In Vitro Evaluation of the Antimicrobial Activity and Physical Properties of Pistacia eurycarpa Yal Gum, An Ethnobotanical Study from Kurdistan Region

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Abstract
The present investigation examined the physical qualities and in vitro antimicrobial potential of Pistacia eurycarpa Yal, From the heritage of folk medicine in Kurdistan. Gum samples were collected from males and females on three different dates, Amada village (36. 7849546°N, 44. 0569152°E) and Banka village (37°09'55.0"N, 43°10'07.4"E) on August 15, September 15, and October 15, 2024 in Kurdistan region. Physical properties considered were gum weight, moisture content and oil content. The antimicrobial activity was assessed with Staphylococcus aureus and Staphylococcus haemolyticus, Escherichia coli, and Klebsiella pneumonia, using the agar well diffusion method. The highest gum yield was recorded on August 15, from male trees in Banka (23.587 g) followed by those from Amada (22.764 g). Subsequently, gum production reduced in later months with male trees always bearing more gum than females. The maximum moisture content on October 15 was 17.333% for female trees from Amada, other samples being generally a bit lower. The result showed the maximum oil content on August 15 in male trees from Amada (1.000%), with moderate variation across the locations and dates. The most potent antimicrobial activity was found in gum from female trees in Amada on September 15, which showed inhibition zones of 11.130 mm for S. haemolyticus, 10.650 mm for S. aureus, 5.466 mm for Klebsiella pneumoniae, and 4.466 mm for E. coli. Antimicrobial activity and physical properties were significantly affected by harvest date, tree gender, and site of collection, with Amada samples showing superior performance overall.
Keywords: Pistacia eurycarpa Yal, Antimicrobial activity, Gum exudates, Ethnobotany.

Introduction

Kurdistan Region of Iraq is part of a biodiversity hotspot in the Zagros Mountains. The region is famous for its plant diversity and rich ethnobotanical traditions. Among the many plants, Pistacia eurycarpa Yal, which is commonly known as "Qezwan" has gained an important place in traditional Kurdish medicine. The gum, which is an exudate of natural resin, is applied by rural populations for the treatment of various ailments, including gastrointestinal problems, wounds, oral infections, and respiratory problems (5.(
The emergence of such pathogens as antibiotic-resistant ones has accentuated the inevitable requirement to discover and develop novel antimicrobial agents from natural sources. Medicinal plants and medicinal values of their derivatives, known for their therapeutic properties, have been recognized for a long time, particularly in traditional health care systems. The most of resins and gums of species from the genus Pistacia have been widely investigated for their broad-spectrum biological activities, including antimicrobial, antioxidant, and anti-inflammatory properties (1&7.(
Pistacia eurycarpa Yal known locally as " Qazwan" in Kurdish dialects, is a wild pistachio species prevalent in mountainous

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areas of the Kurdistan Region. Its gum exudate has been traditionally applied by Kurdish communities for wound healing, dental care, and gastrointestinal ailments, reflecting its deep ethnobotanical relevance (11). Though, scientific validation of those uses is very limited in terms of antimicrobial efficacy, as recorded in ethnomedicine.

This study investigates the in vitro antimicrobial activities of the gum obtained from Pistacia eurycarpa Yal collected in different ecological zones in Kurdistan Region. The study intends to investigate sample variability with respect to bioactivity as a result of different geographical locations.

These four clinically relevant strains from bacterial pathogens will be tested for antimicrobial properties in the current research, Escherichia coli is a Gram-negative enteric pathogen causing gastrointestinal infections and now becoming more associated with urinary tract and extra intestinal infections (12). Enterococcus faecalis is a Gram-positive organism which usually is associated with urinary tract bloodstream and hospital-acquired infections (4). Staphylococcus aureus is responsible for most skin, respiratory, and bloodstream infections, including strains that are methicillin resistant (23), while Staphylococcus haemolyticus a coagulase-negative staphylococci is becoming an important opportunistic pathogen mainly in device-related and nosocomial infections due to its multidrug resistance (6). The pathogens thus represent both Gram-positive and Gram-negative organisms, thus providing a balanced evaluation of the antimicrobial spectrum on the gum.

This study is an effort to aid the fast-growing number of researches that seek to integrate ethnobotanical knowledge with modern microbiological validation, thus establishing the prospects for future applications of Pistacia eurycarpa gum as a natural antimicrobial. The study further emphasizes the need to preserve the medicinal plant heritage of Kurdistan while stimulating pharmacological examination of its native flora .

Material and Methods

Study period and location

The research was carried out at the College of Agricultural Engineering Sciences, University of Duhok, Iraq, from August 15th -2024 to Jun 15th 2025.

Sampling area

This Research was done up in the Kurdistan Region of Iraq in two locations of different altitude. The first site was Amada Village (36. 7849546°N, 44. 0569152°E), lying in the Akre District, approximately for 100 Km east of Duhok city, namely near the town of Akre. The second site was Banka Village (37°09'55.0"N, 43°10'07.4"E) which is near Duhok city.

The two sites differ in altitude: Banka Village is situated at around 1300 m above sea level while Amada Village is located around 850 m.

Table 1: Meteorological data of Banka and Amada locations from Jun –November at 2024.

Month	Average									Sum
	Banka					Amada				
	Evaporation (mm)	Humidity (%)	Temperature (°c)		Rainfall (mm)	Evaporation (mm)	Humidity (%)	Temperature (°c)		Rainfall (mm)
Jun	175.65	24	23.43	34.81	0.4	202.17	17	25.30	39.30	0.08
July	201.03	23	24.73	37.34	0	260.83	14	26.30	40.30	0
August	182.10	21	24.98	38.10	0	196.50	14	28.70	42.80	0.3
September	136.19	33	19.86	32.36	44.6	148.22	17	23.40	36.20	2.41
October	93.09	33	13.21	24.73	6.2	112.48	31	18.50	30.00	29.7
November	48.96	64	6.89	15.76	75.4	49.99	31	10.40	21.40	66.7

Tree Selection and Sampling Procedure

For each location selected for a study, a total of six mature Pistacia eurycarpa trees were picked, comprising three male and three female trees, with the male and female identification based on their floral structures. The selected trees were aged between 50 and 100 years to assure maturity and reliable gum production. For these trees, four sampling points were established

at breast height (130 cm from the ground) for uniformity of collection. These points were situated on the North, South, East, and West aspects of each tree trunk; the gum was collected for three consecutive months to observe seasonal variations in yield and characteristics

Collection occurred at August 15th, September 15th, and15tho of October. The voucher specimen of the named plant (No. 4256) has been lodged at the herbarium of the Forestry Department/ College of Agricultural Engineering Sciences/University of Duhok/Iraq Kurdistan Region.

Soil Analysis

A physical and chemical soil analysis was performed on both studied sites to

assess certain soil conditions that influence wild Pistacia growth as shown in table (2) and compared with the best range for plant growth refer to the reference (47-52.(

Table (2): Physical and Chemical Soil Analysis for both studied locations (Amada and Banka villages.(

Parameters	Best range for plant growth	Banka Sample	Amada Sample	Units
pH	6-7.2	7.63	7.74	----
EC	<2	0.1794	0.1836	dS.m-1
Available Potassium	>110	255	450	mg/Kg
Available Phosphorous	10-20	5.175	3.89	mg/Kg
Available Nitrogen	>140	176.53	238.17	mg/Kg
Organic Matter	>3	4.49	2.25	%
CaC03	<10	7.36	10.90	%
Clay	30	23.35	20.85	%
Silt	40	5.47	35.47	%
Sand	30	ok31	3".6 u	%
Soil Texture	loam	loam	loam	----
Moisture%	-	15.6	7.9	%

The moisture content of Pistacia eurycarpa gum was determined using the method by (3). with some modifications. Fresh samples of 3 grams were collected from the male and female trees under all treatments for both sites on three different sampling dates. Each sample was placed in a pre-weighed crucible (W_0) and weighed with the crucible (W_1). The crucibles holding the samples were then put into a forced draft oven at a temperature of 98-100 °C for 24 hours. After drying, the crucibles
were removed, and weighed again (W_2) after cooling in a desiccator. This drying process was repeated a number of times until constant weight (W_2) were observed to ensure complete moisture removal. This procedure was conducted for all treatments. The formula used to calculate the water content (%) in each sample is as follows:

Moisture content (%) = $[(W_1 - W_2) / (W_1 - W_0)] \times 100$

W0 is the weight of the crucible alone, W1 is the weight of the crucible plus sample before drying and W2 is the weight of crucible plus sample after drying.

Determination of Crude Oil

The determination of crude oil content from Pistacia eurycarpa gum-dried, was done in a Soxhlet as per (3. method with minor modifications. A total of 2.0 grams of gum samples were prepared from male and female trees from both locations on three different extraction dates. Each sample was placed into a labeled extraction thimble, covered with cotton wool, and extracted with 100 ml of 96% hexane in a 250 ml boiling flask. The Soxhlet apparatus was fitted, and extraction was refluxed for 6 hours. The thimbled were removed after extraction, and the boiling flasks were placed in a hot air oven at 70 °C for complete solvent evaporation. The flasks were dried, cooled in a desiccator, and

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weighed. Crude oil content was calculated for all treatments using the below formula.

Crude Fat Content (%) = (Weight of extracted fat (g) / Weight of Sample (g)) × 100.

Where weight of extracted fat is the difference between the final weight of the flask (after solvent evaporation) and the original weight of the empty flask.

Essential Oils for bioassay

The gum exudates of Pistacia eurycarpa were harvested from male and female trees from two sites on three dates. The samples were air-dried, grounded, and stored under sterile conditions until extraction. Approximately 5 g of each sample were dissolved in 10 mL of pure analytical-grade DMSO, briefly vortexed, and stirred at room temperature for 12 hours. The mixture was filtered through Whatman No. 1 paper to eliminate any residues. The DMSO extracts served as stock solutions for antimicrobial assays .

Antibiotic Discs

The antimicrobial susceptibility of human-derived bacterial isolates was evaluated with two methods in this study. At Ghazi Specialized Medical Laboratories, Duhok, Iraq, isolates were tested with the VITEK system against 13 clinically relevant antibiotics (Ampicillin, Cefepime, Ceftazidime, Ciprofloxacin, Clindamycin, Erythromycin, Gentamicin, Imipenem, Meropenem, Tetracycline, Tigecycline, Sulfamethoxazole/Trimethoprim, and Vancomycin(

Bacterial Isolates

The bacterial strains used in this work were isolated from clinical samples collected at Ghazi Hospital Laboratory in the Duhok Governorate of Iraq. The isolates included Gram-positive and Gram-negative bacteria typically found to be associated with human infections. Gram-positive species included Staphylococcus aureus and Staphylococcus haemolyticus, both of which can cause bacteremia, wound infections, and conjunctivitis (18). Gram-negative strains include Escherichia coli and Klebsiella pneumonia, which are often linked with urinary tract infections, pneumonia, bloodstream infections, and gastrointestinal diseases (22). Identification and confirmation of the bacterial strains were carried out according to the standard microbiological and biochemical techniques as per the protocols of Ghazi Laboratory.

Determination of the antibiotic and EOs sensitivity profile

Antimicrobial activity of Pistacia-derived EOs was evaluated on Gram-positive bacteria (Staphylococcus aureus and Staphylococcus haemolyticus) and Gram-negative bacteria (Escherichia coli and Klebsiella pneumoniae). All clinical isolates were confirmed at the Microbiology Laboratories of Central Laboratory, College of Veterinary Medicine, University of Duhok using the VITEK 2 Compact system.

The assay was performed by the agar diffusion method using CLSI guidelines (13). The essential oils derived from pistachio gum were collected under different conditions, including collection date, geography, and the tree's gender, then applied in the pure format. Overnight bacterial cultures were suspended to an approximate 0.5-McFarland turbidity standard (approximately 1.5 x 10^8 CFU/mL). Different agar media were selected according to the growth requirements of each of the bacterial species. Mueller-hinton agar and mannitol salt agar were used for the culture of Staphylococcus aureus and Staphylococcus haemolyticus, while MacConkey agar and blood agar for Escherichia coli and Klebsiella pneumoniae respectively. The respective bacterial suspensions were uniformly spread over the surface of the respective agar plates using sterile cotton swabs. 15 µL of pure EO were deposited directly at the marked spots on the agar surface .

The plates were kept for 24-48 hours at 37 degrees Celsius. The zones of inhibition were measured after incubation using a vernier caliper. For interpretation purposes, CLSI standards were applied, and in the case of human isolates, those from (46) were

followed. Sensitive or resistant classifications were made, whereas the intermediate results in this study were interpreted as resistant.

Statistical Analysis

The gathered data submitted to SPSS software (19) in order to analyze it statistically. Descriptive statistics and Two-way ANOVA were applied. Duncan's Multiple Range test (9) for separating means within each factor were used. However, the same statistical program was used for drawing the charts.

Results and Discussion

The results in (table2) were observed that the weight of gum from Pistacia eurycarpa varied with collection date, site, and tree gender. The male trees produced a maximum record for gum weight on august 15, 23.587 g at Banka, followed by 22.764 g at Amada. Values were generally lower for female trees at both sites, with Amada recording values of 16.696 g whereas Banka evaluated values of 12.877 g. There were also differences on September 15, but for Banka male trees produced more weight of gum (18.828 g) as opposed to females (13.548 g). The Amada figures were of lower yields for both genders, with the males producing 10.237 g and females 7.299 g. By 15 October, the gum yields generally reduced, with the male trees in Amada producing 11.743 g and those in Banka producing 5.126 g, while female trees produced 9.578 g and 3.292 g respectively. Over all the dates and places of study, male trees produced consistently heavier gum exudates than females, and gum weights were generally higher in August than in later months.

Gum yield from Pistacia eurycarpa differed quite extensively with respect to the date, site, and tree gender: it recorded its highest production in mid-August during hot and dry conditions. Male trees produced more gum than females in both sites, particularly in that of Banka (23.587 g), which confirms studies that dry-adapted species produce more resin under water stress and high temperatures (36&27). Though Amada has higher nitrogen and potassium figures, Banka had more elevation (1300 m), which may have offered a more favorable microclimatic and soil condition for gum exudation than others (25). Both sites had loamy soils slightly alkaline (pH ~7.6-7.7), and Amada's increased $CaCO_3$ content might have contributed to a limited phosphorus availability that could negatively affect gum yield (28). The consistent superiority of male trees over females in gum production indicates physiological differences that appear to be internal. These have been observed in other dioecious resin-bearing species as well (26). All in all, results underscore the extent environmental stress, elevation, and gender within the same species affect gum production.

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Table (3): Effect of Collection Date, Location, and Tree Gender on the Weight of Pistacia eurycarpa Gum.

Mean of Dates	Dates& Location	Gender		Location	Dates
		Female	Male		
18.981a	19.730 a	16.696cab	22.764a	Amada	15-Aug
	18.232 a	12.877cadb	23.587a	Banka	
12.478b	8.768 c	7.299cd	10.237cdb	Amada	15-Sep
	16.188 ab	13.548cadb	18.828ab	Banka	
7.435c	10.660 bc	9.578cdb	11.743cdb	Amada	15-Oct
	4.209 c	3.292d	5.126d	Banka	
Location		10.548b	15.381a	Mean of Gender	
		14.786b	23.175a	15-Aug	Effect of Dates& Gender
		10.424bc	14.532b	15-Sep	
		6.435c	8.435bc	15-Oct	
13.053 a	Amada	11.191a	14.914a	Amada	Effect of Location & Gender
12.876 a	Banka	9.906a	15.847a	Banka	

followed the same letter for each factor and interaction do not differ significantly from each other's according to Dun n's Multiple range Test t 5% level.

Table (4) showed that the moisture content of the gum of Pistacia eurycarpa is also that it is variable under the influence of collection date, location, and gender. The highest moisture content, 14.333%, was recorded on August 15 for gum from female trees in Banka, followed by 15.266% for that of male trees of Amada. The moisture levels of gum from male trees in Banka (10.766%) and female ones in Amada (9.266%) were rather lower. But on September 15, moisture content decreased in both locations, ranging from 11.733% in male trees from Amada to 5.466% in female trees from Banka. It is noticeable that on October 15, female trees of Amada were having the highest moisture content of gum (17.333%), with that of male in the same place (12.133%) and that of male trees in Banka (11.633%) following next. This suggests that female trees tended to have higher moisture content in their gum exudates later in the season, especially in Amada, while moisture levels, in general, declined from August to September and rose slightly again in October.

Moisture content in gum from P. eurycarpa was affected by date of collection, location, and gender of the trees; the former two suggest environmental influence, while the latter hints at physiological influence. Seasonal decrease in moisture content was likely governed by rising temperatures and evaporation, while higher moisture content of the gum from female trees in late season points to possible gender effects on gum composition and water retention (26). However, environmental conditions—such as altitude and organic matter in the soilalso play a significant role, with a cooler environment at higher elevations leading to a stabilizing effect on moisture. Plant-derived exudates have varying moisture contents, often due to timing of secretion and environmental stress, as the for moisturizing application, gums must be said to act under detrimental environments (30.)This indicates that gum quality is a result of complex interactions between plant father physiology and local environmental conditions.

Table 4: Effect of Collection Date, Location, and Tree Gender on the Moisture Content of Pistacia eurycarpa Gum

Mean of Dates	Dates &Location	Gender		Location	Dates
		Female	Male		
12.408b	12.266c	9.266g	15.266b	Amada	15-Aug
	12.550b	14.333c	10.766f	Banka	
7.850c	10.166e	8.566h	11.733e	Amada	15-Sep
	5.533f	5.466i	5.600i	Banka	
13.308a	14.733a	17.333a	12.133d	Amada	15-Oct
	11.883d	12.133d	11.633e	Banka	
Location		11.183a	11.194a	Gender	
		11.800c	13.016b	15-Aug	Dates &Gender
		7.016e	8.683d	15-Sep	
		14.733 a	11.883c	15-Oct	
12.388a	Amada	11.722b	13.055a	Amada	Location &Gender
9.988b	Banka	10.644c	9.333d	Banka	

followed the same letter for each factor and interaction do not differ significantly from each other's according to Dun n's Multiple range Test t 5% level.

The oil content of gum from Pistacia eurycarpa trees was influenced by date a, place, and sex of collection as represented in Table (5), but with less variation than its weight and moisture content. On August 15, male trees from Amada produced the highest oil content (1.000%) while slightly lower for female trees (0.900%) from the same location. In Banka, oil content in male trees was lower (0.366%), while female trees had 0.766%. On September 15, oil content was uniform, going from 0.600% to 0.700% in all trees and locations, with differences between genders being minor. On 15 October, female trees in Amada reached the highest oil content of 0.866%, followed by male ones in Amada (0.733%). In Banka, male and female trees produced 0.566% and 0.700%, respectively. Generally, female trees were inclined to yield higher amounts of gum before October; however, during September, oil and moisture levels remained relatively stable across collection dates in comparison with the weight.

Major growth conditions and gum oil levels of Pistacia eurycarpa were drastically influenced by the environmental settings along with soil properties of Amada and Banka villages on the Kurdistan Region of Iraq. Poorly developed nutritional conditions with greater evaporation, owing to lower altitude, are considered conducive to drier with hotter conditions in Amada, while at the higher elevation Banka comes under cooler temperatures with more rainfall especially during the late months. Such climatic differences were mirrored in soil properties that while Amada enjoyed good nutrient availability, Banka had a higher organic content and moisture-retaining capacity. The oil from the gum collected at Amada, during the early collection periods particularly, was higher in oil content than that from Banka even with Banka undergoing more organic stress, implying that environmental stress due to drier conditions may enforce greater oil synthesis. Female trees were found to produce comparatively better oil during early collection periods than their male counterparts

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with lesser distinction noted - implying that while Amada could serve best for short-term to better nutrient conditions and stress-driven oil production, soil conditions at Banka could best support long-term growth. This study gives importance to microclimatic and soil yields due

conditions that maximize both yield and quality in gum-producing species (38.(

Table 5: Effect of Collection Date, Location, and Tree Gender on the Oil Content of Pistacia eurycarpa Gum

Mean of Dates	Dates &Location	Gender		Location	Dates
		Female	Male		
0.750a	0.950a	0.900b	1.000a	Amada	15-Aug
	0.550d	0.766c	0.366f	Banka	
0.658b	0.666c	0.700cd	0.600e	Amada	15-Sep
	0.666c	0.633de	0.700cd	Banka	
0.725a	0.783b	0.866b	0.733c	Amada	15-Oct
	0.650c	0.700cd	0.566e	Banka	
Location		0.761a	0.661b	Gender	
		0.816a	0.683b	15-Aug	Dates& Gender
		0.650b	0.666b	15-Sep	
		0.816a	0.633b	15-Oct	
0.800a	Amada	0.822a	0.777b	Amada	Location& Gender
0.622b	Banka	0.700c	0.544d	Banka	

followed the same letter for each factor and interaction do not differ significantly from each other's according to Dun n's Multiple range Test t 5% level .

Table (6) All given isolates were subjected to antibiotic susceptibility assay against a wide range of antibiotics: E. coli, Klebsiella pneumoniae, Staphylococcus aureus, and Staphylococcus haemolyticus. Total resistance of E. coli was seen against ampicillin (0 mm), tetracycline (5.2 ± 0.3 mm), gentamicin (13.0 ± 0.6 mm), streptomycin (15.3 ± 0.5 mm), and ceftiofur (6.0 ± 0.6 mm). In contrast, it was sensitive to ciprofloxacin (28.1 ± 1.6 mm), ceftriaxone (26.7 ± 2.4 mm), chloramphenicol (26.5 ± 2.5 mm), and amoxicillin-clavulanic acid (23.0 ± 2.5 mm). K. pneumoniae was resistant to tetracycline (5.0 ± 5.0 mm), gentamicin (14.7 ± 1.5 mm), and tylosin (0 mm), but susceptible to ciprofloxacin (32.7 ± 3.2 mm), ceftriaxone (27.7 ± 0.3 mm), streptomycin (16.0 ± 1.5 mm), chloramphenicol (30.0 ± 1.7 mm), amoxicillin-clavulanic acid (25.3 ± 1.2 mm), and ceftiofur (23.2 ± 4.3 mm.(

Staphylococcus aureus was sensitive to all the antibiotics tested: ampicillin (30.7 ± 1.2 mm), ciprofloxacin (22.3 ± 3.1 mm), ceftriaxone (26.7 ± 1.5 mm), tetracycline (26.6 ± 2.3 mm), gentamicin (27.4 ± 2.4 mm), streptomycin (25.5 ± 3.0 mm), chloramphenicol (27.3 ± 1.6 mm), amoxicillin-clavulanic acid (26.0 ± 2.8 mm), ceftiofur (32.5 ± 1.6 mm), and tylosin (34.7 ± 4.6 mm). Staph. haemolyticus, on the contrary, was resistant against ampicillin (11.3 ± 0.3 mm), tetracycline (0 mm), gentamicin (8.0 ± 0.6 mm), ceftriaxone (11.7 ± 0.3 mm), and tylosin (8.3 ± 3.6 mm), but exhibited sensitivity towards ciprofloxacin (22.0 ± 0.5

mm), streptomycin (20.2 ± 1.6 mm), chloramphenicol (22.6 ± 0.3 mm), amoxicillin-clavulanic acid (22.0 ± 0.7 mm), and ceftiofur (26.3 ± 1.5 mm.(

Table (6): Antibiotic sensitivity profiles of human isolates, highlighting resistance and susceptibility patterns across various bacterial strains.

Staph. haemolyticus	*Staph.aureus*	*Klebsiella pneumoniae*	*E. coli*	Antibiotic
R 11.3 ± 0.3	S 30.7 ± 1.2	S 12.0 ± 1.5	R 0	Ampicillin
S 22.0 ± 0.5	S 22.3 ± 3.1	S 32.7 ± 3.2	S 28.1 ± 1.6	Ciprofloxacin
R 11.7 ± 0.3	S 26.7 ± 1.5	S 27.7 ± 0.3	S 26.7 ± 2.4	Ceftriaxone
R 0	S 26.6 ± 2.3	R 5.0 ± 5.0	R 5.2 ± 0.3	Tetracycline
R 8.0 ± 0.6	S 27.4 ± 2.4	R 14.7 ± 1.5	R 13.0 ± 0.6	Gentamicin
S 20.2 ± 1.6	S 25.5 ± 3.0	S 16.0 ± 1.5	R 15.3 ± 0.5	Streptomycin
S 22.6 ± 0.3	S 27.3 ± 1.6	S 30.0 ± 1.7	S 26.5 ± 2.5	Chloramphenicol
S 22.0 ± 0.7	S 26.0 ± 2.8	S 25.3 ± 1.2	S 23.0 ± 2.5	Amoxicillin-Clavulanic (AMC)
S 26.3 ± 1.5	S 32.5 ± 1.6	S 23.2 ± 4.3	R 6.0 ± 0.6	Ceftiofur
R 8.3 ± 3.6	S 34.7 ± 4.6	R 0	R 0	Tylosin

The data represented in Table (7) illustrated that the most treatments caused significant differences in the treatment effect and the response of different bacterial species to the techniques over treatments with an overall mean value of 5.719 ± 0.067 with a 95% confidence interval between 5.587 and 5.851. Effect of treatments among those highest was exhibited by such group as "Amada/15-Sep/F" (11.130 ± 1.11), while no effect was observed in the control mean value of 0.000. Generally, treatments involving Amada isolate gave quite stronger effects compared to those involving Banka isolates-with some Amada treatments, such as "Amada/15-Oct/M" and "Amada/15-Aug/F", scoring high mean values of 9.716 and 8.008, respectively. By contrast, treatments like "Banka/15-Sep/M" and "Banka/15-Aug/F" had substantially lower effects with mean values of 1.793 and 3.261, respectively, and this difference was highly statistically significant ($P \leq 0.0001$). Thus, Staphylococcus haemolyticus had the highest mean response to bacterial species (7.888 ± 1.33), followed by Staphylococcus aureus (7.404 ± 0.46), while E. coli and Klebsiella pneumonia, gave significantly lower responses (means of 3.654 and 3.928, respectively). Such interspecies differences were also statistically significant ($P \leq 0.001$), thus suggesting variability in the susceptibility to or response of the bacteria to the treatment.

Other published studies on extracts of Pistacia vera have reported antimicrobial activity, especially against Gram-positive bacteria, and

in our case showed zones of inhibition comparable to that obtained by us. For instance, a methanolic extract of pistachio skin showed a mean inhibition zone of 17.1 ± 0.7 mm against Staphylococcus aureus using the disc diffusion method, thus corroborating the antibacterial-power of the phenolic-rich fractions (21). Similarly (20) reported inhibition zones in the ranges of 10.75 mm and 11.47 mm against Pseudomonas aeruginosa using aqueous extract of pistachio green hull at 5 mg/ml and 10 mg/ml concentrations, respectively. Polyphenol-rich kernel extracts of P. vera showed bactericidal effects against S. aureus, Listeria monocytogenes, and methicillin-resistant S. aureus in another study, although specific inhibition zone diameters were not provided (14). Also, (10) confirmed significant antimicrobial efficacy of microwave-assisted extracts from pistachio hull against E. faecalis, S. aureus, and Bacillus species.

Table (7) :Effect of Collection Date, Location, and Tree Gender on the Oil Content of Pistacia eurycarpa Gum on Bacterial Species Inhibition Zones.

Overall Mean				
95% Confidence Interval		**± Std.**		
Upper Bound	**Lower Bound**	**Error**	**Mean**	
5.851	5.587	0.067	5.719	
Effect of Treatment				
95% Confidence Interval		**± Std.**		
Upper Bound	**Lower Bound**	**Error**	**Mean**	**Treatment**
.000	.000	.000	0.000 i	**Control**
2.270	1.317	0.464	1.793 h	**Banka/15-Sep/M**
3.737	2.784	1.09	3.261 g	**Banka/15-Aug/F**
4.009	3.056	0.81	3.532 g	**Banka/15-Sep/M**
4.034	3.081	0.813	3.557 g	**Banka/15-Sep/F**
7.927	6.973	1.04	7.450 d	**Banka/15-Oct/M**
9.344	8.391	0.89	8.868 c	**Banka/15-Oct/F**
6.316	5.363	0.24	5.839 e	**Amada/15-Aug/M**
8.484	7.531	1.89	8.008 d	**Amada/15-Aug/F**
6.757	5.804	1.04	6.280 e	**Amada/15-Sep/M**
11.607	10.653	1.11	11.130 a	**Amada/15-Sep/F**
10.192	9.239	1.43	9.716 b	**Amada/15-Oct/M**
5.386	4.433	1.06	4.909 f	**Amada/15-Oct/F**
≤ 0.0001 **				***P Value (Sig.)***
Effect of Bacteria species				
95% Confidence Interval		**± Std.**		
Upper Bound	**Lower Bound**	**Error**	**Mean**	**Treatment**
3.919	3.390	0.469	3.654 c	***E. Coli***
4.193	3.664	0.70	3.928 c	*K.pneumonia*
7.669	7.140	0.46	7.404 b	***Staphylococcus aureus***
8.152	7.623	1.33	7.888 a	***Staphylococcus haemolyticus***
≤ 0.001 **				***P Value (Sig.)***

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Mean's followed the same letter for each factor and interaction do not differ significantly from each other's according to Dun n's Multiple range Test t 5% level.

These findings are in good agreement with our own results, showing strong inhibition against Staphylococcus haemolyticus (mean = 7.888 mm) and S. aureus (7.404 mm), while the inhibition against E. coli (3.654 mm) and Klebsiella (3.928 mm) exhibited the weaker responses. The pattern of greater susceptibility in Gram-positive bacteria compared to Gram-negative strains is in agreement with trends in the literature, confirming that pistachio-derived extracts, particularly skin and hull, have selective antimicrobial activity against clinically relevant pathogens.

The results of the analysis showed that all treatments had significant effects on inhibition of bacterial growth, and also showed variations among different treatment groups and species of bacteria. It was, however, observed that the gum samples collected from Amada were mostly superior in antimicrobial activities when compared to those collected from Banka, thus indicating that other environmental differences such as temperature, humidity, and altitudes have been known to affect the biosynthesis and accumulation of bioactive secondary metabolites in Pistacia species (42&44). Indeed, treatments collected in September and October, particularly from female trees in Amada, were also shown to exhibit a heightened response, probably on seasonal influences on the gum's phytochemical profile, as is consistent with findings indicating that there can be upregulation of synthesized pathways of terpenoids and phenolic compounds under certain climatic conditions (41).With respect to the bacterial response, Gram-positive bacteria generally had more susceptibility than both Staphylococcus haemolyticus and Staphylococcus aureus, and the former was envisioned to hold a wider margin of inhibition than Gram-negative species such as Escherichia coli and Klebsiella pneumoniae. This is consistent with historical reports that indicate more complex structures in the outer membranes of Gram-negative bacteria that obscure the entrance of more hydrophobic antimicrobial agents such as those from the Pistacia gums (43&45) .Thus, all these information have evidence to support hypotheses stating that environmental factors as well as biological factors-along with altitude, harvesting time, and gender of the tree-play very significant roles in determining chemical composition and biological activity in nature for plant resins.

Conclusions

It is proven from this study that the harvest date, tree gender, and collection site of Pistacia eurycarpa gum are the factors that massively influence both physical and antimicrobial properties of the gum. Among the different sources of gum in Amada, the one that is derived from female trees in the mid-September period showed the strongest antimicrobial activity mainly against gram-positive bacteria. Although the antimicrobial power of this substance may be lower than that of conventional antibiotics, key benefits include natural source, low side effects, and high biocompatibility with human body. These properties validate using it in traditional Kurdish medicine and also prove its potential value as a natural anti-inflammatory and antimicrobial agent. Further research on gums from Pistacia eurycarpa may give it to develop into a safe and effective therapeutic agent for infections and inflammatory conditions.

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