

Antibacterial Activity of *Pistacia vera* L. Nuts and Extracted Lipophylic

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الخلاصة

في هذه الدراسة تم التحري عن التأثير المثبط لمستخلصات ثمار الفستق *Pistacia vera* و Lipophylic في أنواع مختلفة من الجراثيم الموجبة والسالبة لصبغة كرام باستخدام طريقة الانتشار بالأقراص . اظهر المستخلص المائي لثمار الفستق تأثيرا معتدلا في الجراثيم المدروسة وكان تأثيره واضحا في ج رثومة *Bacillus cereus* , أما المستخلص الايثانولي فقد كان تأثيره التنبيطي جيدا في جميع الأنواع الجرثومية وبأقطار تنبيط تجاوزت أقطار تنبيط المضادات الحيوية المستخدمة في الدراسة ماعدا الجرثومتين *Serratia marcescens* و *Salmonella typhimurium* فقد قاومتا المستخلصين المائي والايثانولي وبجميع تراكيزه , أما المستخلص الأسيتوني فقد كان تأثيره اقل مقارنة بالمستخلص الايثانولي كما لوحظ أن التركيز الأمثل كان 12.5 ملغم /سم³ في جرثومة *Pseudomonas aeruginosa* , أما ال Lipophylic المستخلص فقد اظهر تأثيرا جيدا في جرثومتي *Staphylococcus aureus* و *Ps. aeruginosa* كما أن التركيز 12.5 ملغم/سم³ كان الأمثل في جرثومة *Ps. aeruginosa* أما الجراثيم *Klebsiella pneumoniae* و *Sal. typhimurium* و *Serr. marcescens* فقد قاومت مستخلص ال Lipophylic بشكل تام.

Abstract

The present study investigated antibacterial activities of different extracts from *Pistacia vera* nuts against Gram positive and negative bacteria using disc diffusion method, The Aqueous extract showed moderate effects against test bacteria specially on *Bacillus cereus*. Ethanol extract showed significant inhibitory effects against most bacteria compared with the standard antibiotics except *Serratia marcescens* and *Salmonella typhimurium* which resisted all concentrations of aqueous and ethanol extracts. Acetone extract showed

less activity against test bacteria compared with ethanol extract, and the optimal concentration was 12.5mg/cm³ against *Pseudomonas aeruginosa*, The lipophylic extract was active against *Staphylococcus aureus* and *Ps. aeruginosa* and also the optimal concentration was 12.5mg/cm³ against *Ps. aeruginosa*, meanwhile no inhibitory effect was seen using lipophylic extract against *Klebsiella pneumoniae*, *Salmonella typhimurium* and *Serr. marcescens*.

Introduction

During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics [1] has lead to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures which overcome the above disadvantages [2,3]. Plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries [4], and almost the exclusive source of drugs for the majority of the world's population [5].

Pistacia vera L., (pistachio) member of *Anacardiaceae* or cashew family, a small deciduous tree, which has fruits with a grayish white, boney, keeled nut-shell which, encloses light yellow to deep green edible kernels with a reddish coat, the genus *Pistacia* contains only 11 species, of which *P. vera* is by far the most economically important [6]. In addition to its economical value, kernels of *P. vera* are remarkably rich in linoleic and linolenic acids, the fatty acids vital for human health [7], the essential oil (lipophylic) from *P. vera* contains about (89.67%) monoterpenes, (8.1%) oxygenated monoterpenes and (1.2%) diterpenes, α -Pinene (75.6%), β -pinene (9.5%), trans-verbenol (3.0%), camphene (1.4%), trans-pinocarveol (1.20%), and limonene (1.0%) [8]. *P. vera* kernels are also reported as a remedy for sclerosis of the liver, abdominal ailments, abscess, bruises and sores, chest ailments, circulation problems [9].

On the other hand, *Pistacia* species were reported to have various biological activities such as anti-parasite [10] antifungal [11], anti-atherogenic, hypoglycemic [12], antioxidant, anti-inflammatory [13], and ant-insect activities [14].

The objective of this study was to elucidate antibacterial activities of *P. vera* nuts using different extracts, including lipophylic extract among various types of bacteria.

Materials and methods

Plant material

Nuts of *P. vera* L. were collected from Mosul, Ninewa province in June 2006 and was identified at Department of Biology, College of Science / University of Mosul.

Preparation of Extracts

Pistachio nuts were dried at room temperature in dark then grinded to powder using an electrical blender.

Aqueous and Ethanolic extracts

The method for preparing aqueous extracts from plant parts has been already described [15], dried nuts material 25gm was stirred in 250 ml of distilled water for 15 min at 90°C followed by rapid filtration through four layers of gauze and then by a more delicate filtration through Whatman filter paper No.1. The resulting filtrate was frozen and freeze-dried. The extract was stored at -18°C in a desiccant until required.

Ethanol extracts were carried out according to [16], which includes mixing 40gm of plant powder with 400 mL ethanol(concentration 95%), after stirring the extract was kept overnight at room temperature, then filtered using Whatman filter paper No.1, and evaporated under reduced pressure using a rotary evaporator at 40°C .The dried extract was stored in sterile bottles until further use.

Acetone extract

Dried and powdered nuts 80gm were extracted with acetone 300 ml in a soxhlet extractor for 10–12 hours continuously. The solvent was removed in vacuum 40°C to give concentrated extract.

Lipophylic extract

The organic solvent extraction of the grinded nuts of *Pistacia vera* 80gm dry weight was carried out in a conventional Soxhlet device using n-hexane as solvent, in the presence of anhydrous Na₂SO₄. The cartridges were introduced in the Soxhlet apparatus containing 300 ml of solvent. The total reflux time was 5 hours for the sample. After cooling, the solvent was separated from the solute using a rotary vacuum evaporator

with a vacuum controller 40°C. The resulting extracts were weighed in an sensitive scale [17].

Preparation of extract concentrations

Several concentrations (200,100,50,25 and 12.5 mg/cm³) of the different extracts were accomplished by dissolving in DMSO (Dimethylsulphaoxide), then were sterilized via pasturalization, the aqueous extract was dissolved using distilled water and sterilized by membrane filtration, then kept under refrigerated conditions until use.

Test Bacteria

The microorganisms used included *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Bacillus cereus*, *Serratia marcescens*, *Salmonella typhimurium* and *Klebsiella pneumoniae* which were obtained from Department of Biology, College of Education, University of Mosul.

Inoculum preparation

Mueller–Hinton Broth (Difco) was applied for growing and diluting of the microorganism suspensions. strains of bacteria were grown to exponential phase in medium (MHB) at 37°C for 18 hours with aeration. The bacterial suspensions used for inoculation were prepared at 10⁸ cfu/ml by diluting fresh cultures by comparing with McFarland density (1.5*10⁸ cfu/ml).

Screening of antibacterial activity

Screening plant extracts for their antibacterial activity was conducted using the disc assay described by [18], One hundred micro liters of prepared culture was spread on surfaces of Mueller–Hinton agar. Sterile filter paper discs (Whatman No.1 : 6mm in diameter) were soaked with different concentrations of plant extracts by adding 0.1 mL of extract/10 paper disc, then placed on the surface of the inoculated media plates slightly, antibiotic discs (Bioanalyse) 6mm in diameter of (Gentamycin 10µg, Cefalexin 30µg and Carbencillin 100µg) were used as positive controls. Spread plates were then kept at room temperature for 30 min to allow diffusion and absorption of extracts prior to incubation at 37°C for 24 hours. At the end of the period, inhibition zones formed on the medium were evaluated in mm.

Result and Discussion

The results revealed various inhibitory effects of different extracts against tested bacteria via disc diffusion method, the inhibitory effect depends on extract type and the bacteria strain, in addition to the used concentration progression. Aqueous extract from *P. vera* nuts showed high inhibitory effect against *B. cereus* compared with Cefalexin with an inhibition zone of 17.2mm in diameter using the concentration 200mg/cm³, the extract showed good inhibitory effect against *Pr. vulgaris* compared with Gentamycin, meanwhile *Ps. aeruginosa*, *S. aureus*, *E. coli* and *Kleb. pneumoniae* showed a medium susceptibility compared with the standard antibiotics, the inhibitory effect had a direct relation with extract concentration against all tested bacteria, the concentrations (25, 12.5mg/cm³) were inactive against tested bacteria this may be due to the low concentration of active components in the extract, the results also revealed the complete resistance of *Serr. marcescens* and *Sal. typhimurium* against the aqueous extract this may be attributed to the defensive agents of these bacteria such as plasmid resistance which can be obtained through modifications in metabolic pathways of enzymes and receptors of antibacterial materials [3]. Many articles have discussed the inhibitory effects of *Pistacia* species like *P. terebinthus* and *P. lentiscus* against gram positive bacteria and proved that the effect is better compared with gram negative bacteria [19], on the other hand it was reported that the isolated gum from *P. lentiscus* has antifungal activity against the yeast *Candida albicans* [20]. In addition most studies have reported that the aqueous extracts processes moderate inhibitory effects against bacteria, the most close explanation is the inability of water in separating essential oils which have high antibacterial activities [21], on the other hand water is active in separating flavonoids and phenolics which exist in *P. vera* and are good antimicrobial components.

(Table 1) showed that the minimum concentrations of ethanol extract had inhibitory effects on tested bacteria, *E. coli* showed high susceptibility to ethanol extract compared with Gentamycin with an inhibition zone 24.1mm in diameter (Figure 1), it was also seen that *Pr. vulgaris* (Figure 2) and *B. cereus* were inhibited via ethanol extract with significant variations compared with the standard antibiotics which are used as positive controls, followed by *S. aureus* and *Ps. aeruginosa*, as for *Serr. marcescens* and *Sal. typhimurium* both bacteria showed complete resistance against all concentrations of ethanol extract. The study results proved the suitability of the ethanol solvent in dissolving active components from plants in addition to natural products, and agrees with [8, 22, 23].

Table 1: Antibacterial activity of Aqueous and Ethanol extracts from *P. vera* L. nuts.

Microorganisms	Aqueous extract concentrations mg/cm ³					Ethanol extract concentrations mg/cm ³					Control		
	200	100	50	25	12.5	200	100	50	25	12.5	CN	CL	Cb
<i>S. aureus</i>	11.3	9.0	7.1	R	R	17.1	14.1	12.3	11.2	10.4	-	-	13.2
<i>Ps. aeruginosa</i>	11.1	9.2	8.1	R	R	17.3	15.2	13.0	11.3	11.0	19.1	-	-
<i>Pr. vulgaris</i>	14.1	9.2	9.0	R	R	23.4	18.7	14.6	12.4	9.1	-	14.1	-
<i>Ser. marcescens</i>	R	R	R	R	R	R	R	R	R	R	18.1	-	-
<i>E. coli</i>	12.1	9.6	8.1	R	R	24.1	19.3	17.1	13.1	11.5	16.3	-	-
<i>B. cereus</i>	17.2	12.2	9.1	R	R	23.3	15.2	14.1	11.4	10.6	-	11.3	-
<i>Sal. typhimurium</i>	R	R	R	R	R	R	R	R	R	R	-	13.1	-
<i>Kleb. pneumoniae</i>	9.2	R	R	R	R	9.1	R	R	R	R	-	14.0	-

R: Resistant, - : Not assessed, CN: Gentamycin, CL: Cefalexin, Cb: Carbencillin



Figure 1(A):Effect of Ethanol extract on *E. coli* using different concentration ,1(200 mg/cm^3)2(100 mg/cm^3)3(50 mg/cm^3)4(25 mg/cm^3)5(12.5 mg/cm^3) CN(Gentamycin).



Figure 1(A):Effect of Ethanol extract on *Pr. vulgaris* using different concentration ,1(200 mg/cm^3)2(100 mg/cm^3)3(50 mg/cm^3)4(25 mg/cm^3)5(12.5 mg/cm^3) CL(Cefalexin).

Our results indicated that acetone extract of Pistachio nuts has potential source of antibacterial effects against all test bacteria except *Sal. typhimurium* and *Serr. marcescens* (Table 2), it was also seen that increasing extract concentration didn't increase the antibacterial effect as in *Ps. aeruginosa*, the optimal concentration was 12.5mg/cm³, meanwhile effect against bacteria decreased using the concentration above 12.5mg/cm³ (Figure 3), the scientific explanation for this situation is that the cytoplasm membrane of bacteria contains pores that prevents high concentrated extracts from insertion, in fact acetone extract was able to breakthrough cell wall and cytoplasm membrane and accomplish antibacterial activity, this was also described by [24] who studied the effect of plant extracts on bacterial cytoplasmic membrane permeability, the membrane permeability changed when incurrence to high concentrations of plant extracts and this also effects on bacterial susceptibility to antibiotics. Its reported that Pistachio trees contain 88 natural chemical compounds among the essential oil these compounds are considered as secondary products and some are secreted as a reaction of Pistachio trees when infected with bacterial, fungal and viral infections such as the secreted gum.

This investigation also showed that lipophylic extract had a moderate activity against *S. aureus* (Figure 4), *Ps. aeruginosa* and *E. coli*, meanwhile other types resisted the extract, this can be due to the scanty of active compounds in lipophylic extracts. The active compounds in plants play a cooperating role in showing inhibitory properties, if one or more of these compounds are separated it may decrease extract activity [3].

From (Table 2) it was also seen that *E. coli* and *Ps. aeruginosa* were more sensitive to the extract concentrations 25 and 12.5mg/cm³ respectively than the other used concentrations. The main antibacterial cause of *P. vera* is due to the existence of compounds such as carvacrol, camphene and limonene which are the main compounds in lipophylic structure (essential oil) [23], and several studies [25, 17, 26 and 7] mentioned that the essential oil of *P. vera* contains a high rate of fatty acids, like myristic, lignopalmatic, oleic, stearic, elaidic and melissic acids, which are responsible of the plant extract antibacterial activity, meanwhile other studies [27, 28, 29, 30 and 31] reported Pistachio species as a remedy for eczema, paralysis, diarrhea, gingivitis, antiviral and antibacterial which was proved in this study.

Table 2: Antibacterial activity of Acetone and Lipophylic extracts from *P. vera* L. nuts.

Microorganisms	Acetone extract concentrations mg/cm ³					Lipophylic extract concentrations mg/cm ³					Control		
	200	100	50	25	12.5	200	100	50	25	12.5	CN	CL	Cb
<i>S. aureus</i>	13.5	8.5	R	R	R	18.3	17.5	14.3	10.2	R	-	-	13.2
<i>Ps. aeruginosa</i>	14.7	14.2	13.3	9.2	15.5	19.6	14.5	12.3	16.5	19.4	19.1	-	-
<i>Pr. vulgaris</i>	15.3	13.5	11.2	9.2	9.0	R	R	R	R	R	-	14.1	-
<i>Ser. marcescens</i>	R	R	R	R	R	R	R	R	R	R	18.1	-	-
<i>E. coli</i>	13.3	10.2	R	11.2	9.1	11.2	12.5	12.1	14.5	10.6	16.3	-	-
<i>B. cereus</i>	18.2	10.3	9.2	R	R	R	R	R	R	R	-	11.3	-
<i>Sal. typhimurium</i>	R	R	R	R	R	R	R	R	R	R	-	13.1	-
<i>Kleb. pneumoniae</i>	10.3	9.2	R	R	R	R	R	R	R	R	-	14.0	-

R:Resistant, - : Not assessed, CN: Gentamycin, CL: Cefalexin, Cb: Carbencillin



Figure 1(A):Effect of Acetone extract on *Ps. aeruginosa* using different concentration ,1(200 mg/cm³)2(100 mg/cm³)3(50 mg/cm³)4(25 mg/cm³)5(12.5 mg/cm³) CN(Gentamycin).



Figure 1(A):Effect of Lipophylic on *S. aureus* using different concentration ,1(200 mg/cm³)2(100 mg/cm³)3(50 mg/cm³)4(25 mg/cm³)5(12.5 mg/cm³) Cb(Carbencillin).

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