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ORIGINAL RESEARCH PAPER

CHEMICAL COMPOSITION AND ANTIBACTERIAL PROPERTIES OF ESSENTIAL OILS OF *Pimpinella Anisum L.* GROWING IN MOROCCO AND YEMEN

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Abstract: We report in this study the chemical composition and antibacterial activities of the seed's essential oils of *Pimpinella anisum* L. collected from Morocco and Yemen. The hydro-distillation technique was used to extract their essential oils, followed by continuous liquid-liquid fractionation using water and ethyl acetate as solvent system. Obtained essential oils were analyzed by gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS). GC and GC/MS results showed that 4-allylanisole was the major compound of *Pimpinella anisum* L. with percentages of 76.70 and 85.28% of Moroccan and Yemen, respectively, in addition to other minor compounds such as limonene (9.75% for Moroccan species and 5.53% for Yemen species) and fenchone (6.16% for Moroccan species and 4.12% for Yemen species). Furthermore, both essential oils were evaluated for their antibacterial activity against a panel of pathogenic microorganisms. The results showed that both essential oils inhibit most pathogenic bacteria tested.

Keywords: *antibacterial, essential oils, food-borne diseases, GC-MS, hydro-distillation, Pimpinella anisum L.*

INTRODUCTION

Plant and herbal preparations have been used numerously throughout history for the treatment of various diseases. The plant *Pimpinella anisum* L. is a well-known annual herb with white flowers and small green to yellow seeds, that grows in India, Egypt, Turkey, Iran, and many other warm regions of the world [1-2]. It was reported that *Pimpinella anisum* had several therapeutic effects such as neurologic, digestive, gynecologic and respiration disorders. In addition, it was demonstrated that the Anise showed ovicidal activity against stored-product insects [3]. In addition, *Pimpinella anisum* displayed another biological activity in the Central Nervous System (CNS) field. Indeed, the extract oil of this plant has been reported to delay the onset of picrotoxin-induced seizures in mice [4] and anethole possesses muscle relaxant effect. *Pimpinella anisum* is primarily grown for its fruits, commercially called "seeds" that are currently used for flavouring. Furthermore, essential oils from *Pimpinella anisum* fruits are valuable in perfumery and in medicine domains [5].
Due to the increasing of human consumption demand for more natural nutrition, the abuse of toxic synthetic food substances and the increasing of resistance of pathogenic microorganisms against antibiotics, natural isolated substances from plants are considered as promising natural sources of preservatives food [6-11]. Consequently, a strong and increasing search for natural substances isolated from plants as antimicrobial agents are growing up. These agents are extracting from aromatic plants and in particularly essential oils [12]. In this direction, phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases including viral infections [13]. Several studies have been carried out to extract various natural products for screening antimicrobial activity but no intensive attention has been focused on the study of combinations of these products [14].
Herein, we have investigated the determination of the chemical composition as well as the antibacterial activity of the essential oils of Anise (or sweet Alice) seeds.

MATERIALS AND METHODS

Plant material

Anise was collected from the North of Yemen (Sa'adh) and middle of Morocco (Meknes) in December 2009. Anise seeds samples were dried at room temperature for 21 days.

Essential oil extraction

The dried seeds were used for essential oils extraction Essential oils were extracted by hydro-distillation technique. The principle consisted of immersing the dry seeds of *Pimpinella anisum* L. (200 g) in distilled water contained in around glass flask (boiling flask 5 L). This mixture was heated until boiling for 3 hours and the produced vapor carrying the volatile substances (essential oil) was then passed through a cooling system (condenser) where condensation occurred. Essential oils were collected from the surface

of water and then stored at a temperature of +4 °C in well-filled, tightly closed glass vials wrapped in aluminum foil to avoid exposure to light and oxygen [15].

GC-MS analysis

About 10 μL of sonicated anise extract in mixture with methanol, chloroform and *n*-hexane was analyzed by GC–MS using Ultra Trace GC (Themo-Fisher Scientific) composed of a VP-5 capillary fused silica column (30 m, 250 µm, 25 µm film thickness) and a Polaris Q Themo-Fisher Scientific as mass spectra detector. The oven temperature was held at 60 °C for 2 min and then programmed with the rate of 16 °C/min to reach 280 °C in 20 min. Additional operating conditions are the following: He (99.99 %) as carrier gas, 76 kP as inlet pressure, linear velocity: 20 cm/s; injector temperature: 220 °C; detector temperature: 300 °C and 1:25 as split ratio. The identification of the components was based on comparison of their mass spectra with those of Wiley and NBS Libraries [16] and those described by Adams [17]. Furthermore, the components relative concentrations were calculated based on GC peak areas without using correction factors.

Antibacterial activity

Evaluation of antibacterial activity was carried out using isolated standard Gram-positive and Gram-negative strains as indicated in Table 1. The microorganisms were stored on Muellere Hinton Agar (Bio-Rad) at 4 °C and were obtained from the culture collection of the Laboratory of Medical Bacteriology, INH, Rabat, Morocco. The nutrient broth (Bio-Rad) and the Muellere Hinton agar were used, respectively, for growing and diluting the microorganism suspensions for the antimicrobial assays. The essential oils were dissolved in Tween 30 %. A 0.1g mL^{-1} of initial concentration was obtained.

Table 1. *Origin of tested bacterial strains*

Bacterial groups	Bacterial strains tested	Origin
Cocci Gram-positive	*Streptococcus pyogenes*	urinary infection
	Streptococcus pneumoniae	urinary infection
	Streptococcus songuins	skin infection
	Staphylococcus epidermidis	urinary infection
	Staphylococcus aureus Methicillin-resistant	nosocomial infection
	Staphylococcus aureus	urinary infection
Bacilli Gram-negative	*Pseudomonas aeruginosa*	urinary infection
	Acinetobacter baumannii	nosocomial infection
	Pseudomonas fluorescens	otitis
	Salmonella enteritidis	food borne illness
	Salmonella enterica typhimurium	food borne illness
	Salmonella aerizonae	food borne illness
	Hafnia alveie	urinary infection
	Yersinia enterocolitica	food borne illness
	Escherichia coli	urogenital infection
	Klebsiella pneumoniae	urinary infection

Antibacterial activity of seed essential oils was assessed using the agar well diffusion method [18], and were prepared in the plates with the help of a cork-borer (0.6 cm). Finally, 50 µL of the tested essential oil was introduced into the well, and then the plates were incubated for 24h at 37 °C. The diameter of the visible zone showed the absence of growth. For each bacterial strain studied, controls were maintained. The result was obtained by measuring the zone diameter and experiments were repeated three times. The mean values are presented. The determination of MIC of the CFS against microbial strains pathogen is performed according to the technique in microtiter plates, and showed in Table 3 [19].

RESULTS AND DISCUSSION

Chemical composition of the essential oil

The essential oils extracted by hydrodistillation from Anise seeds are colorless and present a pungent odor at room temperature. The composition of the essential oils was determined by gas chromatography-mass spectrometry on the basis of the GC retention times as summarized in Tables 2. The structures of the major compounds are presented in Figure 1.

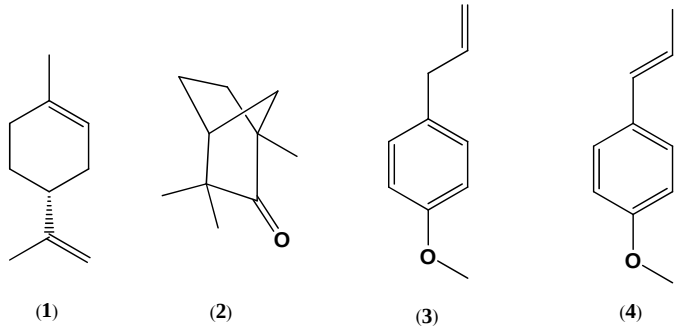

Figure 1. *Chemical structures of Limonene* ***(1)****, Fenchone* ***(2)****, Anisole* ***(3)****, Anethole* ***(4)***

Table 2*. Chemical composition of Pimpinella anisum* L. *essential oil*

Compound	Amount, % (Marocco)	Amount, % (Yemen)	RT(min)
Camphène	trace	trace	8.81
Limonene	9.75	5.53	12.23
Fenchone	6.16	4.12	14.33
4-allylanisole	76.70	85.28	18.53
Anethole	7.40	3.54	21.34
Acide linoléique	tace	trace	40.21

Antibacterial activity

The antibacterial activity of Anise seeds essential oil was determined against different pathogenic bacterial strains. The zone of inhibition, measured in millimeters, including the diameter of the well, was used as the criteria of the antibacterial potency. The essential oils of the two plants with a mean antimicrobial growth inhibition zone of 9.0-30.0 mm against *Streptococcus pyogenes, Streptococcus pneumoniae, Staphylococcus*

epidermidis, Streptococcus songuins, Staphylococcus aureus Methicillin-resistant, Staphylococcus aureus, Acinetobacter baumannii, Hafnia alveie, Yersinia enterocolitica, Klebsiella pneumoniae, Escherichia coli and Salmonella arizonae was observed (Table 3). Under the same experimental conditions, 50 µL (initially at 0.1 g·mL^{-1}), *Pseudomonas aeruginosa, Salmonella enteritidis, Salmonella enterica typhimurium* and *Pseudomonas fluorescens* displayed strong resistance against both tested essential oils, while *Acenitobacter baumannii* was the most sensitive strain. Interestingly, these data indicate that Gram-positive bacteria are the most sensitive strains for the different essential oils with the mean zone of inhibition growth ranking between 9 and 30 mm. Our results are in good agreement with previous works [21] showing a weaker activity of essential oil of *Salvia tomentosa*, which is rich in camphor and carvacrol, against Gram-negative bacteria.

Table 3. *Antibacterial activity and minimum inhibitory concentrations (MIC) of essential oils of Pimpinella anisum L.*

Microorganism	***Essential oils***		
	MIC	***ID*** *(Inhibition Diameter)*	
		Marocco	**Yemen**
Streptococcus pyogenes	50	10	10
Streptococcus pneumoniae	25	26	26
Streptococcus songuins	50	16	16
Staphylococcus epidermidis	50	11	10
Staphylococcus aureus Methicillin-resistant	50	12	12
Staphylococcus aureus	50	14	15
Acinetobacter baumannii	25	30	30
Salmonella aerizonae	50	12	14
Hafnia alveie	50	9	10
Yersinia enterocolitica	50	10	12
Escherichia coli	25	21	20
Klebsiella pneumoniae	100	9	8

Minimum inhibitory concentrations (MIC)

As shown in Table 3, the MIC values of the essential oils against the bacterial strains shoed that both essential oils displayed broad antibacterial effects against all six Gram-positive bacteria: *Streptococcus pyogene, Streptococcus pneumoniae, Streptococcus songuins, Staphylococcus epidermidis, Staphylococcus aureus Methicillin-resistant* and *Staphylococcus aureus,* and six Gram-negative bacteria: *Escherichia coli, Acenitobacter baumannii, Hafnia alveie, Salmonella arizonae, Yersinia enterocolitica and Klebsiella pneumoniae,* with MIC values of 25, 25, 50, 50, 50 and 50 mg·mL^{-1}, respectively.

CONCLUSION

Qualitative and quantitative analysis of essential oils showed the presence of six compounds in anise extract collected from Morocco and Yemen. The major component in both anise essential oils is 4-allylanisole, with a small percentage difference between both essential oils. The bacterial activity against pathogenic strains of bacteria was

performed using the agar well diffusion technique and showed that essential oils displayed very interesting antibacterial activity. These biological activities differ according to the bacterial strain tested. The first interesting results reported in this paper show the antibacterial activity against Gram-positive and Gram-negative strains of essential oil of anise, and open a new interesting approach to develop plants as natural source and preservative for the food industry.

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