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 mirabillis* 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 mirabillis, Enterococcus clocae, 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 prove 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 increased[2]

The focus of this current study is on the seeds of *Cuminum cyminum L.* It is known as cumin that belongs to Family Apiaceae[3], and other seeds belong to the same family, known as fennel (*Foeniculum vulgare* Miller).[4] 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.*[5–7] High performance liquid chromatography (HPLC) is applied for the invention and investigation of active compounds quantitatively and qualitatively in plant extracts.[7] 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-3ethylbenzothiazoline 6-sulfonic acid) $C_{18}H_{24}N_6O_6S_4$, 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 mirabillis, Enterococcus clocae, 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.[3,6]

Estimated Phenolic Components by Hplc Analysis

The crude methanolic extract was subjected to HPLC to estimate quantitative and qualitative phenolic compounds.[11] 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.[6,8]

Preparation McFarland Solution

It has been prepared a solution by mixing 0.5 ml of 1.75% barium chloride $BaCl_2$. $2H_2O$ then it was added to 99.5 ml 1% sulfuric acid H_2So_4. This turbidity is equal to about 1.5×10^8 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 peptone water with turbidity McFarland solution.[9]

Estimation Antibacterial Activity Versus Bacterial pathogen

It has been used diffusion method or CUP assay method.[10] The volume inoculum was adapted to 10^8 CFU/ml for all of the test bacteria. The inoculum suspension was smeared over a Muller-Hinton agar plate and permitted to dehydrate about 15 min. Subsequently, pits had been bored in agar by using sterile cork borer. 25 µl of raw debriefed and gauged. DMSO samples were put inside a hole and the plates were kept up for 30 min at 25–30°C for spread of specimen inside holes. Next, the petri dishes had been brood for 24 hours at 37°C. Then, the inhibition area had been evaluated to the adjacent millimeter (mm) minus 4 mm hole size.

Estimated Antioxidant Activity in Plant Medical Extracts

Antioxidant activity of plant extracts was determined with some modification.[12,13]

Solution No. 1. Dimethyl sulfoxide was used to prepare a series of decimal dilutions of plant extracts between (5–500 µg/ml).

Solution No. 2. Potassium hydroxide KOH (0.1 M) was prepared to dissolve 0.561 gm KOH in some deionized distilled water. Then, it was completed the volume to 100 ml with distilled water.

Solution No. 3. Potassium acetate (0.1) M was prepared to mix 0.6 ml glacial acetic acid with some deionized distilled water. The pH was regulated to 4.7 with KOH (0.1) M. Next, it was completed to 100 ml deionized distilled water.

Solution No. 4. (1 mM) from ABTS radical was prepared to dissolve 0.066 gm Potassium persulfate in some of potassium acetate solution (0.1) M. Then, it was added 0.055 mg ABTS radical to the potassium acetate solution. After that, it has been dissolved absolutely. It was completed to 100 ml deionized distilled water.

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.

$$PI(\%) = [1-(At/Ar)] \times 100^*$$

*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 variation 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 (52.05, 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 quercetin 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.

Table 1. **The active substance in crude seeds extracts by HPLC analysis**

Active substance / Sample	Galic acid ppm	Catechine ppm	Quercetin ppm	Tannin ppm	Rutin ppm
Cumin	33.8	6.36	—	52.05	—
Fennel	10.25	17.36	5.30	20.25	—

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. Figrue 2 shows that the concentration at 100 mg/ml of cumin extract increase inhibitory act towards *Streptococcus agalactiae* (B1), *Proteus mirabillis* (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

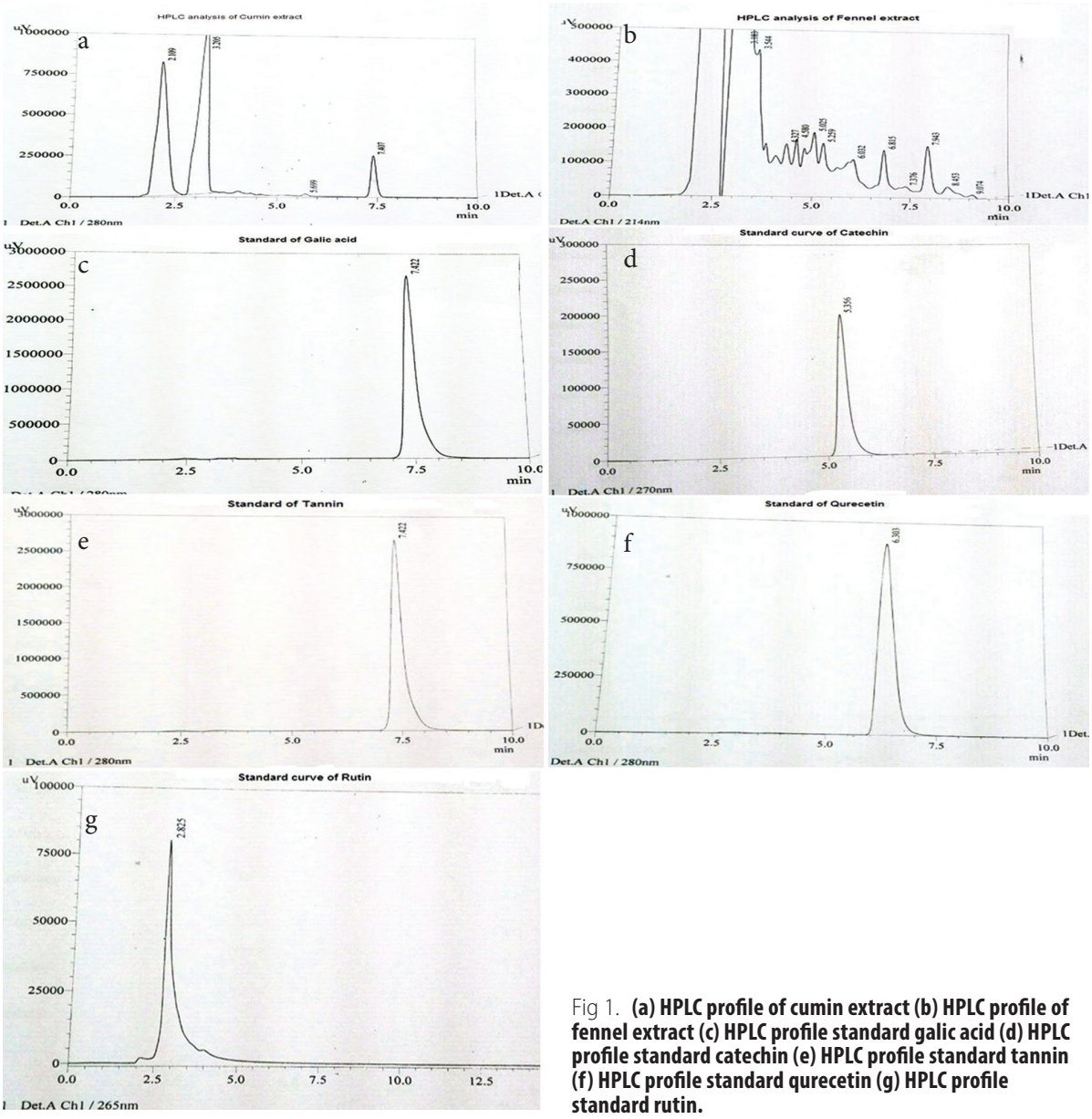

Fig 1. **(a) HPLC profile of cumin extract (b) HPLC profile of fennel extract (c) HPLC profile standard galic acid (d) HPLC profile standard catechin (e) HPLC profile standard tannin (f) HPLC profile standard qurecetin (g) HPLC profile standard rutin.**

display that the subsistence of relevance inter alia the concentricity of separated and the rates of inhibition.

Antibacterial Activity using Fennel Extracts

It has been noted that the differences are not considerable (Figure 3 and Table 3). The *P* value was noted ($P < 0.05$) between the types of the concentration of Fennel extract versus pathogenesis. Figure 3 shows that the concentration at 100 mg/ml of fennel extract increased inhibitory action towards *Streptococcus agalactiae* (B1), *Proteus mirabillis* (B2), *Enterococcus cloacae* (B3), *Escherichia coli* (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.[8] 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.[14]

The outcomes of this search were harmonious[6] who had been observed in his study that the fennel-rich phenolic and

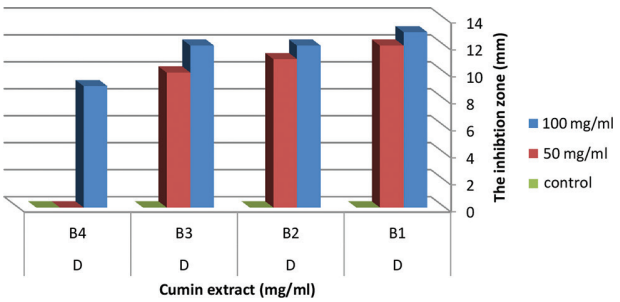

Fig 2. **The antibacterial activity of cumin extracts (D) against pathogenic bacteria (B) B_1; *Streptococcus agalactiae*, B_2; *Proteus mirabillis*, B_3; *Enterococcus clocae*, B_4; *Escherichia coli*.**

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

Bacteria	mm diameter halo of inhibition of bacterial test*		
	Cumin concentration mg/ml		
	Control (DMSO 10%)	50 mg/ml**	100 mg/ml**
Streptococcus agalactiae	0	12	13
Proteus mirabillis	0	11.5	12
Enterococcus cloacae	0	10	11
Escherichia coli	0	0	9
LSD $P < 0.05$		2.44	

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

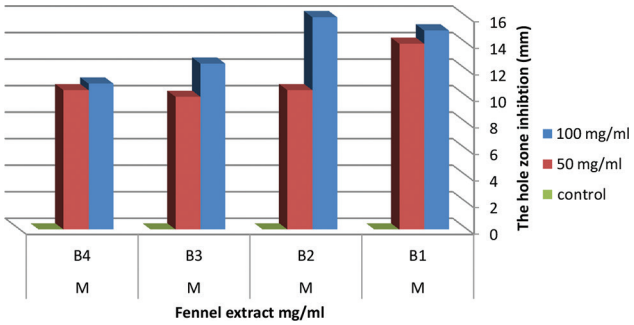

Fig 3. **The antibacterial activity of Fennel extracts (M) against pathogenic bacteria (B) B_1; *Streptococcus agalactiae*, B_2; *Proteus mirabillis*, B_3; *Enterococcus clocae*, B_4; *Escherichia coli*.**

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

Bacteria	mm diameter halo of inhibition of bacterial test*		
	Fennel concentration mg/ml		
	Control (DMSO 10%)	50 mg/ml	100 mg/ml
Streptococcus agalactiae	0	14	15
Proteus mirabillis	0	10.5	16
Enterococcus cloacae	0	10	12.5
Escherichia coli	0	10.5	11
LSD $P < 0.05$		4.57	

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

flavonoid components also[7] 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.[15,16] In addition, these results have the same opinion with the previous result[17] 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,[15–17] 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.[4,7]

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

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[4,6] 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[4,6,8] 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[4] 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.[8,14,16,17] 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.[18] 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.

References

1. Wafaa Kadhim Jasim, Mayada Sahib Hassan, Ghsoon Ghanem Keam. Study the effect of Nigella Sativa on thyroid function and reproductive hormone of female rat. J Contemp Med Sci. 2016;2:67–69.
2. Hamdii, RF. Effect of some plants extracts on some bacterial isolates causing urinary tract infection (UTI). J Sci Al-Anbar University. 2012;3.
3. Chaudhary N, Husain SH. S, Ali M. Chemical composition and antimicrobial activity of volatile oil of the seeds of *Cuminum cyminum L*. World J Pharm Pharm Sci. 2014;3:1428–1441.
4. Abdulhussein TM, Allaithi SH. M, Mahdi AJ, Mouhsen ZM. Evaluation inhibitory efficiency for some aqueous and alcoholic extracts of cumin, fenugreek, sweet fennel and black cumin in the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria. J Ibn Al-Haytham Pure Sci. 2016;In press.
5. AL-Wakeel MI. Extraction and purification of some phenolic compounds from propolis and bee pollen and evaluation their inhibitory activities against some species of Streptococcus isolated from the human mouth. MSc Thesis, College of Science, University of Karbala. 2006.
6. Dua A, Garg G, Mahajan R. Polyphenols, flavonoids and antimicrobial properties of methanolic extract of fennel (*Foeniculum vulgare Miller*). Eur J Exp Biol. 2013;4:203–208.
7. Kulkarni S, Sane A, Bhise K, Patil A, Dhamole P, Desai, SH. Development of extraction methods and quantification of safranal by high performance liquid chromatography from *Cuminum cyminum L*. and studying its antimicrobial properties. Int Cong Environl Biotechnol Chem Eng. 2014;64.
8. Anwer F, Ali M, Hussain AI, Shahid, M. Antioxidant and antimicrobial activities of essential oil and extracts of fennel (*Foeniculum vulgare Miller*). seed from Pakistan. Flavor and Fragrance J. 2009;24:170–176.
9. Arora DS, Kaur GJ. Antibacterial activity of some Indian plants. J Nat Med. 2007;61:313–317.
10. Speak M. Compendium of a method for the microbiological examination of food. 2nd Ed. Washington D.C. USA. 1984.
11. Dua A, Gupta SK, Mahajan MA. Int J Pharm Biol. 2012;3:1110–1116.
12. Budart P, Shotipruk A. Extraction of phenolic compounds from the fruit of Bitter Melon (*Momordica charantia*) with subcritical water extraction and antioxidant activities of these extracts. Chiang Mai J Sci. 2008;35:123–130.
13. Chan KW, Iqbal S, Khong N, Babji AS. Preparation of deodorized antioxidant rich extracts from 15 selected spices through optimized aqueous extraction. J Med Plants Res. 2011;5:6067–6075.
14. Nadeem M, Riaz A. Cumin (*Cuminum cyminum*) as a potential source of antioxidants. Pakistan J Food Sci. 2012;22:101–107.
15. Al-Hashemi FH. Chromatographic separation and identification of some volatile oils, organic acid, phenols from the seeds of *Cuminum cyminun* growth in iraq. Int J Recent Res Appl Stud. 2014;19:80–90.

16. Gupta D. Comparative analysis of spices for their phenolic content, flavonoid content and antioxidant capacity. Am Int J Res Formal Appl Nat Sci. 2013;13:38–42.
17. Christova-Bagdassarian VL, Bagdassarian KS, Atanassova MS. Phenolic compounds and antioxidant capacity in Bulgarian plants (dry seeds). Int J Adv Res. 2013;1:186–197.
18. Bondet V, Brand-Williams W, Berset C. Kinetics and mechanisms of antioxidant activity using the DPPH free radical method, Lebensmittel-Wissenschaft und Technologie. 1997;30:609–615.
19. Wojdyło A, Oszmianr'ski J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem. 2007;105:940–949.