



QUALITATIVE PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND WOUND HEALING ACTIVITIES OF *PISTACIA PALAESTINA* BOISS. EXTRACTS

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The current work was designed to undertake a qualitative phytochemical screening of various plant parts of *Pistacia palaestina*, and estimate antioxidant and wound healing activities for therapeutic applications. The qualitative phytochemical screening of the leaf and the fruit of *P. palaestina* was performed using different chemical tests. The total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity were determined by using Folin-Ciocalteu, aluminum chloride, and DPPH (2,2-Diphenyl-1-picryl hydrazyl) methods, respectively. The extracts with the highest TPC and TFC were examined for their wound healing activity using an incision wound model on rats. The qualitative phytochemical screening revealed the presence of phenols, tannins, flavonoids, carbohydrates, saponins, phytosterols, terpenoids, mucilages, and resins, whereas no coumarins, alkaloids, cardiac glycosides, or anthraquinones were found. TPC and TFC were observed between (144.80 ± 8.81 to 489.79 ± 15.09 mg gallic acid equivalent (GAE)/g extract) and (18.34 ± 1.16 to 36.16 ± 1.05 mg rutin equivalent (RUE)/g extract), respectively. Methanolic extracts of leaves and fruits showed high antioxidant activity with IC_{50} about 5.16 ± 1.22 and 8.21 ± 1.87 µg/mL, respectively, which are slightly higher than ascorbic acid (IC_{50} = 3.52 ± 0.14 µg/mL). The prepared ointments from methanolic extracts significantly accelerated wound healing compared to a negative control group, as the final time of healing was 8.2 ± 1.30 (fruits), 9.6 ± 1.14 (leaves), 12 ± 1.22 (gentamicin ointment 0.1%) and more than 21 (negative control) days. In conclusion, *P. palaestina* could be used for the treatment of skin wounds.

Keywords: *Pistacia palaestina*, Antioxidant activity, Qualitative phytochemical screening, Wound healing, TPC

INTRODUCTION

The medical importance of plants has been done since antiquity and may be considered the basis of modern medicine. Recently, the World Health Organization (WHO) has listed around 21,000 plants that are used for many medicinal purposes worldwide¹. *Pistacia* is a plant genus related to the Anacardiaceae family, which includes about 76 genera and more than 600 species. They are distributed across the Mediterranean area and Central Asia. The most common species of the *Pistacia* are *P. lentiscus*, *P. atlantica*, *P. khinjuk*, *P. vera*, and

*P. palaestina*², which are shrubs or trees with well-developed vertical resin canals. Leaves are deciduous and alternate with 5-7 pairs of leaflets, and rarely with a small odd one. Flowers are often unisexual, small, and radial. Fruits are ovoid-globular and brownish at 5 mm in diameter^{3&4}. In fact, about 3,500 plant species have been found in Syria, hundreds of which have medicinal importance. *Pistacia* genus is one of the most important wild plants in Syria and is used traditionally for its useful effects on human ailments⁵. Previous phytochemical studies on *Pistacia* have shown the presence of many secondary metabolites

such as terpenoids (volatile oil, monoterpenoids, and sesquiterpenoids), phenolic compounds (gallic acid, flavonoids, anthocyanins), and sterols (β -sitosterol, stigmasterol)². Additionally, the importance of this genus comes from being a good source of vitamins, minerals, essential oils (EO), tannins, and resins⁶⁻⁸. These components display an important role in the pharmacological activity of *P. palaestina*, including anti-inflammatory, antifungal, antioxidant, and antimicrobial activities⁹. The aerial parts of *P. palaestina* were used in folk medicines as a stimulant, diuretic, laxative, and aphrodisiac, in addition to its use to treat eczema, cough, gastrointestinal diseases (anti-helicobacter), kidney stones, and hypertension^{7&9}. Some researchers stated the chemical composition of essential oils from leaves and fruits of *P. palaestina* of diverse origins. However, there is only a few number of reports available in the literature. For instance, the composition of volatile oil of *P. palaestina* has been carried out by Awwad et al., 2004, using GC/MS, where the oil from the leaves with hydro-distillation consisted of 29.8% sabinene, 19.8% α -pinene, 13.1% β -pinene, and 10.3% α -terpineol⁷. In another study conducted by Flamini et al., 2020, in the same field, it was reported that the main compounds were myrcene (13.3%) and α -pinene (63.1%) in the leaves and (Z)-ocimene (3.8-13.0%), sabinene (20.3-24.1%), and (E)-Ocimene (33.8-41.3%) in both ripe and unripe fruits¹⁰. Fruits of *P. palaestina* are called in Arabic "Butom". They are eatable and sold in markets where the raw or toasted ripe fruits are mixed with other aromatic plants to obtain the so-called "Zaatar"¹⁰. Historically, it was considered a source of food in ancient times, according to archaeological records in ancient Greeks⁹. Oxidative stress appears when there is a physiological imbalance between the antioxidant system and the formation of free radicals. These generated free radicals damage the major biomolecules in the cell by oxidizing cell proteins, enzymes, membrane lipids, and DNA¹¹. Antioxidants are chemical compounds from synthetic or natural sources that can work in several mechanisms: lowering the localized O² ions to stop the production of hydroxyl radical, scavenging free radicals that propagate peroxidation, and chelating metal ions that increase the oxidation process¹². Bioactive compounds such as polyphenols, flavonoids, and tannins have the antioxidant ability with

potential benefits for health. They could decrease cancer risk due to anti-inflammatory and antioxidant activities¹¹ according to their chemical structures where, the phenolic acids like, protocatechuic acid, gallic acid, ferulic acid, and caffeic acid consist of phenolic ring (C₆H₅OH), carboxylic acid (-COOH) and hydroxyl group (-OH)^{13&14}. Also, flavonoids include at least two phenolic rings attached through heterocycle carbonic ring¹⁵. The antioxidant activity of phenolic compounds is basically due to their redox properties, which act as hydrogen donors, chelate metals, and adsorb or neutralize free radicals¹⁶. A wound is known as a breaking or loss of anatomic and cellular or functional continuity of living tissue. Wound healing is a complex process that aims to restore tissue continuity¹⁷. Many plants have shown a high level of success in wound healing activities¹⁸, *Leea macrophylla* which stimulates the production process of collagen and promotes cell proliferation¹⁹. While flavonoids and phenolic acids present in *Cynodon dactylon* support wound healing activity because of its anti-oxidative activity²⁰. Several *Pistacia* constituents have been confirmed to be effective in wound healing. *P. atlantica* resin extract cured burn wounds by improving angiogenesis, after fourteen days of the treatment process. *P. lentiscus* oil (a dose of 1 mL) was applied for four days to decrease the epithelization period and stimulated wound contraction in the rabbit model. The hydro-ethanolic extract of *P. atlantica* hulls ointment when applied on lesions for three weeks revealed wound healing activity in the rat models. The therapeutic mechanisms of the above-mentioned plants could be explained by their ability to increase the amount of platelet-derived growth factor, fibroblast growth factor, hydroxyproline content, up-regulation of fibroblast proliferation, and neovascularization²¹.

To the best of our knowledge, the literature contains a few papers on the chemical composition and wound healing activity of *P. palaestina* growing in Syria. So, this study aims to determine phytochemical constituents, the TPC, TFC, and antioxidant activity for the leaf and the fruit of *P. palaestina*. Besides, its wound healing activity is carried out in the experimental rats using an incision wound model.

MATERIALS AND METHODS

Material

Chemicals

Rutin (Extrasynthese Genay, France). Folin-Ciocalteu (Sigma-Aldrich, Switzerland). Sodium hydroxide 98% (Medex, UK). Double distilled deionized water (dd. H₂O). Aluminum Chloride Hexahydrate (Scharalau Chemie, Spain). Ethanol (Eurolab, UK). Methanol 99% (Eurolab, UK). Gallic acid (Titan Biotech Ltd., India). Sodium carbonate (Panreac QUIMICA SAU, Spain). 2,2-Diphenyl-1-picryl hydrazyl radical (Sigma-Aldrich, Germany).

Equipment

Ultrasonic apparatus (POWERSONIC 405, Hwashin Technology Co., Korea), Rotary evaporator apparatus (Heidolph instrument, Germany), Spectrophotometer (Shimadzu, Japan). Ultrapure TM water purification system (Lotun Co., Ltd., Taipei, Taiwan), and Sensitive balance (Sartorius TE214, Germany).

Plant material

Fresh fruits and leaves of *P. palaestina* were collected from Aleppo Governorate (36° 12' 0" N - 37° 36' 0" E, Aleppo, north of Syria) in August 2021. The plant was authenticated by an expert at the Faculty of Agriculture, University of Aleppo, Syria. The fruits and leaves were cleaned of dust, washed, and then dried in the shade for ten days. After that, they were crushed to obtain a suitable powder, and subsequently, they were packaged in airtight and opaque containers until use later.

Methods

Preparation of Plant Extracts

Ten grams of powder aerial parts (leaves and fruits) of *P. palaestina* were extracted with 100 mL of water or methanol (60%) through ultrasonic bath apparatus, three times at a temperature of 40 °C for 60 minutes. Each sample was later filtered using filter paper (Whatman No1) and evaporated at 40 °C until they dried under reduced pressure using a rotary evaporator. The dried extracts were weighted to determine the percentage yield²², which was calculated using the formula (1).

$$\% \text{ Yield} = \frac{\text{Weight of dry extract}}{\text{Weight of dry plant}} \times 100 \dots\dots (1)$$

The dried extracts were stored at 4 °C for further investigations.

Qualitative Phytochemical Screening

The qualitative phytochemical screening of aerial parts (leaves and fruits) of *P. palaestina* was carried out by using standard qualitative tests to detect the presence (or absence) of **phenols** (lead acetate and ferric chloride tests)²³, **tannins** (Gelatin and vanillin-hydrochloride tests)^{24&25}, **flavonoids** (Shinoda and Alkaline reaction)²³, **carbohydrates** (Molisch and Fehling's reagent)^{26&27}, **saponins** (foam and olive oil tests)²⁸, **coumarins** (NaOH paper test)²⁶, **alkaloids** (Dragendorff and Mayer's reagent)^{26&23}, **phytosterols** (Liebermann Burshard reaction)²⁹, **terpenoids** (Salkowski reaction)²⁹, **cardiac-glycosides** (Keller-Killani and Baljet's reagent)²⁶, **anthraquinones** (Borntrager reaction)³⁰, **mucilage** (alcohol test)²⁶, and **resins** (acetic anhydride test)²⁶.

Determination of total phenolic content (TPC)

The total phenolic content of *P. palaestina* extracts was determined by using the Folin-Ciocalteu method as described by Lister and Wilson³¹, with mild modification. 0.5 ml of the crude extract (0.25 mg/ml) was added to 2.5 ml Folin-Ciocalteu reagent (10/100 v/v). The mixture was incubated for five minutes and mixed with 2.5 ml of Na₂CO₃ (7.5%, W/V). The prepared samples were incubated in a water bath at 45 °C for 45 minutes. The absorbance was measured at a wavelength of 765 nm against a blank solution (0.5 mL solvent + 2.5 mL 10% Folin-Ciocalteu reagent + 2.5 ml of Na₂CO₃ 7.5%) using a UV-Vis spectrophotometer. A standard of gallic acid was treated in the same way as the samples above at a range of concentrations (0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 mg/mL) and the linearity curve was plotted. The total phenolic content in extracts was expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g extract). All the determinations were performed in triplicates (n=3) and the data were presented as average ± standard deviation (SD).

Determination of total flavonoid content (TFC)

The content of flavonoids of *P. palaestina* extracts was determined using aluminum trichloride method as described by Stanokvic³², with slight modifications. 1 mL of the crude extract (5 mg/mL) was mixed with 1 mL of

(AlCl₃ 2% w/v diluted with methanol). The mixture was incubated in the dark for 60 minutes at 25 °C. After that, the absorbance was measured at a wavelength of 415 nm against a blank (1 mL methanol + 1 mL aluminum trichloride). In the determination of the content of flavonoids, solutions of rutin (0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 mg/mL stock solution in distilled methanol) were used to obtain a linearity curve. The results were expressed as milligrams of rutin equivalents per gram of the dry extract (mg RUE/g extract). All the determinations were performed in triplicates (n=3) and the data were presented as average ± SD.

Evaluation of radical scavenging activity by DPPH

The antioxidant activity of the studied extracts was evaluated by DPPH method and compared with ascorbic acid as a standard³³. Ascorbic acid was diluted in methanol at concentrations of 0.02, 0.05, 0.10, 0.20, 0.50, and 0.75 mg/mL. *P. palaestina* extracts were diluted in methanol at a series of concentrations of 0.0025 to 0.07 mg/mL. DPPH was dissolved in absolute methanol at a concentration of 0.135 mmol/L. Ascorbic acid and *P. palaestina* extracts (1 mL) were mixed with DPPH solution (1 mL). The mixture was incubated at 30 °C in the dark for 30 minutes. The absorbance was recorded at a maximum wavelength of 517 nm against a blank solution of methanol. The percentage of inhibition (I%) was calculated using the formula (2).

$$I\% = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} * 100 \quad \dots\dots\dots (2)$$

Abs_{control}: Absorption of DPPH (0.135 mmol/L) solution.

Abs_{sample}: Absorption of tested extract or ascorbic acid solution.

The free radical scavenging activity of methanol extracts had been interpreted using IC₅₀ (half maximal inhibitory concentration) values. The IC₅₀ value is the concentration that can scavenge 50% of DPPH free radical³⁴. The IC₅₀ was calculated from the logarithmic curve of the graph plotted between the sample concentrations and the percentages of DPPH (I%) free radical scavenging³⁴.

Procedure for preparation of Ointments

(a) First of all, a simple ointment base of 100 g was prepared by placing 5 g of hard paraffin in a beaker and melted on a water bath. Then, 5 g of Cetostearyl alcohol, 5 g of Wool fat, 5 g of Propylene glycol, and 80 g of Yellow soft paraffin were added in descending order of their melting temperature, respectively. All the inactive ingredients were melted over a water bath with constant stirring until homogenization. Finally, the mixture was taken away from the heat source (water bath) and stirred until cool³⁵. (Table 1).

Table 1: Formulation of simple ointment base.

	Name of Ingredient	Quantity to be taken
1	Cetostearyl alcohol	5 g
2	Wool fat	5 g
3	Hard paraffin	5 g
4	Propylene glycol	5 g
5	Yellow soft paraffin	80 g

(b) Second of all, the methanolic extracts of leaves and fruits of *P. palaestina* were used for the preparation of ointments for topical application on incision wounds. These ointments were formulated using the simple ointment base. 5 g from dry extracts were mixed homogeneously with 95 g of a simple ointment base to obtain 5% (w/w) ointment.

In vivo studies

Experimental animals

Twenty Wistar rats (140–200 g; 6 months old) were obtained from the Animal House Center of the Faculty of Pharmacy, Aleppo University. The rats were housed in aerated plastic cages (one rat/cage) and preserved at controlled room temperature (25 °C) and humidity with a 12/12 – hours light / dark cycle. The rats had free access to water and food. The protocol of this study was approved by the Ethics Committee of the Faculty of Pharmacy, Aleppo university, Syria (registration number: 3/I, 2022). All experiments and procedures used in this study were according to the established public health guidelines in the Guide for Care and Use of Laboratory Animals (2011)³⁶.

Induction of incision

Briefly rats were anesthetized with an intraperitoneal injection of ketamine hydrochloride (90 mg/kg). Then The dorsal lumbar skin was prepared by shaving the hair, then the skin was disinfected with (70%) ethanol. Two 15-mm parallel incision wounds were made on the dorsal lumbar area of rats¹⁸. After the wound was made, the experimental animals were divided into four groups. Each group consists of 5 rats:-

- Group I served as a positive control and it was topically treated with gentamicin sulfate ointment (0.1%).
- Group II served as a test group, and it was treated with methanol fruit extract ointment.
- Group III served as a test group, and it was treated with methanol leaf extract ointment.
- Group IV: served as a negative control without any treatment.

Percentage of Wound Contraction (W%) and Healing Period

Ointments were applied topically once daily for 21 days and Wounds were left open. A wound length (L_0) was measured on the 3rd, 6th, 9th, 12th, 15th, 18th, and 21st days. (W%) was calculated from the formula (3), then compared with major wound length¹⁸. Finally, healing times were determined for all experimental groups.

$$W\% = \frac{L_0 - L_x}{L_0} * 100 \dots \dots \dots (3)$$

W%: Percentage of wound contraction. L_0 : Preliminary wound length. L_x : Length of the wound during the follow-up period.

Statistical Analysis

All experiments were replicated in triplicate (n=3), and the results were expressed as mean \pm standard deviation (mean \pm SD) of the three replicates. Differences in mean values were analyzed using one-way variance analysis (one-way ANOVA), and differences were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

Results

Extraction Yield (%)

The dried plant extracts were brown, sticky, and semi-solid consistency. The highest extraction yield was for the methanolic leaf extract ($34.19 \pm 1.33\%$) while the lowest extraction yield was for the aqueous fruit extract ($15.39 \pm 0.76\%$), as shown in Table 2.

Table 2: The yields percentage of solid residue of *P. palaestina*.

<i>P. palaestina</i>	Type of extract	
	Aqueous	Methanol 60%
Leaves	$29.21 \pm 1.05 *$	$34.19 \pm 1.33 *$
Fruits	$15.39 \pm 0.76 *$	$17.17 \pm 0.73 *$

*Express the presence of a significant difference with the aqueous leaf extract at Significant differences at ($P < 0.05$).

Means \pm standard deviation for triplicate results.

Qualitative Phytochemical Screening

The qualitative phytochemical screening was carried out to know the presence (or absence) of the constituents in the leaf and the fruit of *P. palaestina*, which revealed the presence of various bioactive components like phenols, tannins, flavonoids, carbohydrates, saponins, phytosterols, terpenoids, mucilage, and resins in both leaf and fruit. Nevertheless, coumarins, alkaloids cardiac-glycosides, and anthraquinones were absent, as shown in Table 3.

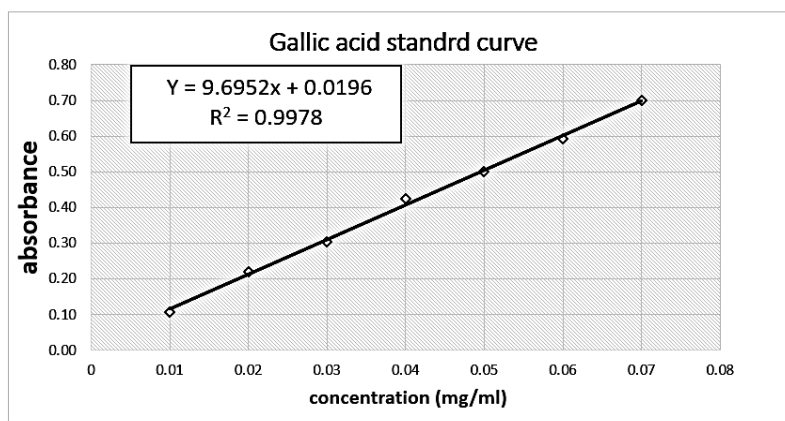
Total Phenolic Content

The total phenolic content of the studied extracts was calculated using a linear equation derived from the standard curve of gallic acid ($y = 9,790x + 0.0196$, $R^2 = 0.9978$), (Figure 1). The highest phenolic content was recorded in the methanolic leaf extract (489.79 ± 15.10 mg GAE/g extract), followed by the aqueous leaf extract (395.17 ± 10.63 mg GAE/g extract), the methanolic fruit extract (293.64 ± 13.31 mg GAE/g extract), and the lowest phenolic content was reported in the aqueous fruit extract (144.80 ± 8.81 mg GAE/g extract), as represented in Table 4.

Table 3: Qualitative phytochemical screening of the leaf and the fruit of *P. palaestina*.

Constituents	Test	The plant part	
		Leaves	Fruits
Phenols	Lead acetate	+++	++
	Ferric chloride	+++	+++
Tannins	Gelatin	+++	++
	Vanillin-hydrochloride	+++	++
Flavonoids	Alkaline	++	++
	Shinoda	+++	++
Carbohydrates	Molisch	++	++
	Fehling	+	+
Saponins	Foam	++	+++
	Olive oil	++	++
Coumarins	NaOH paper test	--	--
Alkaloids	Dragendorff	--	--
	Mayer	--	--
Phytosterols	Liebermann Burshard	++	++
Terpenoids	Salkowski	++	++
Cardiac-glycosides	Keller-Killani	-	-
	Baljet test:	-	-
Anthraquinones	Borntrager	--	--
Mucilage	Alcohol test	++	++
Resins	Acetic anhydride	++	++

(+++) high presence, (++) moderate presence, (+) presence, (-) absence.

**Fig. 1:** Calibration curve of gallic acid standard for determination of TPC.**Table 4:** TPC in the crudes extracts of *P. palaestina* expressed in terms of gallic acid equivalent, mg of GAE/g of extract.

<i>P. palaestina</i>	Type of extract	
	Aqueous	Methanol 60%
Leaves	395.17 ± 10.63 *	489.79 ± 15.10 *
Fruits	144.80 ± 8.81 *	293.64 ± 13.32 *

*Express the presence of a significant difference between all groups at Significant differences at ($P < 0.05$).

GAE: gallic acid equivalent. Means ± SD for triplicate results.

Total Flavonoid Content

The total flavonoid content of different extracts of *P. palaestina* was calculated using a linear equation derived from the standard curve of rutin ($y = 17.623x + 0.014$, $R^2 = 0.9992$), (Figure 2). The highest flavonoid content was found in the methanolic leaf extract (36.16 mg RUE/g extract) while the lowest flavonoid content was recorded in the aqueous fruit extract (18.34 mg RUE/g extract), as shown in Table 5.

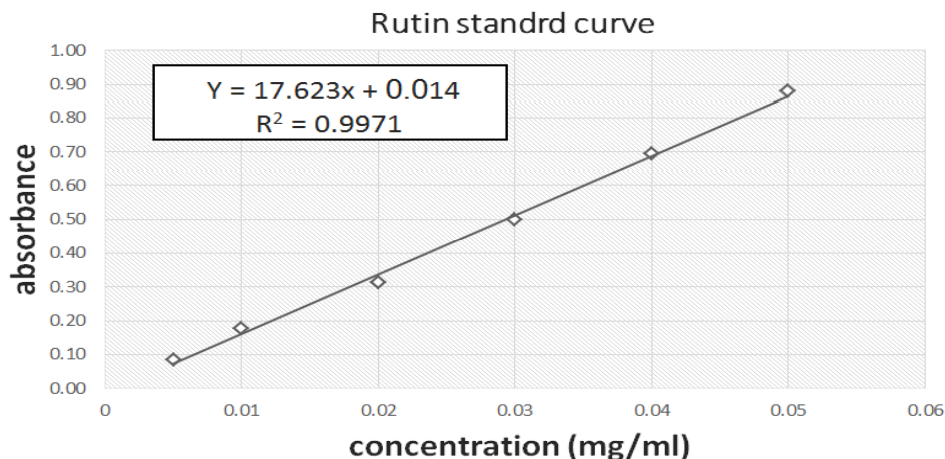


Fig. 2: Calibration curve of rutin standard for determination of TFC.

Table 5: Flavonoid contents in the crudes extracts of *P. palaestina* expressed in terms of rutin equivalent, mg of RUE/g of extract.

<i>P. palaestina</i>	Type of extract	
	Aqueous	Methanol 60%
Leaves	32.66 ± 1.02 *	36.16 ± 1.05
Fruits	18.34 ± 1.16 *	21.05 ± 1.94 *

*Express the presence of a significant difference with the aqueous leaf extract at Significant differences at ($P < 0.05$).

RUE: Rutin equivalent. Means ± SD for triplicate results.

Antioxidant Activity

The capacity of methanolic extracts of *P. palaestina* to scavenge DPPH free radical was recorded as % inhibition, where ascorbic acid could be considered as a positive control in this work. It was found that at 70 µg/mL of concentration, the percentage inhibition of methanolic extracts was $89.58 \pm 1.89\%$ and $79.46 \pm 1.58\%$ for leaves and fruits, respectively. IC_{50} values were calculated using a logarithmic equation derived from the curve in Figure 3. However, these results revealed that leaves extract inhibited DPPH free radicals strongly, with IC_{50} of 5.16 ± 1.22 µg/mL followed by the fruit extract having IC_{50} of 8.21 ± 1.87 µg/mL, while the IC_{50} value of ascorbic

acid was 3.52 ± 0.14 µg/mL, as presented in Table 6.

Wound Contraction and Healing Period

After conducting of wound incision, ointments were applied topically on the rats. Neither changes in rats' behavior nor their death were observed during 21 days (the experimental period). The percentage of wound contraction for all groups is shown in Table 7. The time of healing was recorded and compared with that of group IV (negative control) and group I (Gentamycin ointment 0.1%), where the mean time of healing in the group I, group II, group III, and group IV were 8.2, 9.6, 12, and more than 21 days, respectively. The results showed that extracts-treated wounds healed faster and the rate of wound contraction was significantly ($P < 0.05$) increased in comparison to the negative group, as shown in Table 7.

Table 6: Antioxidant activity of methanolic extracts and ascorbic acid.

Type of extract	DPPH (IC_{50} µg/ml)
Fruits of <i>P. palaestina</i>	8.21 ± 1.87 *
Leaves of <i>P. palaestina</i>	5.16 ± 1.22
Ascorbic acid (standard)	3.52 ± 0.14 *

*Express the presence of a significant difference with ascorbic acid at Significant differences at ($P < 0.05$).

Means ± SD for triplicate results.

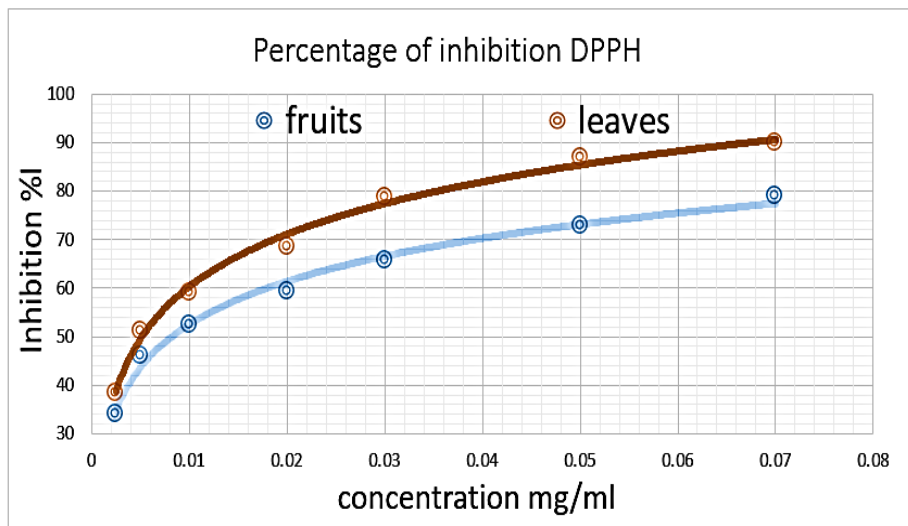


Fig. 3: DPPH free radical scavenging activity for methanol extracts of the leaf and the fruit of *P. palaestina*.

Table 7: Percentage wound contraction and complete wound healing of rats treated with different ointments.

Days	3	6	9	12	15	18	21	final healing period (days)
Group I	17.53 ± 5.42	43.13 ± 6.67	65.2 ± 5.22	100%				12 ± 1.22 *
Group II	28.6 ± 3.34	59.8 ± 5.08	100%					8.2 ± 1.30 *
Group III	24.4 ± 4.11	49.86 ± 6.51	92.76 ± 2.23	100%				9.6 ± 1.14 *
Group IV	12.6 ± 1.73	28.26 ± 5.11	37.93 ± 6.35	46.73 ± 5.85	57.73 ± 4.76	69.4 ± 2.47	75.67 ± 6.32	23.4 ± 3.64 *

*Express the presence of a significant difference with group IV at Significant differences at (P < 0.05).

Values are Mean ± SD of 5 animals in each group.

Discussion

Extraction Yield (%)

The extraction yield of bioactive compounds from plant materials can be affected by many factors such as the extraction methods, the nature of the phytochemicals, the chemical polarity and nature of solvents, and the extraction temperature^{37&38}. According to the results (Table 2), methanol (60%) gave high extraction efficiency of studied plant parts. As phenolic compounds (flavonoids, polyphenols) have polar phenolic hydroxyl group/s, and Hydro-methanol is suitable for their good extraction.

Qualitative Phytochemical Screening

Nowadays, plant extracts from aromatic and medicinal plants are important for the preparation and development of food additives and alternative medicine. The phytochemical

compounds found in medicinal plants are known to have important biological activities (antimicrobial, anticancer, antioxidant, and as a natural antibiotic)^{39&40}. The chemical analysis of plants is a necessary process in qualitative phytochemical studies. Our results were similar to a study conducted by Alhajali et al., 2021, in which they found that leaves of *P. atlantica* contain phenolic compounds, flavonoids, and saponins, but did not have alkaloids or cardiac glucosides⁴¹, and by Barbouchi et al., 2018, in which they concluded that leaves and fruits of *P. lentiscus* have phytosterols, carbohydrates, and phenolic compounds, but anthraquinones were not found⁴². Nevertheless, a study in Morocco differed from our findings, which showed the presence of alkaloids in *P. terebinth*⁴³, and this difference may be due to the change in geographical factors and the genetic combination of these plants⁴²

Total phenolic content

Phenolic compounds are divided into flavonoids, tannins and other compounds which contribute directly to the antioxidative action and are known as potent chain breaking antioxidants because of their hydroxyl groups¹², on the other hand, the basis of the total phenolic assay is the fact that a phenol (C₆H₅-OH) loses an H⁺ ion to form a phenolate (C₆H₅-O⁻) ion, which reduces F-C reagent⁴⁴. Many papers have confirmed that *Pistacia* is rich in phenolic compounds^{2&6}. In this study, TPC was lower than *P. terebinth*, where TPC was 254.59 ± 0.04 and 434.89 ± 1.30 mg GAE/g extract, respectively⁴³. However, in this paper TPC was higher than a study carried out by Bilto et al., 2015, in which they found that TPC was 149 mg GAE/g extract⁴⁵.

Total flavonoids content

The mechanism of the total flavonoid assay is that the aluminum ion (Al⁺³) combines with C-4, and either with C-5 or C-3 hydroxyl, or with ortho hydroxyl groups in the A or B ring of flavonoids structure to form colored complexes³⁹. In this work, TFC values were similar to a study carried out by Alhajali et al., 2021, in which they found that TFC was 31.81 ± 0.26 (mg RUE/g extract)⁴¹. But, our results were lower than a study performed by Bilto et al., 2015, which was carried out on the methanolic extract of leaves of *P. palaestina*, where TFC was 115 (mg RUE/g extract)⁴⁵. The difference in total flavonoids and phenols content among species is influenced by various extrinsic and intrinsic factors, such as the genetic potential of individual species for phenolic biosynthesis, the environmental condition and the maturation stage³².

Antioxidant Activity

Medicinal plants have attracted the attention of scientific researchers, being the potential sources for several antioxidants like polyphenols where free radicals play an obvious role in many pathological manifestations⁴⁶. The principle of the antioxidant capacity assay is that the free radical (DPPH) reacts with the antioxidant compounds in plants due to the essential ability of the antioxidant compounds present in the samples to give hydrogen atoms to DPPH, leading to a color change from purple to yellow. DPPH assay has been used for antioxidant screening activity because it is

sensitive to active constituents at low concentrations, and it can deal with many samples in a short time. The results (Table 5) showed that the antioxidant activity of the methanol leaf extract was higher than the methanol fruit extract due to a high content of phenolic and flavonoid compounds.

Compared to previous papers, our results were better than the study of Bilto et al., 2015, who tested the methanolic leaf extract of *P. palaestina*, where IC₅₀ was 9.5 µg/mL⁴⁵. However, our findings were similar to that a study by Topçu et al., 2007, who found that methanol extracts from Turkish *Pistacia terebinthus* revealed a DPPH free radical scavenging activity of more than 90% at 100 µg/mL⁴⁷. Many studies have confirmed the correlation between antioxidant activity and phenolic content, which agrees with this article⁴⁶.

Wound Contraction and healing Period

Hydrophobic ointments are known to assure a rapid drug release at application sites when they are loaded with hydrophilic compounds as plant methanolic extracts⁴⁸. The phytochemical components play an important role in stimulating the faster healing of the wound. For instance, tannins are a wide group of high molecular weight polyphenolic compounds with the ability to form irreversible and reversible complexes with polysaccharides, proteins, nucleic acids, minerals, and alkaloids⁴⁹. In addition, it can stimulate the wound healing process through three mechanisms: 1) Increasing fibroblasts proliferation and formation of capillary vessels, 2) Their astringent properties promote rapid healing by the production of new tissues in wounds, and 3) Scavenging of reactive oxygen species and free radicals⁵⁰. Flavonoids and polyphenols are also known to improve wound healing activity because of their anti-inflammatory activity (inhibiting the synthesis of prostaglandins), free radical scavenging, and anti-microbial effect, as well as flavonoids increase the viability and strength of collagen fibrils due to inhibiting lipid peroxidation. All these seem to be responsible for the increased rate of epithelialization and wound contraction⁵¹. Saponins are naturally surficial active glycosides that are used in bleeding treatment due to their properties of coagulating and precipitating red blood cells. It also has antibacterial activity⁵². The wound healing

study revealed that the ointments from the methanolic extracts of *P. palaestina* lead to improved wound healing rate and a significant ($P<0.05$) decrease in healing time as compared with the negative control group. These results could be due to the presence of various phytochemical compounds in methanolic extracts of *P. palaestina* such as tannins, terpenoids, flavonoids, polyphenols, and saponins, which may be contributing to the wound healing process independently or by synergistic effects. Supporting evidence explained that this plant has antioxidant and antimicrobial activities⁹, which create a suitable condition for wound healing to take place. Moreover, many studies have shown that *P. atlantica*⁵³ and *P. lentiscus*⁵⁴ have antibacterial and antioxidant activities, and also possess wound healing effects. Compared with the literature, the results are in agreement with other studies. For instance, a study by Tohidi et al., 2011, which was carried out on methanolic extracts of *P. atlantica* and *P. khinjuk*⁵⁵, Also, a study by Hemida et al., 2021, which was conducted on *P. lentiscus* leaf ethanolic extracts⁵⁶.

Conclusion

In this paper, the results of qualitative phytochemical screening of *P. palaestina* have been shown to be rich in a wide spectrum of bioactive compounds. As for the total content of phenols and flavonoids, it was abundant in leaves and fruits. *P. palaestina* extracts exhibited a good antioxidant ability, which could be attributed to polyphenols. Methanolic extracts from *P. palaestina* exhibited effective wound healing activity in rats. This effectiveness is probably due to the presence of phenolic compounds, saponins, tannins, and flavonoids in the studied extracts. Further studies should be carried out to isolate, identify, purify, and characterize the individual active constituents responsible for the antioxidant activity and wound healing of this plant, and to explain the probable mechanism of action of these extracts in the management of wounds and oxidative stress diseases.

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نشرة العلوم الصيدلانية جامعة أسيوط



المسح الكيميائي النباتي النوعي والتحري عن الخصائص المضادة للأكسدة والشافية للجروح في خلاصات نبات البطم الفلسطيني

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يهدف هذا العمل إلى إجراء فحص كيميائي نباتي نوعي لأجزاء مختلفة من نبات البطم الفلسطيني، وتقييم نشاطها المضاد للأكسدة والشافي للجروح من أجل التطبيقات العلاجية. إن المسح الكيميائي النباتي النوعي للأوراق والثمار تم بحته باستخدام اختبارات كيميائية نوعية متعددة. بالإضافة لذلك، اعتمدت الطرق الطيفية لتحديد المحتوى الكلي للفينولات والفلافونويدات والنشاط المضاد للأكسدة حيث استخدم لذلك كاشف الفولين سيكالتو وكلوريد الألمنيوم و DPPH على الترتيب. الخلاصات ذات المحتوى الأعلى من الفينولات والفلافونويدات قيمت فعاليتها الشافية للجروح باستخدام جردان التجربة ونموذج الشق الجراحي. أظهرت نتائج المسح الكيميائي النباتي لأوراق وثمار البطم الفلسطيني احتوائها على (الفينولات، التانينات، الفلافونويدات، السكريات، السابونينات، الستيرويدات النباتية، التربينويدات، والمواد اللعابية والراتنجية، مع غياب الكومارينات والقلويدات والجليكوزيدات القلبية والانثراكينونات). وبالنسبة للمحتوى الكلي للفينولات والفلافونويدات فتراوحت النتائج بين (١٤٤,٨٠ ± ٨,٨١ الى ٤٨٩,٧٩ ± ١٥,٠٩ مغ مكافئ من حمض الغالك / غ خلاصة جافة) و (١٨,٣٤ ± ١,١٦ الى ٣٦,١٦ ± ١,٠٥ مغ مكافئ من الروتين / غ خلاصة جافة) على الترتيب. الفعالية المضادة للأكسدة لخلاصتي الأوراق والثمار وحمض الاسكوربيك (مادة عيارية) كانت ٥,١٦ ± ١,٢٢، ٨,٢١ ± ١,٨٧، ٣,٥٢ ± ٠,١٤ ميكروغرام / مل على الترتيب. أظهرت نتائج الدراسة على الجردان أن المراهم المحضرة من الخلاصات الميثانولية سرعت التئام الجروح بشكل ملحوظ ($P < 0.05$) بالمقارنة مع مجموعة الشاهد السلبي، حيث كان الزمن النهائي للشفاء ٨,٢ ± ١,٣٠ (ثمار)، ٩,٦ ± ١,١٤ (أوراق)، ١٢ ± ١,٢٢ (مرهم جنتاميسين ٠,١%) وأكثر من ٢١ يوم بالنسبة لمجموعة الشاهد السلبي. نستنتج من الدراسة الحالية، أن نبات البطم الفلسطيني يمتلك نشاط مضاد للأكسدة وفعالية في تسريع شفاء الجروح، وبالتالي يمكن استخدامه كدواء محتمل لعلاج الأمراض المرتبطة بالشدة التأكسدية والجروح الجلدية.