RESEARCH ARTICLE

Gas Chromatography-Mass Spectrometry Profiling of *Pimpinella anisum* Oils and its Antimicrobial and Antioxidant Activities

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ABSTRACT

Essential and fixed oils of anise plant *Pimpinella anisum* growing in Iraq have been investigated regarding their chemical components, antioxidant, and antimicrobial properties. Essential oils were extracted using the Clevenger-apparatus, while fixed oil was extracted using a Soxhlet apparatus. Gas chromatography-mass spectrometry (GC-MS) was used for the analysis of the oil components. Six strains of bacteria, namely *S. epidermidis, S. aureus, E. coli, B. cereus, P. vulgaris,* and *S. typhimurium* were tested against the antimicrobial activity of each oil. Anise oil demonstrated a broad antibacterial property range, against gram-positive and gram-negative bacteria, through the inhibition zone. The antibiotic sensitivity test was performed by disk diffusion process against the test organisms. The agar dilution method was used at five different concentrations (12.5, 25, 50, 100, and 200 mg/mL) throughout the test. The minimum inhibitory concentration (MIC) was determined for each volatile and fixed oil. The DPPH radical scavenging assay was used to test the antioxidant activities of essential and fixed oils. Anise oil showed excellent antioxidant activity, in comparison with the reference compounds. Anise oil has the potential to be used as a therapeutic, antimicrobial, and antioxidant agent.

Keywords: Antibacterial, Antioxidant, Essential oil, Fixed oil, GC-MS analysis, *Pimpinella anisum*.

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INTRODUCTION

Umbelliferae (Apiaceae) is the most temperate herbal plants, with umbellate inflorescences, consisting of more than 3,000 species and over 300 genera. There are pairs of fruits or seeds. Plants, as members of this family, are commonly utilized in the food industry and medicine. Many plants in this family, such as, parsley, celery, and carrots, are popular vegetable crops. In contrast, others, such as cumin, anise, and fennel, are known for their therapeutic and fragrant characteristics.[1,2] Anise [*Pimpinella anisum L*. (*P. anisum L*.)], is an annual grassy plant with a white flower, which is grown in warm regions around the globe.[3] About 1.5 to 3.5% of the mass of anise seed are essential oil (volatile oil), consisting of mainly cis- and trans-anethole. The key ingredient of anise (trans-anethole) is used, in the synthesis of different pharmaceutical products, as a substrate.[4,5] Fatty oils (non-volatile) are derived from the Umbelliferae family. The potency of the seed oils obtained from Apiaceae fatty oils depends on the chemical structure of the fatty acid and the composition of minor constituents in the oil.[6] The main fatty acid constituent in oils is petroselinic acid, which is common in the Apiaceae seeds, compared to oleic acid. Aside from oleic acid, a small residue of one more isomer, cis-vaccenic acid is also noticed.[7,8] The volatile oil is applied as a carminative, in cough medicine, particularly for pediatric applications. The significant phenyl-propane, such as, estragole and trans-anethole, has a stabilization effect on the autonomic nervous system.[9] In traditional medicine, anise was applied as a tranquilizer, a diuretic, and appetizer. It was documented that it has various healing properties in various cases, such as, gynecology, respiratory, neurology, and digestive illnesses.[10] Several recent studies showed that oils with plant origin exhibit good antioxidant activity against several microorganisms. Nowadays, plant oils could be used as an alternative to synthetic packing materials that are harmful to human health.[11,12] Moreover, spices are also considered as a source of antioxidants due to the large contents of bioactive compounds, such as, phenolic compounds, flavonoids, tannins, phenolic diterpenes, sulfur-containing compounds, alkaloids, and vitamins. Also, polyunsaturated fatty acid (PUFA) compounds can be obtained from some spices, such as the seeds of Apiaceae.[13,14] Thus, this study aimed to examine the antimicrobial and antioxidant properties and chemical composition of essential and fixed oils in the anise seeds.

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MATERIAL AND METHODS

Plant sample collection of *P. anisum* seeds was carried out in Mosul, Iraq, from a store specialized in seed. Identification of the plant has been done by specialists from the College of Agriculture and Forestry, University of Mosul, Iraq. The plant was washed in order to remove all contaminants and dirts.

Oil Extraction from Seeds of *P. anisum*

Hundreds of dried *P. anisum* seeds were pulverised, using an electric crusher, and the extraction was performed, using a Soxhlet apparatus with 500 mL hexane for 72 hours. Using a rotary evaporator, the extract was sifted and dissipated under the reduced pressure. The extracts were then packed into sterile bottles and placed in a 2 to 4°C refrigerator, for further application.

Isolation of Essential Oil

Essential oils were extracted from clean seeds of *P. anisum* by hydro-steam distillation, using the Clevenger apparatus, and stored in sterile collection tubes. In brief, in the distillation flask (1 L), 100 to 150 grams of the plant was used. The flask was connected to the steam generator by means of a glass tube, and a condenser for oil recovery in a funnel tube. The essential oils are aromatic compounds, extracted from the plant, and dissipated into hot steam, pushing the material away from the plant to separate the essential oil, by not destroying the constituents. To condense the vapor, containing the essential oil, a cooling system was used. The steam was used for 3 hours. After the extracted solution had been deposited, the separation of essential oil was performed. The obtained oil goes under the process of filtration and drying through anhydrous Na_2SO_4. Immediately, the essential oil was collected and stored in a freezer.

Analysis of GC-MS

Samples were analyzed by GC-MS QP 2010 Ultra (Shimadzu) with capillary column Rtx-5MS (30, 0.25 mm; 0.25 µm coating thickness). The Wiley data library was used for both fatty acid and essential oil to identify the chemical components. Experimental criteria are added to fit the specifications of the Wiley Software library.[15]

Microbial Cultures

As test microorganisms, six strains of bacteria were used. Gram-positive bacteria such as *S. epidermidis, S. aureus, B. cereus,* and gram-negative bacteria such as *E. coli, P. vulgaris,* and *S. typhimurium* were included. Clinical isolates were all microorganisms, and defined quite carefully, using normal microbiological methods.

Antimicrobial Testing

On Mueller Hinton broth, the antimicrobial activities of *P. anisum* seed extracts were investigated by the disc diffusion method. A 24 hours bacterial culture was grown at 37°C and reseeded on the Nutrient Broth. The cultures have been adjusted with sterile water to 5.6 × 106 CFU/mL. To achieve a uniform microbial growth on broth control and test surfaces, 1 mL of the suspension was applied to the plates, having Mueller Hinton broth. Seed extracts (essential and fixed oil) have been independently dissolved and sterilized in ethylene glycol and DMSO (30 mg/mL), under aseptic conditions. The empty sterilized disks (Whatman paper, 6 mm diameter thickness) were placed on the agar surface while permeating with 20 µg of seed and oil extracts. The plates are left at room temperature for 30 minutes to allow the extracts of seed or oil to spread, and then incubated at 37°C. The inhibition zone was measured in millimeters after the incubation time (24 hours). The negative controls were prepared with the same solvents. Amikacin and gentamycin have been used for comparison, as standard antibiotics. The values of MICs are classified as the minimal concentration of any agent needed to inhibit the visible growth of organisms on the agar plate.

Analysis of Antioxidants

The DPPH radical scavenging property was conducted by a process described previously.[16] About 0.1 mM of DPPH solution was dissolved in methanol, and 1 mL of this solution was combined with 3 mL of extract solution at different concentrations (8, 40, and 80 µ/mL) and incubated for 30 minutes in the dark. The absorbance was measured at 517 nm. Quercetin has been used as a compound of reference. Higher reaction mixture absorbance suggested the increased free radical scavenging activity. All experiments were performed in triplicate. The radical scavenging activity was determined by the following formula as a percentage of DPPH discoloration:

$$\text{DPPH percentage of radical scavenging} = [(A0 - A1)/A0] \times 100$$

Where A0 is the control's absorption, and A1 is the extract's absorption.

RESULTS AND DISCUSSION

Traditionally, as a medicinal plant, *P. anisum* is used heavily in Iraq. Various studies have been conducted on extracts (essential and fixed oils) of *P. anisum* to determine the chemical compositions and therapeutic characteristics of the herb. Aniseeds have been reported to have different properties, such as, antimicrobial and antioxidant activities. The components were accepted as the main constituents of the oils. The results of GC-MS were scanned against both databases of essential oil and fatty acids, and their main components are shown in Tables 1 and 2. The results of the MIC test are shown in Table 3.

Composition of *P. anisum*

The oil obtained from *P. anisum* seeds was analyzed by GC-MS. About 10 compounds (essential oil) were found in aniseed. Trans-anethole was a major component of essential oil (26.97%), followed by estragole (20.50%), longifolene (11.99%), linalol (11.30%), and unidentified (6.68%), but low levels of other compounds (Table 1). Plant essential oils are combined compositions of volatile organic elements that play important roles in the environment for both humans and the plant itself. The possible biological information kept in essential oil composition may give an insight into the plant's

silent culture, and the roles in defense of these chemical products, communication and attracting of pollinators.[17] The most volatile components of these oils, which vary only in the percentage, are trans-anethole, estragole, longifolene, etc, the different characteristics and distinctive smell of each volatile oil can be related to these compounds.

Fixed Oil Composition of *P. anisum*

The qualitative and quantitative improvements in *P. anisum* fixed oil profile are also shown in Table 2. The seed oils have shown a number of different fatty acids (percent), by comparing the analytical data, whereas, petroselinic acid was a major component of fixed oil (15.54%), followed by synthila (13.92%), anisole (13.59%), and pentadecanoic acid (11.68%), but other compounds were in low levels. The biosynthesis of palmitic and petroselinic acids during maturation was in reverse, which could be attributed to the palmitic acid, as a precursor of petroselinic acid.[18] Yetim *et al.*[19] found the most unsaturated fatty acid (UFA) in aniseed oil (85.64%), consistent with our results. The highest value of petroselinic acid was found in anise (65.18%).[20]

Antimicrobial Activity

The antimicrobial property of *P. anisum* seeds oils are shown, as an inhibition zone (mm) in Tables 3 and 4. The results showed that *P. anisum* essential and fixed oil have significant antimicrobial activity against all six bacterial types. They have had very similar antimicrobial activities in general. The *S. aureus* and *B. cereus* were sensitive to *S. epidermidis* essential oil. The *P. anisum* oil against, *E. coli* and *S. Typhimurium* was equally sensitive, in terms of antimicrobial activity, but the sensitivity was more than *P. vulgaris,* compared to antibiotics (Table 3).

The antibacterial activity of fixed oil*,* against tested bacterial strains, was evaluated *in vitro*, using the disk diffusion method. Fixed oil had an antibacterial effect against tested gram-positive and gram-negative bacteria. *S. aureus* showed the highest sensitivity to the fixed oil, while the sensitivity was equal against *B. cereus* and *P. vulgaris,* as well as, for *S. epidermidis,* and *E. coli*, but it has a minimal effect on *S. typhimurium* compared to antibiotics (Table 4).

Table 1: Essential oils of *P. anisum* seeds

No.	*Compound names*	*%*[1]	*RI*[2]	*RT*[3]
1	Trans-anethole	26.97	1,190	4.347
2	Longifolene	11.99	1,471	9.167
3	Unidentified	2.89	1,403	9.689
4	Alpha-terpineol	4.21	3,267	16.712
5	Cis-anethole	4.36	1,917	19.154
6	Fenchone max	6.53	2,764	21.710
7	Estragole	20.50	1,369	21.843
8	Linalol	11.30	2,085	21.930
9	Longifolene	4.57	2,439	25.503
10	Unidentified	6.68	2,149	26.823

[1] Compound percentage; [2]Retention index; [3]Retention time

The biological type of plant material depends on various factors, such as, the plant geographical source, harvest time, soil status, drying system, moisture composition, post-harvest processing, and storage status. The plant antimicrobial properties of numerous plants are measured and tested,[21,22] and the mechanisms that enable natural herb and spice constituents to fight against the microbes were discussed.[23] The data demonstrate that these mechanisms are changing and widely relying on the plant constituents.[24,25] The antibacterial efficacy of pharmaceutical plants is significantly destabilized, relying on the phytochemical characteristics of subfamilies and families. It is not unexpected to see the difference in effectiveness even when using samples from similar plants, but from two different districts.[26] The medicinal use of aniseed is due mainly to its plant antibacterial effects.[27]

Antioxidant Activity

The DPPH free radical scavenging and quercetin were evaluated as references or positive controls, to determine the antioxidant activity of essential and fixed oils. The maximum radical scavenging activity of DPPH was observed at a concentration of 40 µg/mL, containing 91.18% of the essential oil, whereas the standard highest radical DPPH scavenging activity was 85.32% at the same concentration (Figure 1).

The percentages of inhibition of DPPH radical have been calculated to test the antiradical activity of the extract from fixed oil. The highest radical scavenging activity of DPPH at the concentration of 40 µg/mL was detected in the sample at 87.84 percent. In comparison, the standard highest DPPH radical scavenging activity was 85.32% with the same concentration (Figure 2).

Table 2: Fixed oil content of *P. anisum* seeds

No.	*Compound names*	*%*[1]	*RI*[2]	*RT*[3]
1	Anisole	13.59	1,190	4.527
2	3-Hydroxymandelic acid	1.39	1,460	5.181
3	1-[2-(3,4-Dimethoxy-phenyl)-ethyl]-3-phenethyl-thiourea	1.01	3,026	15.654
4	Palmitic acid	2.50	1,878	17.584
5	Pentadecanoic acid	11.68	1,869	18.440
6	Linoleic acid	4.11	2,093	20.810
7	Petroselinic acid	15.54	2,085	20.972
8	(+)-2-Norcepharanthine	2.51	4,931	21.670
9	Cis-Vaccenic acid	10.11	2,175	21.801
10	Docosyl acetate	1.76	2,574	23.024
11	Dimestrol	3.84	2,304	25.042
12	Benzene, 1,2-dimethoxy-4-[[(4-methylphenyl)sulfonyl] methyl]	6.76	2,465	27.223
13	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl)	11.28	2,788	27.977
14	Synthila	13.92	2,304	28.090

[1]Compound percentage; [2]Retention index; [3]Retention time

Table 3: The antimicrobial activity of essential oil from *P. anisum* extract

Bacteria mg/mL	*S. aureus*	*S. epidermidis*	*B. cereus*	*E. coli*	*P. vulgaris*	*S. typhimurium*
200	28	26	27	24	20	24
100	23	21	23	21	15	20
50	21	16	21	15	12	16
25	16	12	10	11	8	13
12.5	10	9	8	-	-	9
			*Antibiotics**			
Amikacin	22	21	24	25	23	21
Gentamycin	25	22	23	22	24	26

*Disc diameter 6 mm

Table 4: The antimicrobial activity of fixed oil isolated from *P. anisum* extract

200	27	25	26	25	26	20
100	21	21	21	22	20	21
50	18	20	15	20	14	16
25	13	15	13	13	11	12
12.5	10	11	9	11	-	10
			*Antibiotics**			
Amikacin	22	21	24	25	23	21
Gentamycin	25	22	23	22	24	26

*Disc diameter 6 mm

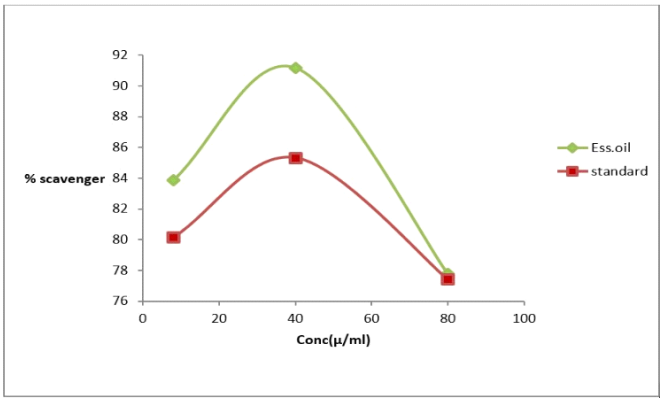

Figure 1: The antioxidant activity of *P. anisum* essential oil

In recent years, it has been growing interest in the antioxidant and anti-cancer activity of the various essential oils, and in many instances, such bioactivities were due to the presence of active compounds.[28,29] Another biological property of great interest is the antioxidant activity of oils, because they can protect food from the toxic characteristics of oxidants.[30] In addition, essential oils that can also scavenge free radicals may play an important role in the prevention of certain disorders, such as brain damage, heart disease, cancer, and weakening of the immune system. There has been growing evidence that cell damage induced by free radicals can cause these complications.[31,32]

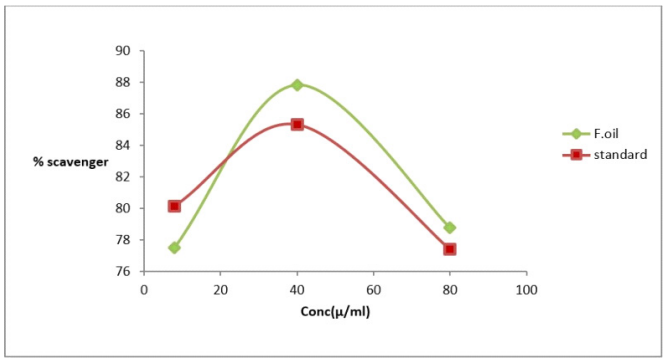

Figure 2: Antioxidant activity of *P. anisum* fixed oil

CONCLUSION

Based on our data, anise seed oils have shown pharmacological properties, such as antibacterial and antioxidant activity, due to their various chemical contents. Anise seeds exhibit remarkable and strong antibacterial and antioxidant activities. Among the various constituents present in the seed of anise plant are the essential oils and fatty acids, which are considered as the most important and active compounds. The bioactive molecules of the anise seed can be used for different drug production and antimicrobial agent synthesis.

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