➔DOI : 10.15740/HAS/AJBS/11.1/12-16
e ISSN-0976-8343 | ■ Visit us : *www.researchjournal.co.in* ■ – AFBSAH-2016 ASIAN JOURNAL OF BIO SCIENCE
Volume **11** | Issue 1 | April, 2016 | 12-16

RESEARCH **P**APER

To study the therapeutic role of Indian spices in the treatment of gastrointestinal disease caused by *Vibrio* species

ARADHANA IRENE CHARAN, AMIT ALEXANDER CHARAN, VINEETA GURNANI **AND** PRATEEK SINGH

Department of Molecular and Cellular Engineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences, ALLAHABAD (U.P.) INDIA
Email : aradhanairenecharan@gmail.com

Article Info : Received : 05.12.2015; Accepted : 5.02.2016

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. In project named, "To study the therapeutic role of Indian spices in the treatment of gastrointestinal disease caused by *Vibrio* species" five of the spices were selected. They were *Hing* (*Ferula assa*), *Jeera* (*Cuminum cyminum*), black pepper (*Piper nigrum*), *Saunf* (*Foeniculum vulgare*), black mustard (*Brassica nigra*) 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. 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 effects of all five spices were obtained using two solvents ethanol and methanol extraction with the concentration of 85 per cent and 100 per cent. 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 study confirmed the antioxidant activity and property of spices extracted.

Key words : *Vibrio*, *Ferula assa*, *Cuminum cyminum*, *Piper nigrum*, *Foeniculum vulgare*, *Brassica nigr*a, *Syzygium aromaticum*

How to cite this paper : Charan, Aradhana Irene, Charan, Amit Alexander, Gurnani, Vineeta and Singh, Prateek (2016). To study the therapeutic role of Indian spices in the treatment of gastrointestinal disease caused by *Vibrio* species. *Asian J. Bio. Sci.*, **11** (1) : 12-16 [Special Issue of AFBSAH-2016].

INTRODUCTION

Over the past two or 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 diseases outbreaks caused by pathogenic micro-organisms. This raises considerable challenges, particularly since there is increasing unease regarding the use of chemicals preservatives and artificial antimicrobial to inactivate or inhibit growth of spoilage and pathogenic micro-organism (Arques *et al.*, 2008). Spices can be added to foods in several forms: as whole spices, as grounded spices, or isolates from their extracts. Spices are some of the most commonly used natural antimicrobial agents in food. Addition of spices in foods not only imparts flavour and pungent stimuli but also provides antimicrobial property (Hirasa and Takemasa, 1998). Spices are aromatic and pungent food ingredients, like herbs, spices can have significant antioxidative effect (Suhaj, 2006). Total equivalent capacities and phenolic contents of 32 spices were measured (Wojdylo *et al.*, 2007). Spices can also have antibacterial effects. Out of 46 spices evaluated, many exhibited antibacterial activity

against food borne pathogens. Gram positives bacteria were generally more sensitive than gram negative resistant bacteria. The antibacterial activity of the extracts was closely associated with their phenolic content (Shan *et al.,* 2007). Natural antimicrobial compounds in spices were found to possess antimicrobial activity. Although some researchers have studied the antibacterial activities of spices against several species of bacteria, few serotypes of salmonella have been tested. In addition, the antimicrobial property of species against may differ depending on the forms of spices added, such as fresh, dried, or extracted forms. Spices are some most commonly used natural antimicrobial agents in foods. Addition of spices in foods not only imparts flavour and pungent stimuli but also provides antimicrobial property (Nevas *et al.,* 2004). Natural antimicrobial compounds in spices were found to possess antimicrobial activity (Kim *et al.,* 2004).

RESEARCH METHODOLOGY

Collection of sample:

Required selected samples of spices were collected from local market of Lucknow in solid form and brought in laboratory. They were: asafoetida (*Ferula assa*), cumin (*Cuminum cyminum*), black pepper (*Piper nigrum*), fennel (*Foeniculum vulgare*), black mustard (*Brassica nigra*) and clove (*Syzygium aromaticum*).

Preparation of extract of spices :

As the polar methanol and ethanol were selected in 85 per cent and 100 per cent concentration. The spices were grounded into powder in a laboratory grinder and sieved into fine powder to be used for extraction. The spice materials were extracted by ethanol and methanol solvents. These solvents were used at a concentration of 85 per cent and 100 per cent separately. About 1g of finely powered spices was weighed separately and extracted with solvents in culture tubes. These spices containing culture tubes were kept in dark place for 24-48 hours at room temperature. The extracts were obtained by filtration using muslin cloth. The perquisite amounts of extracts were stored and kept in eppendorf tubes for its further storage.

Antibacterial test (Agar well diffusion test) :

Firstly, TSA media was prepared and autoclave along with the Petri plates at 15psi and 121^{0}C. Secondly, pouring was done in the Petri plates and allowed to solidify for half an hour. 200 micro litre of each bacterial species was poured in the separate Petri plates and spreading was done using an L shaped glass rod. Wells were prepared with the help of tips and was marked methanol, ethanol and antibiotic (Ampicillin solution at 1000 ppm). For each spices four plates were prepared with each *Vibrio* species in each 20 micro litre of spices extract was poured on the marked well and the solvent was prepared along with the antibiotic. It was kept in incubator for 24 hours to view the bacterial growth and zone of inhibition.

RESEARCH FINDINGS AND ANALYSIS

The spice extracts of asafoetida (*Ferula assa*), cumin (*Cuminum cyminum*), black pepper (*Piper nigrum*), fennel (*Foeniculum vulgare*), black mustard (*Brassica nigra*) and clove (*Syzygium aromaticum*) showed a significant level of antimicrobial activity against *Vibrio cholerae*. The extracts of the spices were prepared in methanol, ethanol. The study also showed that the effect of the spices extracts on above species varied. The concentrations used during the study were 85 per cent and 100 per cent.

Results for clove (*Syzygium aromaticum*) :

The following figures and tables are showing antibacterial activity of clove against ethanol, methanol and antimicrobial solution (yellow and green colonies) (Fig. 1 and 2; Table 1 and 2).

Fig. 1: The antibacterial activity of clove against ethanol, methanol and antimicrobial solution (yellow and green colonies)

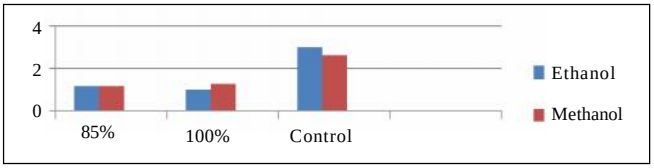

Fig. 2: The antibacterial activity of clove against ethanol, methanol and antimicrobial solution

Table 1: Clove ethanol solvent		
Sr. No.	Percentage of solvent	Zone of inhibition
1.	85%	1.2 cm
2.	100%	1 cm
3	Control	3 cm

Table 2: Clove methanol solvent		
Sr. No.	Percentage of solvent	Zone of inhibition
1.	85%	1.2 cm
2.	100%	1.3 cm
3.	Control	2.6 cm

Results for cumin (*Cuminum cyminum*) :

The following figures and tables are showing antibacterial activity of cumin against ethanol, methanol and antimicrobial solution (Fig. 3 and 4; Table 3 and 4).

Fig. 3: The antibacterial activity of cumin against ethanol, methanol and antimicrobial solution

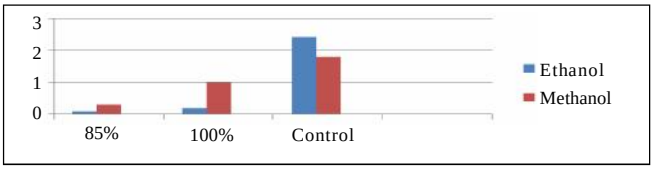

Fig. 4: The antibacterial activity of cumin against ethanol, methanol and antimicrobial solution

Table 3: Cumin ethanol solvent		
Sr. No.	Percentage of solvent	Zone of inhibition
1.	85%	0.1 cm
2.	100%	0.2 cm
3.	Control	2.4 cm

Table 4: Cumin methanol solvent		
Sr. No.	Percentage of solvent	Zone of inhibition
1.	85%	0.3 cm
2.	100%	1 cm
3.	Control	3.4 cm

Results for asafoetida (*Ferula assa*) :

The following figures and tables are showing antibacterial activity of asafoetida against ethanol, methanol and antimicrobial solution (Fig. 5 and 6; Table 5 and 6).

Fig. 5: The antibacterial activity of asafoetida against ethanol, methanol and antimicrobial solution

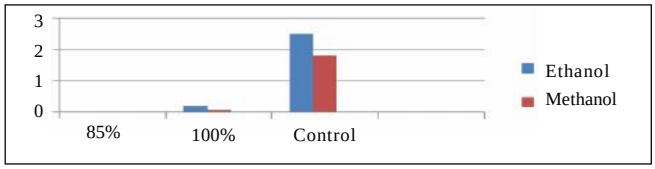

Fig. 6: The antibacterial activity of asafoetida against ethanol, methanol and antimicrobial solution

Table 5: Asafoetida ethanol solvent		
Sr. No.	Percentage of solvent	Zone of inhibition
1.	85%	-
2.	100%	0.2 cm
3.	Control	2.5 cm

Table 6: Asafoetida methanol solvent		
Sr. No.	Percentage of solvent	Zone of inhibition
1.	85%	-
2.	100%	0.1 cm
3.	Control	3.3 cm

Results for mustard (*Sinapis alba*) :

The following figures and tables are showing antibacterial activity of mustard against ethanol, methanol and antimicrobial solution (yellow and green colonies) (Fig. 7 and 8; Table 7 and 8).

Fig. 7: The antibacterial activity of mustard against ethanol, methanol and antimicrobial solution (yellow and green colonies)

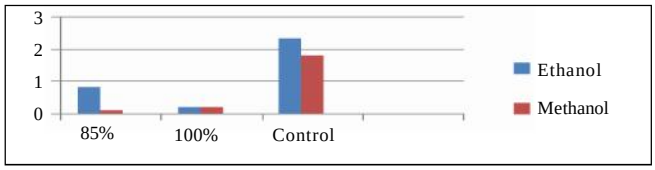

Fig. 8: The antibacterial activity of mustard against ethanol, methanol and antimicrobial solution

Table 7 : Mustard ethanol solvent		
Sr. No.	Percentage of solvent	Zone of inhibition
1.	85%	0.8 cm
2.	100%	0.2 cm
3.	Control	2.3 cm

Table 8: Mustard methanol solvent		
Sr. No.	Percentage of solvent	Zone of inhibition
1.	85%	0.1 cm
2.	100%	0.2 cm
3.	Control	2.4 cm

Results for black pepper (*Piper nigrum*) :

The following figures and tables are showing antibacterial activity of black pepper against ethanol, methanol and antimicrobial solution (Fig. 9 and 10; Table 9 and 10).

Fig. 9: The antibacterial activity of black pepper against ethanol, methanol and antimicrobial solution

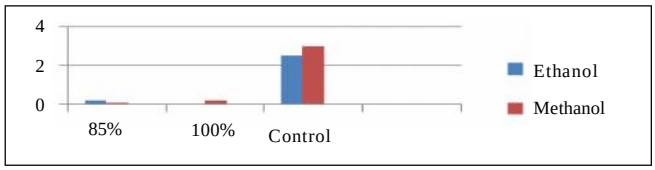

Fig. 10: The antibacterial activity of black pepper against ethanol, methanol and antimicrobial solution

Table 9: Black pepper ethanol solvent		
Sr. No.	Percentage of solvent	Zone of inhibition
1.	85%	0.2 cm
2.	100%	0 cm
3.	Control	2.5 cm

Table 10: Black pepper methanol solvent		
Sr. No.	Percentage of solvent	Zone of inhibition
1.	85%	0.1 cm
2.	100%	0.2 cm
3.	Control	3.0 cm

Results for fennel (*Foeniculum vulgare*) :

The following figures and tables are showing antibacterial activity of fennel against ethanol, methanol and antimicrobial solution (Fig. 11 and 12; Table 11 and 12).

Fig. 11: The antibacterial activity of fennel against ethanol, methanol and antimicrobial solution

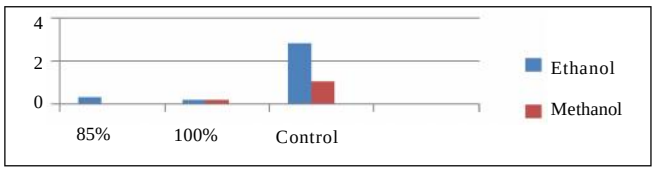

Fig. 12: The antibacterial activity of fennel against ethanol, methanol and antimicrobial solution

Table 11: Fennel ethanol solvent		
Sr. No.	Percentage of solvent	Zone of inhibition
1.	85%	0.3 cm
2.	100%	0.2 cm
3.	Control	2.8 cm

Table 12: Fennel methanol solvent		
Sr. No.	Percentage of solvent	Zone of inhibition
1.	85%	0 cm
2.	100%	0.2 cm
3.	Control	1.0 cm

Conclusion :

Vibrio parahaemolyticus and *Vibrio cholera* can cause large epidemic of cholera with high morality. This study investigated the antibacterial properties of extracts of black pepper, fennel, clove, asafoetida, cumin and mustard against epidemic strains of *Vibrio parahaemolyticus*. Aqueous extracts of all six spices were obtained using two solvents ethanol and methanol extraction with the concentration of 85 per cent and 100 per cent. 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. As in a time now everyone's major concern is health and to be fit with much more being explosive each one knows about the usage of antibiotics and its effects and side effects. So people are much more going into using herbal products that are easily available in order to eradicate their problem. Antioxidant property assessment may require a combination of different method and the result in this study confirmed the antioxidant activity and property of spices extracted.

LITERATURE CITED

Arques, J.L., Rodriguez, E., Nunez, M. and Medina, M. (2008). Inactivation of gram negative pathogens in refrigerated milk by reuterin in combination with nisin or the lactoperoxidase system. *European Food Res. & Technol.,* **227** (1): 77-82.

Hirasa, K. and Takemasa, M. (1998). *Spice science and technology.* Marcel Dekker Inc., NEW YORK, U.S.A.

Kim, H.O., Park, S.W. and Park, H.D. (2004). Inactivation of *E. coli* O157:H7 by cinnamon aldehyde purified from Cinnamon mum cassia shoot. *Food Microbiol*., **21**: 105-110.

Nevas, M., Korhonen, A.R., Lindtrom, M., Turkki, P. and Korkeala, H. (2004). Antibacterial efficiency of Finnish spice essential oils against pathogenic and spoilage bacteria. *J. Food Protec.,* **67**(1): 199-202.

Shan, B., Cai, Y.Z., Brooks, J.D. and Corke, H. (2007). The *in vitro* antibacterial activity of dietary spice and medicinal herb extracts. *Internat. J. Food Microbiol.,* **117** (1) : 112-119.

Suhaj, M. (2006). Spice antioxidants and their antiradical activity. *J. Food Compound Anal.,* **19**(6-7) : 531-537.

Wojdylo, A., Oszmianski, J. and Czemerys, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem.,* **105**(3): 940-949.