Evaluation of Antimicrobial activity of Carum carvi (Seeds) extract against E.coli and Aspergillus niger

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The in vitro antibacterial of alcoholic and aqueous extracts from Carum carvi (Caraway) medicinal herb belongs to family ‘Apiaceae’ was investigated by disc diffusion method against E.coli while antifungal activity was investigated by poisoned food technique against fungus Aspergillus niger. The main components of C. carvi were carvone, limonene, germacrene D and transdihydrocarvone. The activity was particularly high against the high concentration while a lower activity was observed against lower concentration. On the basis of investigation, we can say Carum carvi seeds could be used as a source of new antimicrobial agent for developing drugs to inhibit some pathogens.

Keywords: Antimicrobial Activity, Spice, Herb, Carum carvi, Apiaceae

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [1]. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in-recent years, largely due to in discriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases [2]. The drug resistant bacteria and fugal pathogens have further complicated the treatment of infectious diseases [3].

The increasing antibiotic resistance of some pathogens that are associated with foodborne illness is another concern [4,5,6]. Therefore, there has been increasing interest in the development of new types of effective and nontoxic antimicrobial compounds. There is growing interest in using natural antibacterial compounds such as extracts of spices and herbs, for food preservation [7]. Spices are recognized to prevent the microbial deterioration of food. Antimicrobial activity of spices depends on several factors, i.e. kind of spice, composition and concentration of spice and its occurrence level, substrate composition and processing conditions and storage [8].

Carum carvi L. is a medicinally important plant that belongs to the family ‘Apiaceae’. The seeds of this plant contains number of medicinally important compounds. Seeds possess antimicrobial, antileukogenic, antitumor, antiproliferative and antihyperglycemic. It is grown however less for the medicinal properties of the fruits, or so called ‘seeds’ than for their use as a flavouring in cookery, meat products, sauces and alcoholic liqueurs [9]. Seeds are laterally compressed somewhat horny and translucent, slightly curved and marked with five distinct pale ridges. The present study was carried out to determine the potential antimicrobial agent of methanol and aqueous seeds extracts of Caraway (C. carvi L.) against some human pathogens.

MATERIALS AND METHODS

Plant materials – Seeds of Carum carvi were purchased from the local market for use. Caraway is a biennial with smooth furrowed stems growing 1.5 to 2 feet high. The fruits or seeds are laterally compressed somewhat horny and translucent, slightly curved and marked with five distinct, pale ridges.

Microorganism and culture – The tested pathogens Escherichia coli and the Aspergillus niger were kindly provided by the Department of Microbiology, School of Life Sciences, Dr. B.R. Ambedkar University, Khandari, Agra. The strains were cultured and maintained at 37°C.


Preparation of extracts: Crude plant extracts were prepared by ‘Soxhlet Extraction’ method. About 50gm of powdered material was uniformly packed into a thimble and run in soxhlet extractor. It was exhaustible extract with aqueous and methanol for the period of about 48 hrs and 20-22 cycles for each till the solvent in the Siphon tube of an extractor become colourless. After that extract in rotary evaporator to get the syrropy consistency.

Determination of antibacterial activity: A disc diffusion method was employed for determination of antibacterial activity. Plant extract was dissolved in suitable solvent then different concentration (200mg/ml, 100mg/ml, 50mg/ml) were prepared by serial dilutions. Empty sterile disc having diameter of 6mm were impregnated with 25μl of each serial dilution of extract solution and then incubated for 15 minutes for proper diffusion. Now, aseptically pack up some colonies from the pure culture was mixed in nutrient broth. This broth was inoculated on entire surface of media and wait for 5-6 minutes. With the help of sterile forceps, herbal extract containing disc are placed on inoculated surface of agar plate. These plates were incubated for 24 hours at 37°C and measured the zone of inhibition in mm.
**Determination of Antifungal activity:** A poisoned food technique was employed for determination of antifungal activity. Sterilized Sabouraud agar medium with extract was placed in sterilized petriplates in inoculation chamber. In each petriplate (already have 25µl of sterilized medium) 1ml extract was added in laminar flows chamber under aseptic conditions when medium become semisolid, a small disc of 6mm of fungus (already grown on PDA) was cut with a sterile cork borer from periphery of 8 days old colony and transferred in the center of petri dish containing medium. Suitable checks were kept where culture disc were grown under same condition on medium without extract. Both incubated at 28 ± 1°C for 4 days. Finally % inhibition in mycelia growth was calculated.

\[ \text{% inhibition} = \frac{\text{Diameter of fungal colony in control} - \text{Diameter of fungal colony in extract}}{\text{Diameter of fungal colony in control}} \times 100 \]

**RESULT**

This work consisted of a study on antimicrobial property of *Carum carvi* against *E. coli* and *Aspergillus niger*.

**Antibacterial activity**

*Carum carvi* showed antibacterial property by disc diffusion method, which includes methanolic and aqueous extracts for comparison three concentrations 1250 µg/disc (50mg/ml), 2500 µg/disc (100mg/ml) and 5000 µg/disc (200mg/ml) was chosen. Both extract of *Carum carvi* seeds are least active against isolate of *E. coli* showing zone of inhibition ranges between 6mm to 9mm. Methanolic extract showed maximum inhibition zone 8.1mm at 200mg/ml concentration while aqueous extract showed maximum inhibition zone 7.00mm at 200mg/ml concentration (Table -1).

**Table -1: Inhibition zone in mm of different extract of *Carum carvi* (seeds) against *E. coli.***

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Plant part used</th>
<th>Extract</th>
<th>Code given on plate</th>
<th>Cons./ disc</th>
<th>Growth inhibition zone in (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methanolic</td>
<td>1250 µg/disc</td>
<td>2500 µg/disc</td>
<td>5000 µg/disc</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aqueous</td>
<td>1250 µg/disc</td>
<td>2500 µg/disc</td>
<td>5000 µg/disc</td>
</tr>
<tr>
<td></td>
<td>Cefotaxime</td>
<td>Drug</td>
<td>30 µg</td>
<td></td>
<td>19 ± 0.14</td>
</tr>
</tbody>
</table>

**Table -2: Antifungal activity of *Aspergillus niger* with different extracts of *Carum carvi.***

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Plant part used</th>
<th>Extract</th>
<th>% Mycelial growth</th>
<th>Drug (Macamazole)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus niger</em></td>
<td>Seeds</td>
<td>Methanolic</td>
<td>38.5</td>
<td>45.8</td>
</tr>
<tr>
<td></td>
<td>Aqueous</td>
<td></td>
<td>36.3</td>
<td>41.8</td>
</tr>
</tbody>
</table>

**DISCUSSION**

*Carum carvi* spice and herb extracts tested in this study. High concentration of both extracts exhibited high antimicrobial activity against the bacteria, *E. coli* and the fungus, *Aspergillus niger*. To some extent, these results were similar to those of previous studies. Many previous studies have reported the antibacterial activity, phenolic content and antioxidant activities of spices and herbs. But it was not easy to compare directly the result of different studies and to establish reasonable relationship between antibacterial activity, phenolic content and antioxidant activity because of the low number of spice and herb samples tested, different determination methods and different bacterial strains used. Spices offer a promising alternative for food safety and plant protection. Inhibitory activity of spices and their derivatives on the growth of bacteria, yeasts, fungi and microbial toxin synthesis has been reported [10, 11]. It was reported that various powder concentration of mint, sage, bay, anise and red pepper significantly inhibited the growth of *Aspergillus parasiticus* [12]. Chilli, coriander, pepper, cumin and asafetida were found to inhibit food spoilage moulds [13]. Several scientific reports describe the inhibitory effect of spices on a variety of microorganisms, although a considerable variation in resistance of different microorganisms to different spices has been observed [14]. Similar results were also obtained in the present study. Spices are frequently used as an active ingredient in certain medicines and reported to possess a number of pharmacological effects to treat different human ailments [15]. Several investigations have been directed towards their antibacterial properties [16]. Previous research studies have documented that *E.eoli* are known to be multidrug resistant [17,18]. The most active constituents (essential oils) of many spices having wide spectra of antimicrobial activity are aromatic phenolic compounds, such as thymal and carvacrol in oregano and thyme, eugenol in clove and cinnamon and cinnamic aldehyde in cinnamon [19, 20]. These bioactive principles in the related dietary spices and medicinal herbs were also identified in previous studies [21,22]. *C. cuminum* and *C. carvi* essential oils inhibited the growth of *Aspergillus parasiticus* and yeast and Gram-positive and Gram-negative bacteria [23]. Thus, according to our investigation caraway can be used as a potent antimicrobial agent for human pathogens.

**Antifungal activity**

Antifungal activity of selected plant of family ‘Apiaceae’ was studied by poisoned food technique. In the present study spice plant *Carum carvi* as powder and methanolic and aqueous extracts showed inhibitory effect on the fungal growth increasing with their concentration. Methanolic extract showed maximum 47.3% mycelia growth inhibition at 200mg/ml while aqueous extract showed the maximum 45.4% mycelia growth at 200mg/ml as shown in Table -2.

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Ankur Gupta, *et al*.: Evaluation of Antimicrobial activity of *Carum carvi* (Seeds) extract against *E.coli* and *Aspergillus niger*.
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