Antimicrobial effect of ajwain seed ethanolic extract against food borne pathogenic bacteria

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Abstract

Ajwain (Trachyspermum ammi) is a source of medicinally active compounds and has various pharmacological effects and therapeutic uses. The aim of this study was to evaluate the antimicrobial activities of ajwain seeds ethanolic extract against different pathogenic bacteria. Ethanolic extraction of ajwain seeds was carried out by supercritical fluid extraction method and GC-MS of the extract showed the presence of thymol as its major component (71.06%) along with o-Cymene (3.37%), γ-Terpinene (3.83%), 2-methyl-5-(1-methylethyl)-phenol (0.51%). The extract was analyzed for its ethanolic DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging potential, showing increase in percent inhibition (50% to 83%) with increase in concentration (50 µg/mL to 250 µg/mL). The antimicrobial test results revealed that the pure ethanolic extract of ajwain seeds is significantly active against the food-borne pathogenic Gram positive and Gram negative bacterial strains (range of inhibition, 15-19 mm) and was compared with that of the broad-spectrum antibiotic, chloramphenicol (range of inhibition, 33-37 mm). Results concluded that ajwain seeds contain high amount of phenolic compound (mainly thymol) and have high antioxidant and antimicrobial activity and, hence, can be used as an excellent bio-preservative and for medicinal purpose, and can be useful for various perishable and fat rich food products by enhancing their shelf-life.

Keywords

Antioxidant
Antibacterial
Pathogenic bacteria
Trachyspermum ammi

Introduction

Nature has been as a rich source of medicinal floras for a long period of time to maintain human health, having a tremendous sanative potential to heal many infectious maladies, without any side effects (Shelef, 1983). Apart from providing aroma and savor, spices have been recognized for their properties of preserving foods and medicinal values due to the presence of bioactive compounds (Papp et al., 2007). Resistance among pathogenic microbes against various antimicrobial drugs has been an increasingly important and most appalling problem, globally. Synthetic chemicals can be toxic in nature; hence, the plant extracts containing phytochemical, which have both antimicrobial and antioxidant properties, must be taken to control this problem (Agaoglu et al., 2007). The antioxidant activity of several polyphenolic extracts and compounds derived from different plant parts, such as foliage, fruits, seeds, bark or skin, roots and oil-rich seeds has been extensively analyzed and documented (Naz et al., 2008; Li et al., 2012). It has been found that spices are rich in antioxidant activity as compared to fruits, cereals and nuts.

Ajwain (Trachyspermum ammi) is one the natural botanical sources, having several testified officinal properties. Being rich in constituents with promising bioactivity, Ajwain is an extremely reputable plant source (Bairwa et al., 2012). The essential oil is employed in the treatment of gastrointestinal ailments, lack of appetency and bronchial problems, at therapeutic level. Thymol being the major phenolic compound present in Ajwain has been reported to be a germicide, spasmolytic and antimycotic agent (Nagalakshmi et al., 2000). Thymol has antibacterial (Kumar et al., 2011), antifungal (Paul et al., 2011), and antioxidant (Moein et al., 2015) effects. Depending on the concentration used, such phenolic compounds can act as either bactericidal or bacteriostatic agents (Ashrafi Tamai et al., 2013). The concentration of the polyphenols and carotenoids in ajwain leaves extracts has been found to influence the antioxidant activity (Raza et al., 2015).

The foods containing such phytochemicals have proved their strong protective effects against many diseases and are increasingly being gaining importance (Orhan et al., 2010; Phapale et al., 2010; Özçelik et al., 2011; Guest et al., 2012). The main aim of this study was to evaluate the antimicrobial activity of the Trachyspermum ammi seeds ethanolic extract against some of the important food borne pathogenic bacteria.
as well as its antioxidative properties for its potential use as a natural food additive.

Materials and Methods

Sample preparation

Ajwain seeds were purchased from the local market of Chennai, India. The seeds were ground using electronic grinding machine, to get fine and uniform powder. The sample was stored at -20°C until further use.

Supercritical fluid extraction (SFE)

Extraction was carried out in a pilot-plant scale supercritical fluid extractor (Thar Technology), comprising a 2 L cylinder extraction cell and a separator (of 0.5 L capacity) with temperature and pressure control. Recirculation system was also included in the extraction device, where CO₂ was condensed and pumped up to the desired extraction pressure. For each extraction cycle, the extraction vessel was filled with 5 g of ground ajwain seed. Parameters were set as per Table 1. Each cycle was run for 60 minutes. Solvent was removed using rotary evaporator at 40°C. Pure extract was collected, weighed and stored at 4°C for future use. Percentage Yield was calculated on w/w basis (g extract/g sample load) (Mónica et al., 2011). Greater yield cycle was continued for further extract collection.

Qualitative analysis of the extract using GC-MS technique

The analysis of ajwain seed extract was carried out in Agilent 7890B GC System (Agilent Technologies) connected to 5977A mass spectrometer detector, comprising a split/splitless injector, G4513A auto injector, and data was analyzed using Mass Hunter software. The column used was an Agilent HP-5MS 5% Phenyl Methyl Silox -60°C-325°C capillary column (30 m × 250 μm i.d. × 0.25μm phase thickness).

Parameters were set as described by Villanueva et al. (2015), with some modifications. Oven temperature was programmed to 60°C isothermal for 4 min then increased to 106°C at 2.5°C/min and from 106°C to 130°C at 1°C/min and finally from 130°C to 250°C at 20°C/min and this temperature was kept constant for 10 min. Sample injections (1 μL) were performed through auto-sampler in split mode (1:10). The chromatographic separation was carried out at constant pressure of 20.74 psi and Helium (99.996 mass %) was used as a carrier gas (He flow rate= 2mL/min.). Injector temperature was 250°C, whereas, mass spectrometer ion source and interface temperatures were 230°C and 280°C, respectively. Mass spectrometer was used in total ion current (TIC) mode and sample scanning was done from 40 to 500 amu. Thymol was identified by comparison with the mass spectra from NIST (The National Institute of Standards and Technology) library.

DPPH radical scavenging activity

The free radical scavenging activity of the sample was measured in vitro by DPPH assay (Sarker et al., 2006) with minor modifications. Ajwain seed ethanolic extract was taken and 0.1mM solution ethanolic DPPH solution (0.04g/L) was prepared. Different concentrations of working solutions (50, 100, 150, 200 and 250μg/mL of pure extract using absolute ethanol) were prepared and left in dark, at room temperature, for 30 minutes incubation. Absorbance was measured at 517 nm. Absolute ethanol without DPPH was used as Blank and ethanolic DPPH was used as Control. Butylated hydroxytoluene (BHT) was taken as reference.

Equation 1 was used to express the result as percentage of inhibition of the DPPH radical.

\[
I(\%) = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100
\]

Where, \(A_{\text{blank}}\) is the absorbance of the control and \(A_{\text{sample}}\) is the absorbance of the test compound. The sample concentration showing 50% inhibition (IC\(_{50}\)) was calculated by plotting graph of inhibition percentage vs sample concentrations. IC\(_{50}\) value and its 95% confidence interval were calculated.

Antimicrobial property of the extract

The food borne pathogenic bacterial strains were obtained from MTCC, and were sub cultured every 30 days in nutrient agar medium at 30°C. The susceptibility of antibiotics was carried out using disc diffusion method on Muller-Hinton (MH) agar (Luangtongkum et al., 2007). MH agar plates were prepared and the strains of S. aureus, Shigella, Salmonella and E.coli were taken. On the other hand, antimicrobial discs (~9 mm) were prepared.
using the pure extract and 250 µg/mL ethanolic concentration. One untreated disc was used as control. Chloramphenicol (50 mg/mL) antibiotic disc was used as standard to compare the results. Wells were bored (~9 mm diameter) in the agar plates, the discs were placed gently and the plates were incubated at 37°C for 48 hours. Later, the diameter of the zone of inhibition was measured, which gave the extent of microbial inhibition.

**Statistical analysis**

Standard deviation was applied for determination of significant difference between mean values of zone of inhibition used to calculate antimicrobial property (Hernández-Carranza et al., 2013).

**Results and Discussion**

Extraction of thymol from ajwain seeds using SFE technique

The efficiency of supercritical fluid extraction was similar to that obtained by conventional liquid extraction, but the quality of the super critically extracted oil was higher, equivalent to a degummed liquid – extracted oil. In SFE, the stages like solvent distillation and oil refining can be omitted; hence, it is competitive with conventional liquid extraction (Gomez et al., 1996).

SFE result showed that at 45°C temperature and 250 bar pressure the yield was greater than at lower temperature and pressure, as shown in Table 1, even though the CO₂ flow rate and Co-solvent flow rate remained constant. This showed that with increase in temperature and pressure the percent yield of the extract increased. Hence, Set 2 cycle was continued for further extract collection. These results were in accordance with study that said both high temperature and pressure helped in increasing extraction efficiency (Langenfeld et al., 1993).

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Retention Time (s)</th>
<th>Compound Name</th>
<th>Molecular Formula</th>
<th>Molecular Weight (Da)</th>
<th>Percentage of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.986</td>
<td>o-Cymene</td>
<td>C₁₀H₁₆</td>
<td>134</td>
<td>3.388</td>
</tr>
<tr>
<td>2</td>
<td>9.469</td>
<td>γ-Terpinene</td>
<td>C₁₀H₁₈</td>
<td>136</td>
<td>3.826</td>
</tr>
<tr>
<td>3</td>
<td>21.817</td>
<td>Thymol</td>
<td>C₁₀H₁₄O</td>
<td>160</td>
<td>71.064</td>
</tr>
<tr>
<td>4</td>
<td>22.008</td>
<td>Phenol, 2- methyl-5-1-methylethyl</td>
<td>C₁₀H₁₆O</td>
<td>150</td>
<td>0.512</td>
</tr>
</tbody>
</table>

Table 2. GC-MS result showing the presence of thymol and other phenolic compounds in the extract

![Figure 1. GC-MS result of ajwain seed ethanolic extract](image)

**Qualitative analysis of the extract using GC-MS technique**

GC-MS result showed the presence of a mixture of components, mainly thymol together with a small amount of other volatile compounds. Four main components were identified, which represented 99.97% of the total oils: Thymol (71.06%), o-Cymene (3.37%), γ-Terpinene (3.826%) and 2-methyl-5-(1-methylethyl)-phenol (0.512%) were found as main phenolic components, as shown in Table 2.

Figure 1 shows the GC-MS result in the form of peaks. From the figure it can be observed that the highest peak is of thymol. Here the peaks depict the abundance of that particular compound in the sample at that period of time. For example, the presence of thymol appeared at 21.817 seconds. In the figure, the presence of other non-phenolic compounds can be observed. Some previous studies have already reported that thymol is the major component of this oil but its concentration varies due to time of plant growing, preparation process, cultivar differences and geographical location from which the plants were collected (Saei-Dehkordi et al., 2010).
Antioxidant property using DPPH radical scavenging activity

It was observed that with increase in concentration from 50 µg/mL to 250 µg/mL, the percentage inhibition increased from 50% to 83%. IC$_{50}$ was calculated and its 95% confidence interval (CI) value came out to be between the lower endpoint 5.484 and the upper endpoint 17.53188. The graphical representation, in Figure 2, depicts the activity of the sample extract compared with BHT (Butylated hydroxytoluene) standard.

Antimicrobial property of the extract

Agar well diffusion method showed the antimicrobial activity of the pure ajwain seed extract with zone of inhibition of 15.33±0.35 mm, 19.02±0.34 mm, 16.29±0.32 mm and 16.46±0.13 mm against Salmonella, S. aureus, E. coli and Shigella, respectively, whereas, the control discs did not show any zone of inhibition. The diameter of zone of inhibition recorded includes the size of filter paper discs. This showed that the ajwain seed extract has antimicrobial properties against various pathogenic and food spoilage microbes as shown in few studies (Wadhwa et al., 2010; Masih et al., 2012; Hassanshahian et al., 2014). In Figure 3, the graph shows the zone of inhibition of different concentrations against the pathogenic bacteria. Here the control and 250 µg/mL ethanolic concentration of the extract did not show any inhibitory activity against the microbes.

Conclusion

From this research work, it was concluded that ajwain seeds’ ethanolic extract, obtained via supercritical fluid extraction method, is rich in phenolic compounds (mainly thymol) and it has high antioxidant and antimicrobial activity. DPPH radical scavenging activity showed that the extract has comparative antioxidant property as that of the BHT standard. The disc diffusion method showed the effectiveness of the extract against various Gram positive and Gram negative bacteria. Hence, this extract can be used as an excellent bio-preservative and for medicinal purpose. It can also enhance the shelf-life of various perishable and fat rich food products, even at room temperature.

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References


Hassanshahian M., Bayat Z., Saeidi S. and Shiri Y. 2014.


