Antibacterial Activity of Pistacia Khinjul Fatty Acids Extract on Some Pathogenic Bacteria

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Abstract

Background: Some bacteria have resistant against antibiotic, so several trail made to use plant extract as antibacterial against some pathogenic bacteria

Objective: To compare and evaluate the difference between two species of *Pistacia khinjuk* (P. khinjuk) oil extract present in Kurdistan (Iraq) marked in their contain and antibacterial effect against some pathogenic bacteria

Patients and Methods: Essential oil was extracted from two species *Pistacia khinjuk* present in Kurdistan (Iraq) marked during the period of February to April, 2016 and evaluated the difference between two sizes components and their antibacterial effect against some pathogenic bacteria. Their activity is reasonably due to their ability to complex with extracellular and soluble proteins also to complex with bacterial cell walls. Also, known that *P. khinjuk* had anthocyanin pigment within its component Extraction was done using methanol and direct pressing methods. The essential oil extract analysed by HPLC (High-performance liquid chromatography) and tested against different pathogenic isolated bacteria (Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa). The activities were considerably dependent on concentration of two types of seed.

Results: Successfully essential oils of locally big and small *Pistacia khinjuk* (*P. khinjuk*) seed were extracted . Both big and small seed oil gave a similar component of fatty acid (oleic, linoleic and lauric acid), while the small seed had more oil extract. P. khinjuk essential oil extract inhibited growth of the three isolated bacteria (Staphylococcus aurous, Escherichia coli and Pseudomonas aeruginosa). Both big and small seed oil extract had the same PH (about 4). Results showed that maximum absorption to extract pure pigment anthocynin from P. khinjuk oil was at wavelength 479 nm which has reached the highest concentration of the dye. The reason can be attributed to the purity of the color and the presence of some of the other pigments.

Conclusion: Extracted essential oil noted antimicrobial activity and was characterized mostly by the occurrence of flavonoid and flavonoid glycosides. Difference between the absorbency of pure pigment anthocynin was attributed to the purity of the color and the presence of some of the other pigments.

Key words: Plants extracts, Pistacia khinjuk, Plant oil.

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Introduction

The fruits of Stocks are composed of antioxidant, highest total phenol and flavonoid in different parts of the fruit. There is a higher correlation between the various antioxidant activity and total phenol and flavonoid contents. Whatever the phenol compounds mainly responsible for

the antioxidant power of the fruit extracts [1]. Studies investigated the largest producer of Pistacia species in the world is Iran, with over 44% of the world production [2], and a few places such as the Zagros Mountains, where wild pistachio persists in natural and extensively managed stands [3].It's growing slowly in tough and drought condition in Kurdistan Mountains in -Iraq [4].There are two species of P. khinjuk exist one of them is granule known as Bnawsh of Qazwan, and the other biggest size known as only Qazwan [5].

Pistacia is a genus of flowering plants and belongs to the family of Anacardiaceous which comprises 11 species [6]. Reviews suggested that plants have long provided humankind with a source of medicinal agents, including natural products once serving as the origin of all drugs [7].

The Pistacia species have been used in folk medicine as antibacterial, antiviral, antifungal, anti-inflammatory, and antipyretic agents and as astringents in the treatment of diarrhea, throat infections, and disorders of the liver, kidney, heart, and respiratory system [8-10].

The primary goal of this present research was to compare and evaluate the difference between two species of P. khinjuk oil extract present in Kurdistan (Iraq) marked in their contain and antibacterial effect against some pathogenic bacteria.

Patients and Methods

Two types of *P. khinjuk* samples were collected from Erbil locally marked and were classified in collage of Agriculture University of Salahddin -Erbil, Iraq during the period of February to April, 2016.

Extraction methods

Methanol extraction method:

Two hundred fifty gram of *P.khinjuk* samples were immersed in methanol (250 ml) and incubated at room temperature for three days. Later were filtered across what-man filter papers (No.1).

Finally extracted oil was concentrated by evaporation using hot plate stirrer at 40°C. Extracted oil dissolved in concentration 0.025 gm /milliter of dimethyl sulfoxide as stock standard solution.

Direct pressing extraction:

The *P. khinjuk* were pressed at room temperature using a commercial machine compress the extract oil was concentrated by evaporation using hot plate stirrer at 40oC.Laterkept away from light, placed in Refrigerator [11].

Characteristic of both products:

P. khinjuk oil extract were characterized, measuring final volume, pH, color and odor.

- HPLC-UV analysis [12].

Antibacterial test:

The in vitro antibacterial activity of different extracts of P.khinjuk oil extract at 25, 50 and 75 mg/ml was studied by disk diffusion method against three standard strains of bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*). The bacterial strains were cultured on Muller Hinton Agar and disks placed for comparison. The Petri dishes were incubated at 37°C for 24 h. After 24 h, inhibition zones appearing around the disks were measured and recorded in mm [13-14].

-Concentration of Anthocyanin:

Determination of Anthocyanin pigment in *P. khinjuk* oil was done using 70% alcohol and 3% acetic acid. Concentrated at 45oC using rotary evaporator, later cooled to 5oC and calculation [15].

Results

Results in Table (1) presented oil extraction by both methanol and direct pressing method of *P. khinjuk*. Results

showed that percentage of *Pistacia khinjuk* oil extract using direct pressing (14.34%, 16.14%) in both big and small seed respectively were more than that of methanol extraction method (12.04%, 13.31%).While the small *P. khinjuk* seeds gave more oil extraction than the big seed. The calculate yield percentage of both products by: % oil extraction = weight of oil extract × 100 / total weight of *P. khinjuk* [2] .Those differences may be due to higher impurity in pressing method.

Table (1): Percentage of P.khinjukusing methanol extraction and direct pressing extraction.

Size of Pistaciakhinjuk	%oil extraction from solvent	%oil extraction from pressing
Big shape	12.04	14.34
Small shape	13.31	16.14

Both big and small essential P.khinjukoil pH was measured andgave similar degree (about 4).

HPLC analysis shows that there are three major peaks with different retention times, for both samples as in figure (1) for small seeds and figure (2) for big seeds. Where, the retention times in figures (1 and 3) refer to the samples as follows(Table 2):

Table (2): Different retention timesforHPLC analysis.

Retention time	5.71	6.55	8.53
Sample	Lauric acid	Linoleic acid	Oleic acid

From the HPLC charts we can recognize the difference in the peak height between the two samples, the highest peaks in figure (1) refer to estimated high concentration of fatty acids in small size seeds, this result satisfy the percentage of small seeds using oil extraction from solvent compare with direct pressing extraction as in table (1).

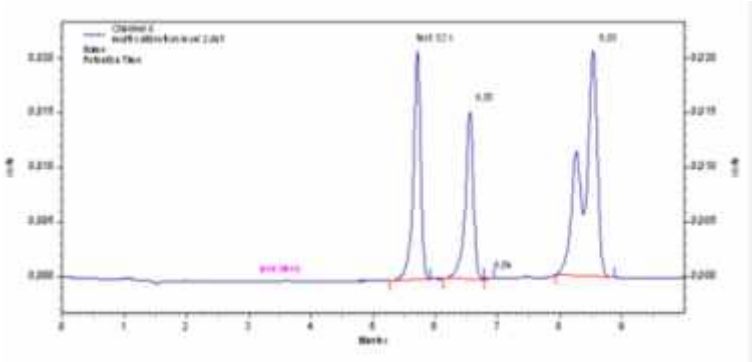

Figure (1): High-performance liquid chromatogram *P. khinjuk* small seed extract oil**.**

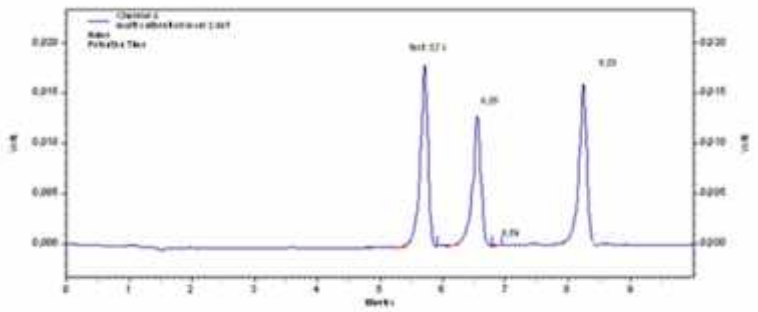

Figure (2): High-performance liquid chromatogram *P. khinjuk* big seed extract oil.

Table (3): Antimicrobial activity of *P. khinjuk* essential oil.

Bacteria species	Inhibition zone diameter(mm.) in different percent of oil					
	25%		50%		75%	
	Small	large	Small	large	Small	large
S.aureus	7	5	10	8	13	11
E.coli	8	6	12	9	16	13
Pseumonus aerogenosa	7	5	10	7	14	12

Table(3) represented the antibacterial results showed that the Pistaciakhinjuk essential oil extract inhibited the three bacteria (Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus and the activities were considerably dependent upon concentration of oil and sizes of Pistacia khinjuk. The extract with the highest antimicrobial activity was that of P. khinjuk (inhibition zone 13-16 mm in small and 11-13 in large sizes). They are characterized mostly by the occurrence of flavonoid and flavonoid glycosides[16].

Table(4) showed that maximum absorption to extract dye from P.khinjuk oil was at wavelength 479 nm.whichhas reached the highest concentration of the dye.

Table (4): Concentration of Anthocynin pigment in P.khinjuk essential oil.

Wavelength(nm)	400	420	440	480	500	520	550
Anthocyanin Absorption	0.251	0.202	0.22	1.65	0.105	0.062	0.157

Discussion

Essential oil wasextracted from two species *P. khinjuk* and evaluated, results showed that the small *P. khinjuk* seeds gave more oil extraction than the big seed. Those differences may be due to higher impurity in pressing method. The results were satisfying antimicrobial characterized mostly because of the occurrence of flavonoid and flavonoid glycosides which is effective antimicrobial substances against a wide array of micro-organisms. Their activity is reasonably due to their ability to complex with extracellular and soluble proteins also to complex with bacterial cell walls [17].

Results showed that maximum absorption to extract dye from *P. khinjuk* oil has reached the highest concentration of the dye. The reason can be attributed to the difference between the absorbency of pure pigment anthocynin.

The purity of the color and the presence of some of the other pigments in the extract do not resolve the impact of transactions Thermal extraction and concentration in the stability of the dye, as well as the effect of light, oxygen and oxidizing enzymes.

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