ANTIBACTERIAL ACTIVITY OF CAROM SEED/ THYME (TRACHYSPERMUMAMMI), ANISE (PIMPINELLAANISUM) AND DRY GINGER (ZINGIBER OFFICINAL) AGAINST V. CHOLERA, V. PARAHAEOMOLYTICUS AND V. VULNIFICUS

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ABSTRACT
This study aims to highlight the antibacterial property of three common spices which are- carom seed (Trachyspermumammi), anise (Pimpinellaanisum) and dry ginger (Zingiber officinal) against epidemic strains of V. parahaemolyticus, V. cholera and V. vulnificus. Aqueous extracts of all three spices were obtained using solvents methanol, water and acetone. Antimicrobial effects of acetone extracts of Anise (Pimpinellaanisum) was observed to be highest against Vibrio cholera (20 ± 0.22 mm). The acetone extract of Dry ginger (Z. officinale) demonstrated moderate antibacterial activity (23 ± 0.35), and water extract showed strong inhibitory effects (30 ± 0.34) against Vibrio parahaemolyticus. A similar strong inhibitory effect was seen in acetone (30 ± 0.30) and water extract (30 ± 0.34) of thyme (Trachyspermumammi) against Vibrio cholera and Vibrio vulnificus respectively.

KEYWORDS: Antibacterial activity, thyme, anise and dry ginger, zone of inhibition, therapeutic.

INTRODUCTION
Since Vibrio cholerae is autochthonous to the aquatic environment (Colwell and Spira.1992, West and Lee, 1982; Xu et al., 1982), monitoring this bacterium in water sources is important for control of cholera. A selective medium, such as thiosulfate citrate bile salts sucrose (TCBS) agar, eliminates most nontarget bacteria in clinical samples but is not satisfactory for
environmental samples because many bacteria present in natural water sources can produce colonies on TCBS agar whose appearance is similar to the appearance of V. cholerae colonies. Furthermore, the series of biochemical tests commonly used to identify V. cholerae (Baumann and Schubert, 1984; Farmer et al., 1985; Kay et al., 1994; West and Colwell, 1983) was originally designed for clinical samples in order to specifically detect pathogenic vibrios. Molecular methods, including PCR and DNA-DNA hybridization performed with probes specific for V. cholerae, provide more reliable identification (Chun et al., 1995; Nandi et al., 2000).

The beneficial health effects of many plants, used for centuries as seasoning agents in food and beverages, have been claimed for not only preventing food deterioration but also acting as antimicrobials against pathogenic microorganisms. A few studies have been carried out in a systematic manner, although phytochemical and pharmacological investigations of several plants have already led to the isolation of some of the natural antimicrobials (Iwu et al., 1999). Scientists are searching for natural products that can be used in large scale to reduce diarrhoea caused by vibrios. Some natural compounds have been examined to act against bacterial growth whereas little is known about specific influence on their virulence regulation (Shinji et al., 2011). Plant-derived drugs remain an important resource, especially in developing countries, to combat serious diseases. Some plants contain bioactive compounds such as glycosides, alkaloids and terpenes which may be used as drugs and antimicrobial agents (Kurita et al., 1982). Many extracts and essential oils have been derived from plants and found to have antibacterial, fungicidal and insecticidal properties (Hänsel et al., 1999).

The essential oil of thyme (Thymus vulgaris) is utilized as a flavour enhancer in a wide variety of foods, beverages, confectionery products and in perfumery for the scenting of soaps and lotions (Arora and Kaur, 1999). It possesses some antiseptic, bronchiolytic, antispasmodic and antimicrobial properties that make it popular as a medicinal herb and as a preservative for foods Briozzo et al; Cosentino et al., 1999). The therapeutic potential of thyme rests on its contents of flavonoids, thymol, eugenol, aliphatic phenols as well as saponins, luteolin and tetramethoxylated flavones (Deans and Ritchie, 1987; Dorman and Deans, 2000).

Pimpinella anisum (aniseed) is an herbaceous and the composition of anise varies considerably with foundation and farming method. The main constituents of aniseed are protein(18%),fatty oil( 8-23%) ,essential oil( 2-7%), starch( 5%),N-free extract( 22-28%) and
crude fibre (12-25%). (Pruthi, 1976; Gerard, 1997) Aniseed also contains anethole, a phytoestrogen so aniseed has been used for treatment of menstrual cramps (Albert-Puleo, 1980). Pimpinella anisum oil exhibited in vitro strong inhibitory activities against the growth of a wide spectrum of bacteria and fungi known to be pathogenic for man and other species (Muller-Schwarze, 2006; Elgayyar et al., 2001).

Ginger (Zingiber officinale), a member of the Zingiberaceae family, is a well-known spice used in the daily diet in many Asian countries (Demin and Yingying, 2010). It is a rhizomatous plant grown throughout South-eastern Asia, China and in parts of Japan, Austria, Latin America, Jamaica and Africa. It has been used as a spice and medicine in India and China since ancient times. It was known in Germany and France in the ninth century and in England in 10th century for its medicinal properties (Sasidharan and Menon, 2010). Over three quarters of the world population still rely on plants and plant extracts for health care (Sasidharan and Menon, 2010). Ginger compounds are active against specific type of diarrhea which is leading to cause death in infant in developing countries. Moreover, it has been found that ginger is effective in treating nausea caused by sea sickness, morning sickness and chemotherapy, though it was found superior over a place for post operative nausea (Sebiomo et al., 2011). In addition, it has been reported that the main ingredients of ginger like volatile oil, gingerol, shogaol and diarylheptanoids work as antioxidant, anti-inflammatory, anti-lipid, anti-diabetic, analgesic, antipyretic and anti-tumor (Demin and Yingying, 2010; Sasidharan and Menon, 2010; Sebiomo et al., 2011; Lee et al., 1986; Penna et al., 2003; Kadnur and Goyal, 2005; Islam and Choi, 2008; Kim et al., 2008; Isa et al., 2008; Wang et al., 2009; Shim et al., 2011).

MATERIALS AND METHOD
Preparation of spice extract
Three spices namely thyme (Trachyspermum ammi), anise (Pimpinella anisum) and dry ginger (Zingiber officinalis) were collected from vendors in Lucknow. Extraction was done at 10% (w/v) of spices and solvents (RANKEM Chemicals) for 48 hours, the extract were filtered using cheese cloth and filtrate was used for further work. The solvents used for extract preparation were acetone, methanol and distilled water.
Maintenance of bacterial culture
The stock culture of the identified three vibrio species (*V. cholera, V. parahaemolyticus and V. vulnificus*) in previous part of this work (*Shukla and Shankar, 2016*) was maintained at 4°C in Tryptone soya agar slants.

Antimicrobial Assay of three spices against *V. cholera, V. parahaemolyticus and V. vulnificus*
The antimicrobial analysis method was performed using Agar well diffusion method (Bauer and Kirby, 1966) to evaluate the antimicrobial properties of three spices: thyme (*Trachyspermum ammi*), anise (*Pimpinella anisum*) and dry ginger (*Zingiber officinal*). Tryptone soya agar plates were prepared to evaluate the antimicrobial activity of spices extract, against selected *Vibrio* species.

Bacterial culture was uniformly spread on nutrient agar plates, after five minutes 6mm diameter well was bored in the plates, spices extract; standard antibiotic solution (as positive control) and solvents (as negative control) were poured into the well. The plates were incubated at 37°C for 24hr. and after incubation plates were observed for the zone of inhibition (mm).

RESULT AND DISCUSSION
The first part of this work has described the identification, using multiplex PCR, of three of the pathogenic species i.e., *V. cholera, V. parahaemolyticus or V. vulnificus* in different pond waters collected from different regions of Uttar Pradesh. Also three common spices, Black cardamom (*Amomum subulatum*), Mustard seed (*Brassica nigra*) and Liquorice (*Glycyrrhiza glabra*) extracts were analyzed for their antimicrobial activity against these three vibrio species.

The antimicrobial assay result of spices showed a maximum activity by aqueous extract Liquorice (*Glycyrrhiza glabra*) of 23 ± 0.52 (mm) against *V. vulnificus* followed by Mustard seed (*Brassica nigra*) of 22.1 ± 0.60 (mm) against *V. vulnificus*. Methanolic and water extracts of mustard seed also showed significant activity against *V. cholera* of 20.1 ±0.62 (mm) and 19 ± 0.59 (mm) respectively (*Shukla and Shankar, 2016*).
Table 1: Identified vibrio species- *V.cholerae*, *V.parahaemolyticus* and *V.vulnificus* in different pond water samples as reported in earlier part of this work (Shukla and Shankar, 2016)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Location</th>
<th>G_C*</th>
<th>Y_C*</th>
<th>G_C and Y_C</th>
<th>V.V.</th>
<th>V.C.</th>
<th>V.P.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Indira Nagar</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Vaishnopuram</td>
<td>P</td>
<td>A</td>
<td>-</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Sidhari</td>
<td>P</td>
<td>A</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Charbagh</td>
<td>A</td>
<td>P</td>
<td>-</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Kakadeo</td>
<td>A</td>
<td>P</td>
<td>-</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Khatima chauraha</td>
<td>P</td>
<td>A</td>
<td>-</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Rawatpur</td>
<td>P</td>
<td>A</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Geetanagar</td>
<td>P</td>
<td>A</td>
<td>-</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Kalyanpur</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Pratapgarh</td>
<td>A</td>
<td>P</td>
<td>-</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A- Colony absent; P-Colony present

V.V.-Vibrio vulnificus; V.C.-Vibrio cholera; V.P.-Vibrio parahaemolyticus

The second part of this study aims to highlight the antibacterial property possessed by three more spices which includes thyme (*Trachyspermum ammi*), anise (*Pimpinella anisum*) and dry ginger (*Zingiber officinal*). Extracts were prepared in three solvent- Acetone, methanolic and water and agar well diffusion method (*Bauer and Kirby, 1966*) was used for the assay. The results observed are shown below in Table 1.

*Figure 1: Antibacterial activity of spices by agar well diffusion method; Media used-Tryptone

Soya Agar media; well size-4 mm. (a) carom seed extract activity against *V.parahaemolyticus* (b)Anise extract against *V.cholerae* (c) Anise extract against *V.haemolyticus*

Antimicrobial effects of acetone extracts of Anise (*Pimpinella anisum*) was observed to be highest against *Vibrio cholerae* (20 ± 0.22 mm) contrary to activity reported by *Gulcin et al.*
where study was done against 10 bacterial species and also *Candida albicans* with disc diffusion method. In the study, ethanolic extract showed significant inhibitory activity against all tested bacteria but not effective on *Candida albicans*. However, the antimicrobial effect of water extract was not detected against Gram-negative bacteria, *Pseudomonas aeruginosa*, and *Escherichia coli*, but it was effective against *Candida albicans* (Gulcin et al., 2003). The alcoholic extracts of *Pimpinella anisum* seeds also showed antibacterial activity against *Micrococcus luteus* and *Mycobacterium smegmatis* (Ates and Erdogrul, 2003). It was also observed that methanolic extract showed no significant effect against any of the test bacterial strains of vibrio, with no inhibitory zone against *Vibrio parahaemolyticus* and *Vibrio vulnificus* (0.0 ± 0.0) and low inhibitory zone against *Vibrio cholera*.

Table 2: Antimicrobial activity of thyme (*Trachyspermum ammi*), anise (*Pimpinella anisum*) and dry ginger (*Zingiber officinal*) extracts in 3 different solvents against pathogenic *Vibrio* species

<table>
<thead>
<tr>
<th>Zone of Inhibition (mm)</th>
<th>Spices used</th>
<th>V.C</th>
<th>V.P</th>
<th>V.V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyme (Trachyspermumammi)</td>
<td>Act.</td>
<td>30 ± 0.30</td>
<td>0.0 ± 0.0</td>
<td>7 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>Met.</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Wat.</td>
<td>20 ± 0.24</td>
<td>19 ± 0.20</td>
<td>30 ± 0.34</td>
</tr>
<tr>
<td>Anise (Pimpinellaanisum)</td>
<td>Act.</td>
<td>20 ± 0.22</td>
<td>16 ± 0.14</td>
<td>8 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>Met.</td>
<td>11 ± 0.32</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Wat.</td>
<td>12 ± 0.34</td>
<td>18 ± 0.32</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Dry ginger (Zingiber officinal)</td>
<td>Act.</td>
<td>23 ± 0.35</td>
<td>9 ± 0.12</td>
<td>19 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>Met.</td>
<td>14 ± 0.16</td>
<td>0.0 ± 0.0</td>
<td>19 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>Wat.</td>
<td>15 ± 0.57</td>
<td>30 ± 0.34</td>
<td>20 ± 0.52</td>
</tr>
</tbody>
</table>

The acetone extract of Dry ginger (*Z. officinale*) demonstrated moderate antibacterial activity (23 ± 0.35), and water extract showed strong inhibitory effects (30 ± 0.34) against *Vibrio parahaemolyticus*. The effect of ethanolic and methanolic extracts of ginger were studied by Bhargava et al. (2012), and have demonstrated similar MIC values of ethanolic extract against tested bacteria. Taura et al. (2014) have showed that the ethanolic extract of ginger was more effective on *S. aureus* (MIC at 100 µg/ml), but it did not act against *E. coli* and *P. aeruginosa*. In another study done by Naji and Jassemi (2010) ethanolic extract of ginger showed the best effect against *P. aeruginosa* and *E. coli*. It was also observed that methanolic extract showed no significant effect against these gram negative bacteria, with no inhibitory zone against *Vibrio parahaemolyticus* (0.0 ± 0.0) and low inhibitory zone against *Vibrio cholera* (14 ± 0.16).
Ivanovic et al. (2012) reported significant activity of the extract and essential oil of thyme against *E. coli* and *Salmonella* strains, with MIC of 640 µg mL⁻¹. Such activity was attributed to the high concentration of thymol in the extract (39.7%) and in the essential oil (48.49%). It was also reported antimicrobial activity of the essential oil of thyme against *E. coli* 5% (V/V) and other food borne bacteria (Silva et al., 2013). A similar strong inhibitory effect was seen in acetone (30 ± 0.30) and water extract (30 ± 0.34) of thyme (*Trachyspermum ammi*) against *Vibrio cholera* and *Vibrio vulnificus* respectively. Methanolic extract showed no such activity against vibrio spp.

**CONCLUSION**

It was showed that the different solvent extracts of Thyme (*Trachyspermum ammi*), Anise (*Pimpinella anisum*) and Dry ginger (*Zingiber officinal*) had variation in their antibacterial effect against three pathogenic vibrio species i.e., *Vibrio cholera*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*. Water extract in all the three spices had shown moderate to strong inhibitory effect against these gram negative bacteria. Methanolic extract showed poor activity with minimum or no zone of inhibition. These finding also highlights the potential antibacterial activity against pathogenic species which are majorly responsible for water borne diseases. The bacterial activity against pathogenic strains of bacteria was performed using the agar well diffusion technique and showed that significant antibacterial activity. Therefore focus must be shifted to identification of active compounds in these potentially rich spices which are commonly used for their flavours and taste.

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