COMPARATIVE STUDIES ON THE ANTIMICROBIAL ACTIVITY OF SELECTED SPICES

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ABSTRACT
In the present study, a total of twelve extracts of four spices namely cumin, mustard, fenugreek and asafoetida extracted with two solvents such as ethyl alcohol and ethyl acetate were evaluated for their antibacterial activity. It was measured by agar disc diffusion method. All the extracts showed antibacterial activity against all the test bacterial isolates. According to the zone of inhibition observed in the entire agar plates, the combination of alcoholic extracts of asafoetida-mustard and asafoetida-fenugreek produced very good zone of inhibition such as 29.5 mm and 23.5 mm respectively against Staphylococcus aureus. Then the combination of asafoetida-cumin and mustard-fenugreek produced the zone of inhibition 23.5 mm and 24 mm respectively against Bacillus subtilis. The effect of asafoetida-fenugreek combination against Pseudomonas aeruginosa produced 22 mm of zone of inhibition. The combination of ethyl acetate extract of cumin-mustard and cumin-fenugreek produced the zone of inhibition 20.5 mm & 19 mm respectively against E.coli. Again the same mixture of extracts produced very good effect against Staphylococcus aureus also and their zones of inhibition are 24.5 mm and 19.5 mm. According to results, these extracts may be an alternative to chemical preservatives and used as natural antimicrobial preservatives to reclaim the shelf-life of food. Further research may be carried out for the identification of bioactive molecule present in the two extracts and in vivo efficacy against food spoilage microorganisms

KEYWORDS: Antimicrobial activity, Pathogens, Spices extract, Agar disc diffusion

INTRODUCTION
According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs. Since ancient times, plants have been model source of medicines as they are a reservoir of chemical agents with therapeutic properties. The general population is increasingly using herbal medicines as dietary supplements to relieve and treat many different human disorders [1]. The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities and low toxicity [2]. At present, it is estimated that about 80% of the world population rely on botanical preparations as medicines to meet their health needs. Herbs and spices are generally considered safe and proved to be effective against certain ailments [3]. The development of drug resistance in human pathogens against commonly used antibiotics has
necessitated a search for new antimicrobial substances from other sources, including plants. Plants used for traditional medicine contain a wide range of substances that are used to treat chronic diseases. The typical Indian spices and herbs like cumin, black cumin, mustard, fenugreek, ajowan, asafoetida, curry-leaf, nutmeg and henna are usually used in curries, pickles, sauces etc. These spices are also known to have some ethno-medicinal or anti-microbial properties [4].

Spices can be defined as any dried, fragrant, aromatic or pungent vegetables or plant substances in whole, broken or ground forms, which contribute flavor, whose primary function in food is seasoning rather than nutrition and that may contribute relish or piquancy of foods and beverages [5]. Spices are plant products used in flavoring foods and beverages. For thousands of years, aromatic plant materials have been used in food preparation and preservation, as well as for embalming [6]. Medicinal and spice plants are renewable raw materials. Their production is an alternative to the overproduction of traditional crops in agriculture. They also have an increasing economic importance.

Although as natural substances spices and herbs are easily absorbed by our bodies and generally do not have any adverse effects, spices as medicine should be used judiciously. This is because substances being derived from a plant do not mean it is always harmless. Antibacterial activities of extracts of different plants against various microorganisms have been reported by many scientists [7]. Some medicinal herbs have also been assessed. Some spices were specifically tested for anti-microbial activities [8]. But there are little reports on some of the Indian spices and herbs [9]. Some studies claim that the phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects [10].

In the present study, we have evaluated the synergistic antibacterial effect of the extracts of four widely used spices in South India such as Mustard (Brassica nigra), Cumin (Cuminum cyminumliinn), Asafoetida (Ferrulafoetida Regel), and Fenugreek (Trigonella foenum-graecum) against 4 different species of Gram-positive and Gram-negative Bacteria. The inhibitory effect of these spices was compared with that of antibiotic tetracycline and the results are discussed. The findings from this study may justify the usage of these spices for their medicinal purposes as well as nutritional supplements.

**MATERIALS AND METHODS**

**Materials**

**Instruments**
The instruments used for the work are Incubator (37°C), Refrigerator (4°C to -18°C), Laminar air-flow system, Autoclave, Hot air oven, Precision electronic balance, Micropipette (100 to 1000 μl), Inoculating loop, needle etc.

**Chemicals**
The chemicals used for the work are Ethyl acetate, Ethanol, Peptone, Agar, Sodium chloride and Beef extract, Mueller-Hinton Agar, (Hi Media, Mumbai, India)

**Consumables**
The consumables used for the work are sterile cotton, sterile paper discs with 5mm thickness (autoclaved), sterile cotton fabrics and Cotton plugs.

**Methods**

**Extraction and storage of plant material**
The freshly collected plant parts were thoroughly washed under tap water followed by sterile distilled water separately. The washed plant material was dried independently in an oven at 50°C for 48 hrs followed by grinding in to a fine powder. The powdered materials of selected spices were stored in air tight jars and refrigerated separately at 4°C. Separately two solvents i.e., ethanol (95%) and ethyl acetate were used for the phytochemical extraction of four plant materials and collected a total of 8 extracts. Ethyl acetate extracts of 4 plant materials were prepared by dissolving 25gm of powdered material in enough of the solvent to make 100ml of ethyl acetate extract (25% w/v). The mixture was kept undisturbed at room temperature for 24 hrs in a sterile flask covered with aluminum foil to avoid evaporation and subjected to filtration through sterilized Whatman no.1 filter paper. After filtration, the extract was evaporated in water bath to get 25 ml of extract in the container. Ethanol extracts of 4 plant materials were prepared by dissolving 25gm of powdered material in enough of the solvent to make 100ml of ethanol extract (25%
w/v). The mixture was kept undisturbed at room temperature for 24 hrs in a sterile flask covered with aluminum foil to avoid evaporation and subjected to filtration through sterilized Whatman no.1 filter paper. After filtration, the extract was evaporated in water bath to get 25 ml of extract in the container.

### Preparation of inoculum

A loopful of inoculum was taken from a pure culture of E. coli bacteria and inoculated into 10 ml of Mueller Hinton broth (Hi Media, Mumbai, India). Similar procedure was adopted to prepare the inoculum of other bacterial species i.e., S. aureus, B. subtilis & P. aeruginosa. The broth suspension was then incubated at 37°C for 3 hrs and utilized for antibacterial assays.

### Agar disc diffusion techniques

Filter paper discs of 5 mm diameter were prepared and sterilized under UV light for 5 min. These discs were dipped aseptically in respective combination of spices extract (1:1 ratio) and placed over Mueller – Hinton Agar plates seeded with respective pathogens. The plates were incubated in an upright position at 37°C for 24 h. The diameter of inhibition zones formed was measured in mm and the results were recorded. Discs with 7 mm diameter are considered as having no antibacterial activity. Diameter between 7 and 12 were considered as moderately active and those with > 12 mm were considered as highly active.

For alcoholic extracts, alcohol was used as negative control and for ethyl acetate extracts; ethyl acetate was used as negative control. In both the cases antibiotic tetracycline (Broad spectrum antibiotic) was used as positive control.

### RESULTS AND DISCUSSION

Microorganisms are the concealed enemies to the mankind. These microscopic organisms cause a very profound damage in human bodies as well as in other living organisms. There has been an increasing consumer demand or foods free or with low, if any, added synthetic preservative because synthetic preservatives could be toxic to humans. Concomitantly, consumers have also demanded for wholesome and safe food with long shelf lives. These requirements are often contradictory and have put pressure on the food industry for progressive novel of chemical preservatives and adoption of natural alternatives to obtain its goals concerning safe food with long shelf lives.

Ethanol and ethyl acetate and spices extracts of 4 different species were mixed in 1:1 ratio. The antimicrobial activity of alcohol and ethyl acetate extracts of spices combination against staphylococcus were given in tables 1&2. The zone of inhibition was measured and tabulated against each organism and the antibacterial effect of each combination of the extracts in ethanol and ethyl acetate against different organisms were plotted taking combination of spice extracts on x-axis and the zone of inhibition in millimeter on y-axis and explained in figure 1&2. Photographs of the effect of ethyl acetate extract of spices on pseudomonas aeruginosa and effect of alcohol extract of spices on staphylococcus aureus were given in figure 3 & 4.

### Table-1 Antimicrobial activity of alcohol extracts of spices combination against Staphylococcus aureus.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Code</th>
<th>Name of the spices</th>
<th>Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A+C</td>
<td>Asafoetida &amp; Cumin</td>
<td>16.5 mm</td>
</tr>
<tr>
<td>2</td>
<td>M+F</td>
<td>Mustard &amp; Fenugreek</td>
<td>7.0 mm</td>
</tr>
<tr>
<td>3</td>
<td>C+M</td>
<td>Cumin &amp; Mustard</td>
<td>6.8 mm</td>
</tr>
<tr>
<td>4</td>
<td>A+F</td>
<td>Asafoetida &amp; Fenugreek</td>
<td>23.5 mm</td>
</tr>
<tr>
<td>5</td>
<td>C+F</td>
<td>Cumin &amp; Fenugreek</td>
<td>18.5 mm</td>
</tr>
<tr>
<td>6</td>
<td>A+M</td>
<td>Asafoetida &amp; Mustard</td>
<td>29.5 mm</td>
</tr>
<tr>
<td>7</td>
<td>E</td>
<td>Ethanol</td>
<td>12.5 mm</td>
</tr>
<tr>
<td>8</td>
<td>T</td>
<td>Tetracycline</td>
<td>25.5 mm</td>
</tr>
</tbody>
</table>
Table-2 Antimicrobial activity of ethyl acetate extracts of spices combination against *Staphylococcus aureus*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Code</th>
<th>Name of the spices</th>
<th>Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A+C</td>
<td>Asafoetida &amp; Cumin</td>
<td>10.5 mm</td>
</tr>
<tr>
<td>2</td>
<td>M+F</td>
<td>Mustard &amp; Fenugreek</td>
<td>11.0 mm</td>
</tr>
<tr>
<td>3</td>
<td>C+M</td>
<td>Cumin &amp; Mustard</td>
<td>19.5 mm</td>
</tr>
<tr>
<td>4</td>
<td>A+F</td>
<td>Asafoetida &amp; Fenugreek</td>
<td>13.5 mm</td>
</tr>
<tr>
<td>5</td>
<td>C+F</td>
<td>Cumin &amp; Fenugreek</td>
<td>24.5 mm</td>
</tr>
<tr>
<td>6</td>
<td>A+M</td>
<td>Asafoetida &amp; Mustard</td>
<td>17.5 mm</td>
</tr>
<tr>
<td>7</td>
<td>E</td>
<td>Ethyl acetate</td>
<td>13.0 mm</td>
</tr>
<tr>
<td>8</td>
<td>T</td>
<td>Tetracycline</td>
<td>16.5 mm</td>
</tr>
</tbody>
</table>

Figure-1 Effect of asafoetida & Fenugreek alcohol extracts against different organisms

![Figure-1](image1)

Figure-2 Effect of Cumin & Fenugreek ethyl acetate extracts against different organisms

![Figure-2](image2)
According to the zone of inhibition observed in the entire agar plates, the combination of alcoholic extracts of asafoetida-mustard and asafoetida-fenugreek produced very good zone of inhibition such as 29.5 mm & 23.5 mm respectively against *staphylococcus aureus*. Then the combination of asafetida-cumin and mustard-fenugreek produced the zone of inhibition 23.5 mm and 24 mm respectively against *Bacillus subtilis*. The effect of asafoetida-fenugreek combination against *Pseudomonas aeruginosa* produced 22 mm of zone of inhibition. The combination of ethyl acetate extract of cumin-mustard and cumin-fenugreek produced the zone of inhibition 20.5 mm & 19 mm respectively against *E.coli*. Again the same mixture of extracts produced very good effect against *Staphylococcus aureus* also and their zones of inhibition are 24.5 mm and 19.5 mm.
The graphs explained that, the combination of asafoetida-fenugreek alcoholic extract produced very good effect against both Staphylococcus aureus and Pseudomonas aeruginosa and similarly the mixture of cumin-mustard ethyl acetate extracts were active against both E.coli and Staphylococcus aureus.

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REFERENCES

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