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Identification of Anise Seed Oils and their Antimicrobial and Antioxidant Activities

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Abstract

Anise (*Pimpinella anisum*, Apiaceae) is an annual herbaceous plant used traditionally in folk medicine in Sudan. The objectives of this work were to identify the composition of oil extract of anise seed, and study its antimicrobial and antioxidant activity. Anise seeds were collected randomly from El-Obeid local market. Using gas chromatography/mass spectrometry 29 compounds including Anesole, Enisole P.allyl, Limonone, Mionene and Palmicinic acid were identified in the extracted oil. The diffusion method was used for assessing the antimicrobial activity using five different concentrations of the oil (10%, 5%, 2.5%, 1.25%, 0.63%) against four bacteria species (*Staphylococcus aurous*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*) and two fungi species (*Candida albican* and *Aspergillus niger*). The results showed that 10% concentration exhibited the highest activity against *P. aeruginos* with inhibition zone diameter of 20.22 mm. The 5%, 2.5%, 1.25% concentrations showed a comparable activity against *E. coli* and *B. subtilis* with inhibition zone diameter in the range of 17 to 18mm. The concentration 0.63 % showed weak activity against all the tested microorganisms except against *P. aeruginosa* that showed an inhibition zone of 17 mm diameter. The inhibition zone of *C. albican* was higher than that of *A. niger*. The antioxidant activity of the extracted oil was evaluated using the standard 2,2 diphenyl - 1 - picrylhydrazyl (DPPH) 0.5 ml. The antioxidant activity of the extracted oil was 22.84±1.0.

Introduction

Plants have played a significant role in providing human and animal feeds and availing traditional medicines (Craig, 1999). Aromatic plants and their

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essential oils have antibacterial and antifungal effects and are used in cosmetic industries (Singh *et al*., 2002). According to (Lopez-Bote *et al*., 1998) they posses antioxidants properties

Anise (*Pimpinella anisum* L.), is an annual herbaceous plant whose seeds are used in traditional medicine (Sengul, 1994; Amin, 2005; Özcan and Chalchat, 2006; Al-Mofleh *et al*., 2007). According to Sengul (1994); Zargari (1996) and Özcan and Chalchat, 2006) these seeds are rich in mineral and important compounds such as anethole, p-anisaldehyde, anise alcohol, acetophenone, pinene and limonene glycerol.

The seeds are an excellent source of many essential B-complex vitamins such as pyridoxine B6, niacin, riboflavin, and thiamin (Basher, 1997).

The medicinal use of anise seeds is largely geared to pharmaceutics (Ahmad *et al*., 2004); perfumery, food and cosmetic (Ross, 2001).

It is extracted oil is for combating microorganisms, insects and nematodes (Elgayyar *et al*., 2001) and as antioxidant (Tunc and Sahinkaya, 1998; Özcan and Chalchat, 2006). It is also used for flavorings because of its aromatic characteristics (Sengul, 1994; Zargari, 1996). In some parts of India people believe that anise seeds are helpful in cancer prevention and treatment (Zaid *et al*., 2010).

The present work aimed to extract, identify the chemical constituents of the essential oil, and test their antimicrobial and antioxidant activities of the extracted oil.

Materials and Methods

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The anise seeds were purchased from the local market in dry form.

Microorganisms:

Gram +ve bacteria (*Bacillus subtilis* NCTC 8236 and *Staphylococcus aurous* ATCC 25923); Gram -ve bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) and fungi (*Candida albicans* ATCC 7596 and *Aspergillus niger* ATCC 9763) used in the study were obtained from the stock culture of the Medicinal and Aromatic Plant Researches Institute.

Methods

Gross chemical composition

Moisture, protein, oil, and ash % were determined following AOAC (1994).

Extraction of anise seeds essential oil

In a 1000 ml round bottom flask, 100gm of anise seeds were mixed with 500 ml distilled water and extracted using a Clevenger apparatus (Duran West Germany). The Clevenger apparatus was heated to 100°C for about 4 hours till the volume of oil above water layer in the receiver is constant. Using a pipette the oil was collected, dried under anhydrous sodium sulphate and stored in a dark container at 4°C.

Preparation of agar media

2.5g nutrient agar were dissolved in 100 ml distilled water, sterilized by autoclave at 121°C for 15 minutes and kept in a refrigerator. 3.2g sabouraud dextrose agar were dissolved in 100ml distilled water and treated similarly.

Preparation of bacterial suspensions

One ml aliquots of a 24 hours broth culture of the tested organisms were aseptically

ISSN: 1858-7658 (Online)
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distributed onto nutrient agar slopes and incubated at 37 °C for 24 hours. The bacterial growth was harvested and washed with 100 ml sterile normal saline, to produce a suspension containing about 10^8-10^9C.F.U/ ml, the suspension was stored in the refrigerator at 4 °C till used.

Preparation of fungal suspension

The fungal cultures were maintained on sabouraud dextrose agar incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally the suspension was stored in the refrigerator till used.

Testing essential oil for antimicrobial activity

The disc diffusion method was used to screen the antimicrobial activity of the essential oil and performed by using Muellar Hinton Agar (MHA). One hundred micro liters of bacterial suspension were swabbed uniformly on the surface of MHA and the inoculums were allowed to dry for 5 minutes. Sterilized filter paper discs were placed on surface of the MHA and soaked with 20µl of essential oil, the inoculated plates were incubated at 37°C for 24 hours in the inverted position. The experiment was carried out following 1999 standards of the National Committee for Clinical Laboratory standards.

Antioxidation activity

The antioxidant activity of the essential oil was measured *in vitro* using 2,2-diphenyl-1-picrylhydrazyl(DPPH**)** according to the method of Shimada *et al*. (1992).

Results and Discussion

ISSN: 1858-7658 (Online)
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The gross chemical composition of anise seeds

The results of chemical composition study of anise seeds are shown in Table (1). The results showed that it has high protein and low oil content. This is reflected in its high digestibility due to low fat to protein ratio. The essential oil was 2.5%. A range between 1.5% and 6% was reported by Sengul (1994). The calories in anise seeds should not be ignored on the grounds that small amounts of it are taken (www.calories.info/food/herbs-spices, 2016). The calculated calories 3.19/g is comparable to the value of 3.5 given by nutritiondata.self.com/facts/spices-and-herb/184/2, 2016.

Table 1. The chemical composition of anise seeds

Content	Unit
Moisture	14.7 %
Protein	42.8 %
Oil	2.5 %
Starch and Fibers	30 %
Ash	10 %
Fat : Protein	0.06:1.0 ratio
Calorific value	319 per 100g

The retention data and chemical composition of 29 essential oils in the anise seeds extract are presented in Fig. 1 and Table 2.

The essential oils composition determined in our study showed

1. Pentaborane (11) exhibited the lowest area and molecular weight.

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2. Benzene 1-mothoxy-4-(1-propenyle) anethole showed the largest area.
3. The least and highest retention time was shown by Limonene [1-methyl-4(methylethenyl) and Hexadecamic acid, respectively.
4. Butanoic Acid 4-methozy-2-(3-methylozirane)phenyl ester showed the highest molecular weight.
5. Similarity in molecular weight was found between: Limonene [1-methyl-4(methylethenyl) and Benzaldehyde(4 methoxy) Benzaldehyde(4 methoxy); AnisoleP.allyl and Benzene 1-mothoxy-4-(1-propenyle) anethole, and between Naphthalene and Zengiberene (Table 2).

Table 2. Identified of some compounds of essential oil of anise seeds determined by GC/MS.

ISSN: 1858-7658 (Online)
ISSN: 1858-7690 (Print)

Peak	Area	RT	Formula	MW	Name of compound
1	0.66	14.059	$C_{10}H_{16}$	136	Limonene [1-methyl-4(methylethenyl)
2	3.47	19.433	$C_{10}H_{12}O$	148	AnisoleP.allyl
3	1.10	21.146	$C_8H_8O_2$	136	Benzaldehyde(4 methoxy)
4	67.63	22.032	$C_{10}H_{12}O$	148	Benzene 1-mothoxy-4-(1-propenyle) anethole
5	0.32	22.217	B_5H_{11}	66	Pentaborane(11)
6	2.41	27.085	$C_{15}H_{24}$	204	Naphthalene
7	1.13	27.380	$C_{15}H_{24}$	204	Zengiberene
8	0.64	30.733	$C_{15}H_{24}O$	220	Decahydro-1,1,trimethyle-4-methylene(-)-pathulenol.
9	15.39	35.236	$C_{15}H_{20}O_3$	248	2-(-E propenyle.2.(1E.propenyle)-4-methoxyphenyle2-mothybunnoate
10	5.60	36.361	$C_{15}H_{20}O_4$	264	Butanoic Acid 4-methozy-2-(3-methylozirane) phenyl ester
11	1.64	37.571	$C_{16}H_{32}O_2$	256	Hexadecamic acid

Anise seeds contains at least 29 compounds such as Butanoic Acid 4-methozy-2-(3-methylozirane)phenyl ester, Limonene [1-methyl-4(methylethenyl) and Benzene 1-mothoxy-4-(1-propenyle) anethole. Its richness in compounds has been indicated by Sengul (1994); Zargari (1996); Basher, 1997and Özcan and Chalchat, 2006).

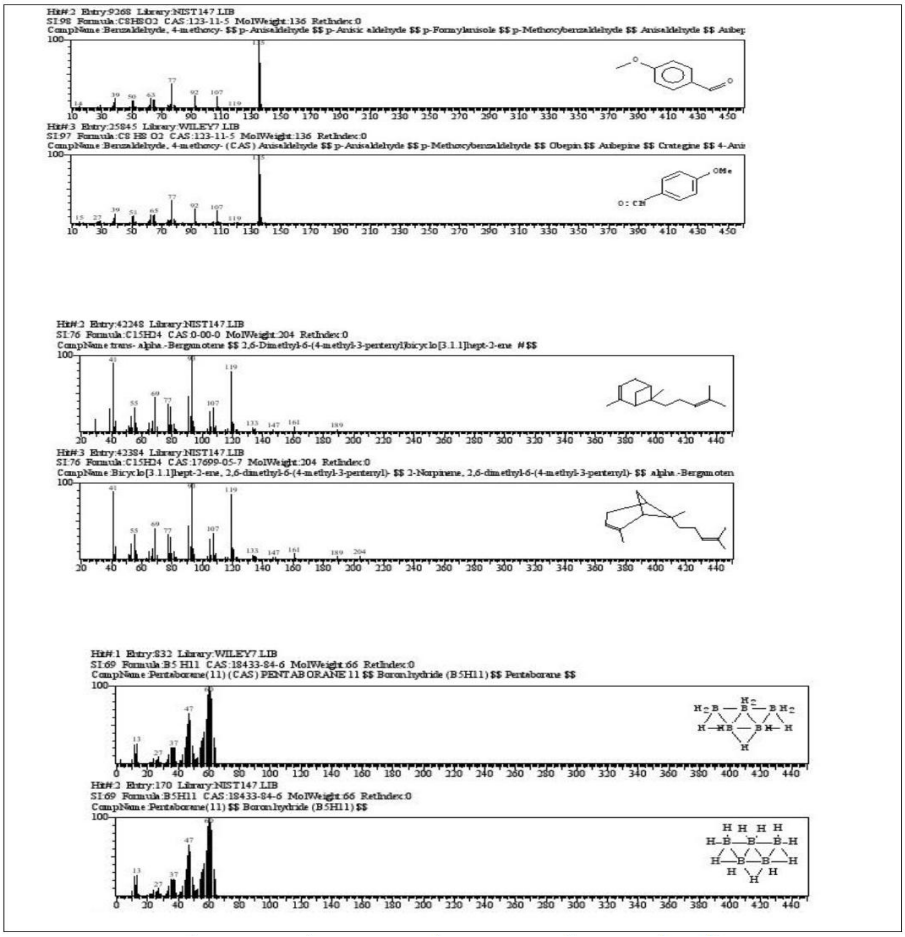

Fig. 1. Gas chromatography-spectra of some anise oils

Antimicrobial activity:

All essential oil obtained from anise seed showed antibacterial activity against *B.subtitles*, *S. aureus*, *E. coli*, *P. aeruginosa* and antifungal activity against *C.albicans* and *A. niger* (Fig. 2). The strongest antibacterial and antifungal effect of anise oil extract was attained by 10%

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concentration. The present findings confirmed the findings of Tunc and Sahinkaya (1998), Singh *et al*. (2002) and Özcan and Chalchat (2006) regarding the antimicrobial effects of this herb.

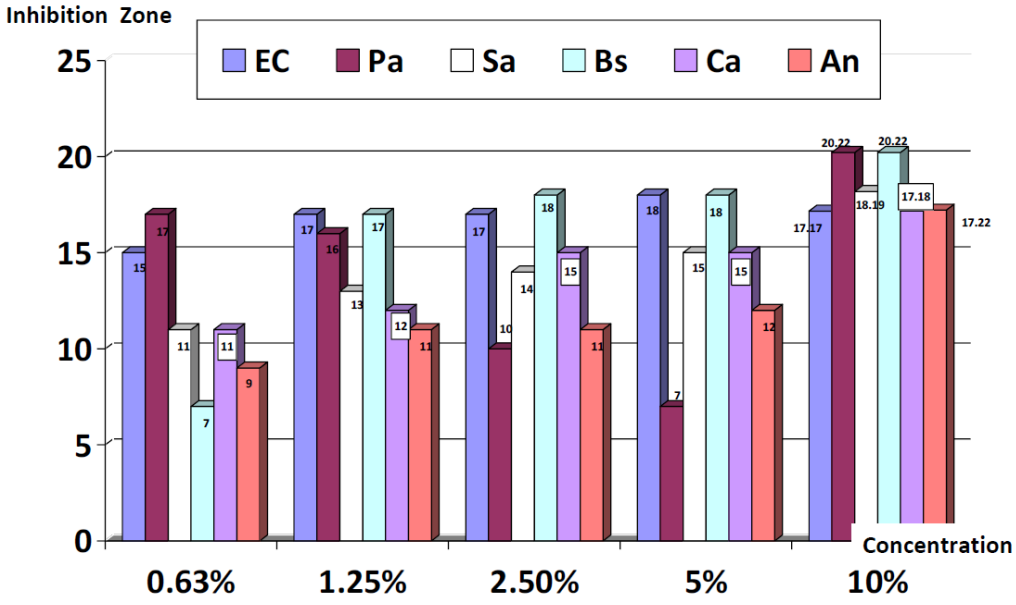

Fig. 2. Inhibition zone in mm of different essential oil concentrations against bacteria and fungi

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Antioxidant activity:

The absorbance of the essential oil, the Propyl Gallate standard (STD) and DMSO without 2,2-diphenyl-1-picrylhydrazyl (DPPH**)** was higher when DPPH added (Table 3). The absorbance was comparable in case of DMSO. The percentage of DPPH radical scavenging activity (RSA) of essential oil was 25% lower than STD (Table). The results indicated that anise seeds contain anti-oxidant compounds. This is in agreement with (Lopez-Bote *et al*., 1998; Tunc and Sahinkaya, 1998; Özcan and Chalchat, 2006). Basher (1997) attributed this antioxidant activity to its content of vitamin-C and vitamin-A. Zaid *et al*. (2010) based on traditional medicine practices in India suggested that consumption of anise seeds is of value in cancer prevention and treatment.

Table 3. Absorbance of essential oil, STD and DMSO without the addition of DPPH and RSA activity

Material tested	Mean absorbance value with DPPH		RSA
	Not added	Added	
Essential oils	1.392008	0.510598	22.84099
STD	1.317894	0.079574	86.79785
DMSO	1.301691	1.301490	

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