSS₂₀ version reveled significant variation ($p < 0.05$) between mean zone of inhibition of all five spice samples extracted in three different solvents i.e. ethanol, methanol and acetone.

4. CONCLUSION

The results of present study confirm that green cardamom (*Elettaria cardamomum*), cumin seed (*Cuminum cuminum*) and cinnamon (*Cinnamomum verum*) possessed higher antimicrobial activity against *Vibrio cholera* microbes, whereas lower antibacterial activity was exhibited by black pepper and coriander. *Vibrio cholera* causes cholera a gastrointestinal disease. These spices act through their natural inhibitory mechanisms by either inhibiting or killing the pathogens completely. The antimicrobial activity of spices varies widely, depending on the type of spices, culture medium and microorganism. 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. This type of study opens up the possibility for the search of new antimicrobials as an alternative to the antibiotics.

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