Chemical compound of cumin and fennel seed extracts against some types of pathogenic bacteria
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Objective  This objective of the study was to find antimicrobial capability from two aromatic plants (Cumin and Fennel seeds) which returns to Apiaceae family.

Methods  The chemical aromatic compounds were extracted from seeds by methanol alcohol by concentration of 70%. The aromatic plant seeds were investigated for their phenols and flavonoids content by HPLC analysis which proved the existence of each gallic acid, quercetin, catechin, and tannin compared to standards. The effectiveness of extracts was estimated as antibacterial activity using CUP assay method and natural antioxidant activity by 2,2’-azino-bis < 3-ethylbenzothiazoline-6-sulphonic acid > (ABTS) assay.

Results  The results show that the inhibitory ability to the fennel extract has been better than the cumin extract versus bacterial pathogen. The highest rates for inhibition of Fennel extract at a concentration 50,100 mg/ml against Streptococcus agalactiae, and Proteus mirabilis reaching (14 and 16 mm), respectively, in comparison with control which did not show any effect against bacterial test. While the highest rates inhibitory of Cumin extract at concentration 50,100 mg/ml against Streptococcus agalactiae reaching (12 and13 mm), respectively, in comparison with control. Although the antioxidant capacity estimated by 2,2’-azino-bis < 3-ethylbenzothiazoline-6-sulphonic acid > (ABTS) assay, the plant extracts showed no susceptibility antioxidant for 2,2’-azino-bis < 3-ethylbenzothiazoline-6-sulphonic acid > (ABTS) Radical.

Conclusions  In this paper, medicinal plant seeds from the Apiaceae family that were Cuminum cyminum L. (Cumin seeds) and Foeniculum vulgare Miller (Fennel seeds) are studied. There has been appraised antibacterial activity against some of pathogenic bacteria Streptococcus agalactiae, Proteus mirabilis, Enterococcus cloaca, Escherichia coli, while we did not find any effective antioxidant using ABTA. Radical assay despite it contains extracts on different percentages of active ingredients, which are characterized as antioxidants. So we recommend conducting further studies to the effectiveness of antioxidants for these extracts using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

Keywords  medicinal plants, phenolic compounds, HPLC, pathogenic bacteria, ABTS

Introduction  
In recent years, there has been an increasing interest in herbs and species as medicinal plants in addition to the increased use of nutrients and preservative materials. There have been occupied important roles in the agricultural and industrial production is the main source of medical plant drugs or as efficient materials which go into the preparation of the drug out of effective extracts, and medical plants are the most substantial item in the pharmaceutical and cosmetic industry.1 It has been demand for medical plants for frequent side effects of chemical drugs and risks in addition to the medical plants contain many active compounds such as phenols, flavonoids, alkaloids, saponins and many of the components that require lengthy explanations has increased1. 

The focus of this current study is on the seeds of Cuminum cyminum L. It is known as cumin that belongs to Family Apiaceae2, and other seeds belong to the same family, known as fennel (Foeniculum vulgare Miller).3 The majority of compounds in these seeds are volatile oil, a variety of phenolic, alcohols, glycosides, aldehydes, and phenols. All these factors combined raised the inhibitory action of plant extracts in the direction of a number of pathogens known, such as Staphylococcus aureus, Escherichia coli and Candida albican.4,5 High performance liquid chromatography (HPLC) is applied for the invention and investigation of active compounds quantitatively and qualitatively in plant extracts.6 The goal of this article was to review recent research into the antibacterial efficacy of cumin and fennel extracts and to detect active chemical compounds by HPLC. The object of this essay is to discover the relevance in the middle of the inhibitory ability of chemical combinations in medicinal plant and its impact on the prevention of the growth of some types of pathogenic bacteria.

Materials and Methods  
Sample Collection  
Cumin and Fennel seeds have been collected from the local market (herbalists) in 2016 at Kerbala city. It was cleaned of impurities and ground and preserved in sealed can and stored in the refrigerator.

Chemicals, Solvent, and Media  
Gallic acid, acetonitrile, methanol, Dimethyl sulfoxide (DMSO), ABTS (2,2-azinobis-3-ethylbenzothiazoline 6-sulfonic acid) C<sub>12</sub>H<sub>17</sub>N<sub>3</sub>O<sub>5</sub>S<sub>2</sub>, potassium hydroxide KOH, glacial acetic acid, potassium persulfate, were acquired from Hi-media, India. 

Muller Hinton Agar and Muller Hinton Broth (Hi-media) has composed the media as commanded by the production company.

Basis of Pathogens  
Strains of Streptococcus agalactiae, Proteus mirabilis, Enterococcus cloace, Escherichia coli were isolated from stools of some patients and purified on selective media such as MacConkey Agar, and XLD Agar. Bacterial isolates were diagnosed by API...
20E of Enterobacteraceae family. It has been obtained from the Laboratories of the Department of Health Surveillance at a holy city of Karbala. Strains of bacteria were grown on nutrient agar (Hi-media) at 37°C used for 24 hours.

**Preparation of Methanolic Extract of Cumin and Fennel Seeds**

20 g of powdered seeds has been added to 70% methanol in a shaker at room temperature for 72 h. The remainder was extracted with 70% methanol again for 24 h. Assembled extract was separated through double-layered muslin cloth after that centrifuged at 6000 rpm for 3 min to get obvious supernatant. The extract was run into the tray, and it was dried in an oven at 45°C to evaporate the solvent. Then it was scraped from the tray and collected in cans and closed. They were kept in a refrigerator at 4°C.

**Estimated Phenolic Components by Hplc Analysis**

The crude methanolic extract was subjected to HPLC to estimate quantitative and qualitative phenolic compounds. The HPLC system (Shimadzu Company, Japan) was equipped with UV detector at 348 nm, C18 column-ODS (25 cm × 4.6 mm × 5 µm). Standards and specimen extracts were analyzed by following gradient program. It was mobile phase (A = MeOH:A:A:DW (10:2:88), and B = MeOH:A:A:DW (90:3:7), A(0–4 min) 40%, A(5–8 min) 50%, A(8–10 min) 60%). Flow rate was 1.0 ml/min and injection size was 50 µl. Detection was done at 280 nm of a specimen of cumin and 214 nm for a specimen of fennel. Peak area (280 or 214 nm) of the specimen are an index of the amount of content, and retention time of unique peaks is used to detect phenolic compounds by comparing with standard polyphenols (gallic acid, quercetin, rutin, catechine, tannin).

**Preparation of Antibacterial In Two Concentrations From Seed Extracts**

It has been prepared concentrations by weighing 1 gm from crude extract and dissolved in 10 ml from DMSO. It was getting concentration 100 mg/ml. While it has been prepared concentration 50 mg/ml by using 1 gm from crude extract and dissolved in 20 ml of DMSO.

**Preparation McFarland Solution**

It has been prepared a solution by mixing 0.5 ml of 1.75% barium chloride BaCl₂, 2H₂O then it was added to 99.5 ml 1% sulfuric acid H₂SO₄. This turbidity is equal to about 1.5 × 10¹⁰ colony forming unit CFU/ml. A loopful of the colony of pathogenic bacteria was taken and put in 4 mL peptone water and continued transport until equal turbidity.

**Estimated Antioxidant Activity in Plant Medical Extracts**

Antioxidant activity of plant extracts was determined with some modification. Antioxidant activity of plant extracts was estimated with dilution the solution No. 4. by potassium acetate buffer (Solution No. 3). Subsequently, it was reset the spectrophotometer with potassium acetate buffer. Until it was getting absorption 0.7 ± 0.02 at a wavelength 734 nm. Then, it has been added 3 mL from above solution (ABTS dilution) to 0.3 ml for each of concentration of extract under study.

The tubes were rocked by using a vortex device and incubated the mixture at 25–30°C for 10 minutes. Next, it was measured absorption at a wavelength 734 nm. The percentages of inhibition of ABTS radical had been predestined in keeping with next neutralization.

\[
PI(\%) = \left[1 - \left(\frac{At}{Ar}\right)\right] \times 100^* 
\]

*As the At and Ar are absorptions for each specimen and ABTS, respectively.

**Statistical Analysis**

GenStat 2008 program was used in the statistical analysis of the data, and was performed by analysis of variance (two way ANOVA table no blocking) to analyze the effect of various transactions in the traits and the significance level of 0.05%.

**Results**

**Yields of Methanolic Extract of Cumin and Fennel Seeds**

Yields (g/100g) of cumin and fennel extracts were found to be 16.2 gm, 15.06 gm, respectively. Table 1 shows an overview of the effective phenolic compounds for each of cumin and fennel extracts. It has been observed that the quantity of tannin and gallic acid of cumin extract had been upper than fennel extract amounting to (32.05, 33.8) ppm, respectively, while their amount of fennel extract were (20.25, 10.25) ppm, respectively. Table 1 shows the comparison of results for the presence of catechin and quercitin in fennel extract at a higher rate than cumin extract (17.36, 5.30) ppm, respectively, for
cumin extract was (6.36, 0.0) ppm, respectively, while the absence of rutin for all of the treatments.

**Predictable Phenolic Components using HPLC Analysis**

The results are indicated in Fig. 1. A comparative study among to the cumin (a) and fennel (b) extracts and standard phenolic components, it was identified as gallic acid, catechin, quercetin and tannin according to HPLC analysis. The recognition of polyphenolic compounds was done by matching retention time of the peaks with that HPLC profile standard compounds. They were recognized in the profile of cumin and fennel extracts. It has been noted from Figure 1 (a) that the emergence of four peaks to the crude extract of cumin, three of them are gallic acid, catechin, and tannin, when compared to fennel extract, is observed the emergence of eleven summits four of them are a quercetin, gallic acid, catechin, and tannin.

**Antibacterial Activity using Cumin Extracts**

It can be noted from Fig. 2, and Table 2, there were significant differences $P < 0.05$ between the types of the concentration of cumin extract against pathogeneses. Figure 2 shows that the concentration at 100 mg/ml of cumin extract increase inhibitory act towards *Streptococcus agalactiae* (B1), *Proteus mirabilis* (B2), *Enterococcus cloacae* (B3), *Escherichia coli* (B4) reaching (13, 12, 11, 9) mm, respectively (Note: the dishes were destroyed after taking the measurement of diameter inhibition zone), while a halo diameter of inhibition zone for the concentration at 50 mg/ml of cumin extract towards the same of bacteria were (12.0, 11.5, 10.0, 0.0). Figure 2 and Table 2

<table>
<thead>
<tr>
<th>Active Substance in Crude Seeds Extracts by HPLC Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Cumin</td>
</tr>
<tr>
<td>Fennel</td>
</tr>
</tbody>
</table>

![Fig 1](image-url). (a) HPLC profile of cumin extract (b) HPLC profile of fennel extract (c) HPLC profile standard gallic acid (d) HPLC profile standard catechin (e) HPLC profile standard tannin (f) HPLC profile standard quercetin (g) HPLC profile standard rutin.
In addition, these results have (B3), (B4) amounting (15, 16, 12.5, 11) mm, respectively. (Note: the dishes were destroyed after taking the measurement of diameter inhibition zone), while an inhibition zone for the concentration at 50 mg/ml of fennel extract towards the same of bacteria were (14, 10.5, 10, 10.5 ) (Table 3).

**Antioxidant Activity in Plant Medical Extracts**

There have been no results for cumin or fennel extracts as antioxidant activity.

**Discussion**

The outcomes have been in accordance with the novel studies, which mention the yields of 80% methanolic extract of fennel-powdered seeds as it was 12.11 gm. In another study, it was found that the yields of methanolic extract of different varieties of cumin seeds ranged from 4.1 to 53.6 mg/g. The outcomes of this search were harmonious who had been observed in his study that the fennel-rich phenolic and flavonoid components also showed in their study that the cumin consists of many active phenolic compounds.

These results give the impression to be consonant of modern studies, which found the gallic acid in cumin extract was the superior than others. In addition, these results have the same opinion with the previous result noticing that tannin and gallic acid in seeds of fennel extract. Also, these differences of the quantities of the effective phenolic compounds have been due to a class of crops, place of agriculture and also the season. Although, these results consistent with some published studies, they are found to differ from their extracts content of rutin and quercetin.

It can be noted from Fig. 2 and Table 2 that there were no significant differences P < 0.05 between the types of the concentration of cumin extract against pathogens. This is caused by the results of HPLC analysis of cumin seed extract which contain a number of active phenolic compounds such as tannins, catechins, and gallic acid which will be responsible for the effectiveness of inhibitory to bacteria. Cumin extracts in different solvents have many effective chemical compounds, which possess the inhibitory ability against a large number of bacteria tests. It seems from the results, all the bacteria are sensitive to the concentrations of cumin methanolic extracts except *Escherichia coli* that is resistant at concentration 50 mg/ml. This may be as a result of the possession of the bacteria to a resistant plasmid which gave a recipe-resistant bacteria.

**Table 2. The antibacterial activity of cumin extracts against some pathogenic bacteria**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Cumin concentration mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (DMSO 10%)</td>
</tr>
<tr>
<td><em>Streptococcus agalactiae</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Enterococcus cloace</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>0</td>
</tr>
<tr>
<td>LSD P &lt; 0.05</td>
<td>2.44</td>
</tr>
</tbody>
</table>

*A halo inhibition is not including the 4 mm diameter. **a methanolic extract cumin w/v.

**Table 3. The antibacterial activity of Fennel extracts against some pathogenic bacteria**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>mm diameter halo of inhibition of bacterial test*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (DMSO 10%)</td>
</tr>
<tr>
<td><em>Streptococcus agalactiae</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Enterococcus cloace</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>0</td>
</tr>
<tr>
<td>LSD P &lt; 0.05</td>
<td>4.57</td>
</tr>
</tbody>
</table>

*A halo inhibition is not including the 4 mm diameter. **a methanolic extract cumin w/v.

**Fig. 2.** The antibacterial activity of cumin extracts (D) against pathogenic bacteria (B): *Streptococcus agalactiae*, *P. mirabilis*, *Enterococcus cloace*, *E. coli*.

**Fig. 3.** The antibacterial activity of Fennel extracts (M) against pathogenic bacteria (B): *Streptococcus agalactiae*, *P. mirabilis*, *Enterococcus cloace*, *E. coli*.
towards inhibitory proficiency of effective components for the cumin extract. This result has not been agreeing with previous literature\(^4\), that there are inhibitory ability of cumin water and alcoholic extracts towards of \(E.\ coli\) at concentration 20 mg/ml were (11, 19.5) mg/ml, respectively.

Figure 3 and Table 3 show the concentrations of the extract and its rapport with the proportions of inhibition. This is due to the results of HPLC analysis of fennel seed extract which contain a number of effective phenolic compounds such as tannins, catechins, gallic acid and quercetin, which are responsible for the efficacy of inhibitory to bacteria. The outcomes of this study congruent with other searches\(^5,6\) displaying fennel extracts in various solvents have many efficient chemical compounds, which own the inhibitory ability of many pathogenic bacteria. The results indicate that all bacteria were sensitive to fennel extract, including \(E.\ coli\). This could be attributable to the entity of quercetin in fennel extract, its absence in the cumin extract, is define that one of phenolic compounds and plus other components. These results match those observed in earlier studies\(^7,8\) who have found in their studies that fennel extracts for various solvents have the high inhibitory ability versus pathogenic bacteria as well as pathogenic yeasts and fungus.

Also Tables 2 and 3 provide the results gained from the preceding test of methanolic extracts for each of cumin and fennel seed extracts. It can be noted from the Tables that the inhibitory ability of methanolic extract of fennel seeds was higher than the inhibitory ability of cumin seed extract for both concentrations as shown in the above results. This might be attributable to the ratio of catechins and quercetin in fennel extract that was higher than cumin extract, and this may lead to raising the efficiency of inhibitory of fennel extract against pathogenic bacteria. These results are different from other research outcome\(^9\) who have found in their study that the inhibitory ability of aqueous and ethanolic extracts of fennel at concentration 10–20 mg/ml were (1, 1, 5, 16) mg/ml for an aqueous extract, while ethanolic extract were (2, 16, 25.5, 18) against \(E.\ coli\) and \(S.\ aureus\) which is less than the ability of inhibitory aqueous and ethanolic cumin extracts amounting (11, 1, 13, 13.5) mg/ml for aqueous extract and were (19.5, 13, 26, 23) mg/ml for ethanolic extracts against the same bacteria. This possibly is caused by the efficiency of methanol to the extraction of phenolic compounds, which have had a significant impact in the inhibition process for the bacterial test.

The actual study exhibits phenolic compounds of the extracts as antioxidant materials. Although, all conditions of the experiment adjusted and it was returned more than once we did not get any results despite many studies have shown that extracts of cumin and fennel seeds possess antioxidant properties.\(^5,6,16\) It is difficult to explain this case, but it might be related to the method of analysis where the estimated effective antioxidant by colorimetric assay using the (ABTS) radical. This likely is because of the ABTS is a positively charged ion (cation) generated by enzymes or biochemical reactions.\(^10\) In addition, when the preparation must be under refrigerated conditions, it has been disintegrated by high temperature during preparation. Therefore, an efficient ABTS radical will be less for the estimation of antioxidants, which are naturally found in herbal medicinal plants.

**Conclusions**

Cumin and fennel seeds can be used as a possible resource for different kinds of phenolic compound extraction as its diverse therapeutic application. Methanol alcohol at concentration 70% was a suitable method to extract active chemical compound. As it is amounted to the final outcome of cumin seeds that were 41.58 mg/250 gm, while fennel seeds amounted were 37.65 mg/250 gm. The results obtained proved that cumin and fennel seed extracts have antimicrobial activity. We stand in front of an enormous wealth of medicinal plants that can be used for treatments in humans. So, we hope to take the advantage of this wealth in the field of medical treatments, and graduated from the laboratory into practical application areas, especially that Iraq has hundreds of medicinal herbs and plants, which have not been studied so far.

**Conflict of Interest**

None.

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**References**

5. AL-Wakeel MI. Extraction and purification of some phenolic compounds, which have not been studied so far from propolis and bee pollen and evaluation their inhibitory activities. Pakistan J Food Sci. 2012;22:101–107.