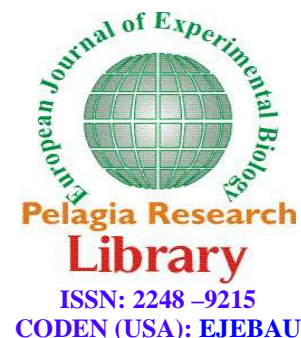




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Antibacterial activity of spices against *Vibrio* species isolated from pond water

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ABSTRACT

There are various human disease caused by *Vibrio* species which are pathogenic in nature these diseases are mainly water-borne. The study "Antibacterial Activity of Spices against *Vibrio* species isolated from Pond water" aims to identify role of certain spices which have significant activity against *Vibrio* species. Members of the genus *Vibrio* are defined as Gram-negative, asporogenous rods that are straight or have a single, rigid curve. They are motile; most have a single polar flagellum, when developed in liquid medium. Most produce oxidase and catalase, and ferment glucose without producing gas. Three species *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*, are well-documented human pathogens. *Vibrio* species account for a significant proportion of human infections from the consumption of raw or undercooked shellfish. The spices Black pepper (*Piper nigrum*) Cumin seed (*Cuminum cyminum*) Coriander (*Coriandrum sativum*) Cinnamon (*Cinnamomum verum*) and Green cardamom (*Elettaria cardamomum*) against epidemic strains of *V. parahaemolyticus*. Aqueous extracts of all five spices were obtained using three Solvents ethanol, methanol & acetone at a concentration of 70%, 85% and 100%. Antibacterial studies were investigated using agar well diffusion method to determine the effect of these spices against the *Vibrio* species.

Keywords: *Vibrio cholera*, *Vibrio parahaemolyticus*, Antibacterial, Cumin, Green cardamom, Cinnamon, Dried ginger, Coriander.

INTRODUCTION

One the past two to three decades many beneficial effects of the common food spices on the health have been understood. There are also new concerns about food safety due to increasing occurrence of new food-borne disease outbreaks caused by pathogenic micro-organisms. This raises considerable challenges, particularly since there is increasing unease regarding the use of chemical preservatives and artificial antimicrobial to inactivate or inhibit growth of spoilage and pathogenic micro-organism [1]. Spices can be added to foods in several forms: as whole spices, as ground spices, or isolates from their extracts. Spices are some of the most commonly used natural antimicrobial agents in foods. Addition of spices in foods not only imparts flavor and pungent stimuli but also provides antimicrobial property [2].

Spices are aromatic and pungent food ingredients, like herbs, spices, can have significant anti-oxidative effect [3]. Total equivalent antioxidant capacities and phenolic contents (Folin-Ciocalteu) of 32 spices was measured. Spices can also have antibacterial effects. Out of 46 spice extracts evaluated, many exhibited antibacterial activity against

food borne pathogens. Gram-positives bacteria were generally more sensitive than Gram-negative bacteria resistant. The antibacterial activity of the extracts was closely associated with their phenolic content [4]. Natural antimicrobial compounds in spices were found to possess antimicrobial activity. Although some researchers have studied the antibacterial activity of spices against several species of bacteria, few serotypes of salmonella have been tested. In addition, the antimicrobial property of spices against may differ depending on the forms of spices added, such as fresh, dried, or extracted forms. Spices are some of the most commonly used natural antimicrobial agents in foods. Addition of spices in foods not only imparts flavor and pungent stimuli but also provides antimicrobial property [5]. Natural antimicrobial compounds in spices were found to possess antimicrobial activity [6]. Although some researchers have studied the antimicrobial activity of spices against numerous species of bacteria. In India, spices have been usually used since ancient times, for the preservation of food products as they have been reported to have antiseptic and disinfectant properties. In this respects, a preface screening for antimicrobial activities of 35 different Indian spices has been carried out. Of the spices surveyed, the results indicate that clove, cinnamon, bishop's weed, chilli, horse raddish, cumin, tamarind, black cumin, pomegranate seeds, nutmeg, garlic, onion, tejpat, celery cambodge have been potent antimicrobial activities against the test organism *Bacillus subtilis*, *Escherichia coli* and *Saccharomyces cerevisiae*. The results also establish the traditional use of spices as food preservatives, disinfectants and antiseptic.

India has been recognized all over the world for spices and medicinal plants. Both exhibit a wide range of physiological and pharmacological properties. Current biomedical efforts are focused on their scientific merits, to provide science-based evidence for the traditional uses and to develop either functional foods or nutraceuticals. The Indian traditional medical systems use turmeric for wound healing, rheumatic disorders, gastrointestinal symptoms, de worming, and rhinitis and as a cosmetic. Studies in India have explored its anti-inflammatory, cholekinetic and anti-oxidant potentials with the recent investigation focusing on its preventive effect on pre-carcinogenic, anti-inflammatory and anti atherosclerotic effects in biological system both under in vitro and in vivo condition in animals and humans. Both turmeric and cur cumin were found to increase detoxifying enzymes, prevent DNA damage, improve DNA repair, decrease mutation and tumor formation and exhibit anti-oxidative potential in animals. Limited clinical studies suggest that turmeric can significantly impact excretion of mutagens in urine in smokers and regress precancerous palatal lesions. It reduces DNA adducts and micronuclei in oral in oral epithelial cells. It prevents formation of nitroso compounds both in vivo and in vitro. It delays induced cataract in diabetes and reduces hyperlipidemia in obese rats. Recently several molecular targets have been identified for therapeutic/preventive effects of turmeric. Fenugreek seeds, a rich source of soluble fiber used in Indian cuisine reduces blood glucose and lipids and can be used as a food adjuvant in diabetes.

MATERIALS AND METHODS

Isolation of Bacteria- Bacteria isolated from pond water sample. Water samples were collected from different areas of Lucknow after that isolation of *Vibrio* species was done by Spreading pond water over the TCBS media to get the colonies and subculture on TSA media by Streaking plate for broth preparation,.

Collection of Spices - Required selected samples of spices were collected from local market of Lucknow, (U.P.) India area in solid form and brought in laboratory and they were:- Black pepper (*Piper nigrum*), Cumin seed (*Cuminum cyminum*), Coriander (*Coriandrum sativum*), Cinnamon (*Cinnamomum verum*) and Green cardamom (*Elettaria cardamomum*).

Preparation of Extract of Spices- The spices were purchased from the local market Black pepper (*Piper nigrum*), Cumin seed (*Cuminum cyminum*), Coriander (*Coriandrum sativum*), Cinnamon (*Cinnamomum verum*) and Green cardamom (*Elettaria cardamomum*). About 30 g of finely powdered spices was weighed separately and extracted with 300 ml solvents each in culture tubes. The spice materials were extracted by Ethanol Methanol and Acetone solvents at different concentration of 70%, 85% and 100% each separately.

Evaluation of Antimicrobial Activity (Agar well diffusion method)- The antimicrobial analysis method was performed to evaluate the antimicrobial properties of indian spices: *Piper nigrum*, *Cuminum cyminum*, *Coriandrum sativum*, *Cinnamomum verum* and *Elettaria cardamomum* with the help of the agar well diffusion plate method. Nutrient agar plates were prepared to evaluate the Antimicrobial Activity of ethanol extract, methanol extract & acetone extract against selected *Vibrio* species. 200µl inoculums of each selected bacterium was uniformly spread on nutrient agar plates with the help of glass spreader, after five minutes 6mm diameter well was bored in the plates.

20µl of spices extract and standard antibiotic ampicillin were poured into the well with the help of sterile syringe. The plates were allowed to diffuse in a refrigerator for about 30min and then transferred to bacterial incubator. The plates were incubated at 37°C for 24hr. and after incubation plates were observed for the zone of inhibition (mm).

RESULTS AND DISCUSSION

Isolation of *Vibrio* species from Fresh water sample- Isolation of *Vibrio* species from fresh water was done on selective TCBS media by Spread plate method having various yellow *Vibrio* colonies.

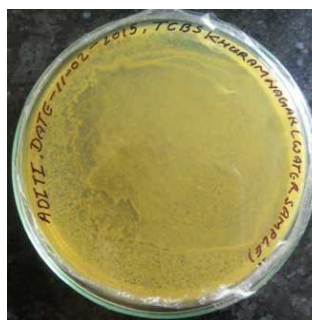


Fig 1. Colonies of *Vibrio* species (Yellow colonies) grown on TCBS media



Fig 2: Colonies of *Vibrio cholera* cultured on TSA media

Inoculation of *Vibrio* colony to Broth media- After isolation of *Vibrio* species bacteria on TSA media inoculated in TSA broth for broth preparation.

Extract preparation- Extract preparation of spices on Ethanol, Methanol & Acetone in 70%, 85% & 100%.

Well diffusion (zone of inhibition) for Indian Spices extraction against *Vibrio* species- For the antibacterial susceptibility test of 5 Indian Spices *P. nigrum*, *C. cyminum*, *C. sativum*, *C. verum*, *E. cardamomum* against *Vibrio* species were done by well diffusion plate method. Black pepper (*Piper nigrum*) was checked against *Vibrio* species and inhibitory effect was seen on TSA media. A maximum inhibitory zone on 100% ethanol and methanol extract were 15 mm and minimum at 70% ethanol extract of spices was 8 mm.

Antibacterial activity of cumin seed (*Cuminum cyminum*) was checked on *Vibrio* species and inhibitory effect was seen on TSA media. A maximum inhibitory zone on 85% ethanol extract was observed to be 15 mm and minimum at 100% ethanol, methanol, acetone extracts were 10 mm inhibition zone. Coriander (*Coriandrum sativum*) antibacterial activity was checked on against *Vibrio* species and inhibitory effect was seen. Maximum inhibitory zone was seen in 85% ethanol extract which was 35 mm and minimum at 70% ethanol extract which was 7 mm.

Antibacterial activity of Cinnamon (*Cinnamomum verum*) was checked which showed an inhibitory effect against *Vibrio* species. A maximum inhibitory zone was observed on 85% as well as 100% acetone extract giving a 25 mm inhibitory zone and minimum at 70% ethanol extract (7mm).

Green Cardamom (*Elettaria cardamomum*) also showed inhibitory effect against *Vibrio* species but to susceptible range, thus a very significant effect. Maximum inhibitory zone on 100% ethanol extract (50 mm) and minimum at 70% methanol as well as acetone extract (10 mm). Our result showed similarity with **Praveen *et al.*, 2013** result with 70% conc. of all solvents.

S.No	Solvents	<i>P. nigrum</i>	<i>C. cyminum</i>	<i>C. sativum</i>	<i>C. verum</i>	<i>E. cardamomum</i>	
1	Methanol	70%	-	10	-	10	
		85%	-	10	10	15	
		100%	15	10	15	10	
		+ve control	35	40	30	30	40
2	Ethanol	70%	8	10	7	7	15
		85%	6	12	35	12	10
		100%	15	10	30	15	50
		+ve control	30	30	30	30	30
3	Acetone	70%	10	-	-	20	10
		85%	20	15	15	25	-
		100%	-	10	10	25	-
		+ve control	45	30	35	30	35

- = absent, +ve control=Amoxycillin antibiotic



Fig 3. The antibacterial activity of black pepper against vibrio cholera (a)Ethanol (b)Methanol (c)Acetone extract



Fig 4. The antibacterial activity of Cumin seed against Vibrio species (a) Ethanol (b) Methanol (c) Acetone extract

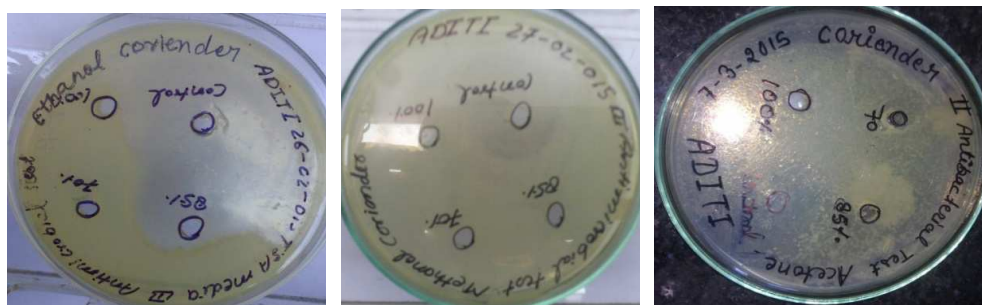


Fig 5. The antibacterial activity of Coriander against Vibrio species Ethanol (b) Methanol (c) Acetone extract



Fig 6. The antibacterial activity of Cinnamon against *Vibrio* species (a) Ethanol (b) Methanol (c) Acetone extract



Fig 7. The antibacterial activity of Green cardamom against *Vibrio* species (a) Ethanol (b) Methanol (c) Acetone extract

CONCLUSION

On the basis of experimental result, it was concluded that are extract which are obtain from different Indian spices - *Cuminum cyminum*, *Elettaria cardamomum*, *Corianderum sativum* *Piper nigrum* and *Cinnamomum verum* show inhibitory effect on the *Vibrio* species which cause gastrointestinal diseases. Antimicrobial studies were investigated using agar well diffusion method to determine the effect of these spices against the *Vibrio* species. Black pepper (*Piper nigrum*) showed maximum antibacterial activity zone on 100% ethanol and methanol extract and minimum at 70% ethanol extract of spices. Coriander (*Coriandrum sativum*) showed maximum antibacterial activity zone on 85% ethanol extract and minimum at 70% ethanol extract. Cinnamon (*Cinnamomum verum*) showed maximum antibacterial activity zone was observed on 85% as well as 100% acetone extract and minimum at 70% ethanol. Green Cardamom (*Elettaria cardamomum*) also showed a very significant effect against *Vibrio* species. Maximum inhibitory zone on 100% ethanol and minimum at 70% methanol as well as acetone. Thus these spices possess potential bioactive compounds which could be further isolated and identified by advanced analytical techniques. And these compounds can be used to curb the growth of bacterial species including vibrio.

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