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Contribution to the study of the biological activities of pomegranate peel

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Abstract

This study investigates the wound-healing efficacy of pomegranate (Punica granatum L.) bark powder, a fruit historically revered for its medicinal properties. Conducted over 14 days using Wistar rats, the research compared a control group, two test groups (treated with 0.5 g and 1 g of powder), and a reference group using a commercial ointment. The experimental design focused on evaluating skin irritation and monitoring macroscopic wound contraction. The results demonstrated that pomegranate bark powder significantly accelerates the healing process without causing skin irritation. Remarkably, treated wounds reached near-complete recovery in approximately four days, a much faster rate than the untreated control group. This enhanced regeneration is attributed to the bark's bioactive compounds, which possess potent antioxidant and regenerative properties. Ultimately, the study concludes that pomegranate bark is a promising natural source for developing pharmaceutical and nutraceutical applications aimed at improving tissue repair and human health.

Keywords: Antioxidant Activity, Creatinine, Pomegranate peel, Skin irritation, Wistar rats.

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1. Introduction

Fruits have long served as a common source of therapeutic agents, often utilized in the form of traditional preparations. According to WHO [1] estimates approximately 80% of the global population relies primarily on traditional medicines to meet their primary healthcare needs. Among the various plants employed in both traditional and modern medicine, the pomegranate *(Punica granatum L.)* stands out as one of the earliest cultivated fruits, alongside figs and grapes. Pomegranates are renowned for their diverse properties and have been extensively used in both dietary and therapeutic contexts. Traditionally, they have been employed to alleviate ailments such as diarrhea and abdominal colic. Over time, numerous additional health benefits have been identified, establishing the pomegranate as a valuable natural remedy [1]. The pomegranate is widely cultivated across tropical and subtropical regions and is recognized as a rich source of antimicrobial, anticancer, antiviral, antioxidant, and antiproliferative compounds. These bioactivities have made it the focus of extensive scientific research. The fruit contains significant amounts of phenolic compounds, including anthocyanins, ellagic acid, punicalin, punicalagin, pedunculagin, and other flavanols [1].

Pomegranate peel (*Punica granatum* L.), often considered a by-product of fruit processing, has recently attracted growing scientific interest as a valuable source of bioactive compounds with notable medicinal and nutritional properties. Accounting for approximately 30–50% of the total fruit weight, the peel is particularly rich in polyphenols such as tannins, flavonoids, and phenolic acids, as well as dietary fibers, vitamins, and essential minerals [2].

Several studies have investigated the distribution of antioxidant bioactive compounds within different parts of the pomegranate (*Punica granatum* L.), revealing that the highest concentrations are present in the peel. In particular, pomegranate peel extract has been shown to enhance free radical scavenging activity, confirming its potent antioxidant potential[3]. Owing to its abundance in natural antioxidants with significant medicinal and therapeutic properties, pomegranate peel extract can be applied in a wide range of fields. Notably, a 5% preparation of the extract, containing approximately 44% phenolic compounds, demonstrated a marked wound healing effect in Wistar rats after ten days of treatment [3, 4].

The pomegranate peel, also referred to as *Malicorium*, accounts for nearly 50% of the total fruit weight. Typically measuring 2 to 3 mm in thickness and displaying bright red or yellow hues depending on the variety, the peel is composed of about 80% water, 8% complex polysaccharides, and 5% soluble polysaccharides. The latter fraction mainly comprises pectins and hemicelluloses. In addition, the peel contains essential minerals such as sodium, potassium, phosphorus, calcium, and magnesium [5].

Due to its richness in antimicrobial and antioxidant constituents, the peel plays a protective role, shielding the fruit against microbial invasion and ultraviolet radiation [6]. These bioactive compounds have been associated with diverse pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects [7]. Recent advances further underscore the potential of pomegranate peel extract in various biomedical applications such as drug delivery, tissue repair, and regenerative medicine highlighting its promising use as a functional ingredient in foods, nutraceuticals, and pharmaceutical formulations [8-10]. This study aims to characterize the biochemical and physicochemical properties of the peel, highlighting its bioactive compounds and their interaction with skin tissues.

2. Materials and Methods

2.1. Ethics Statement

All experimental procedures involving animals were conducted in strict accordance with the ethical standards and guidelines for the care and use of laboratory animals approved by the Algerian Institutional Ethical Committee for Animal Research (Ministerial decree n° 991 of December 10,2020).

2.2. Vegetal Material

The collection of pomegranates (*Punica granatum* L.) was conducted in the Ghardaïa region of Algeria, known for the exceptional quality of its fruits. The pomegranates were carefully selected based on their ripeness and organoleptic characteristics, ensuring a richness in bioactive compounds. After harvesting, the fruits were peeled to isolate the peels, which are particularly rich in polyphenols, flavonoids, and ellagic acid compounds recognized for their antioxidant and anti-inflammatory properties.

To prevent the degradation of metabolites sensitive to light and moisture, the pomegranate peels were air-dried under shaded conditions. This drying step is critical for reducing moisture content to an optimal level, thereby ensuring the preservation of the peel's pharmacologically active constituents. Once completely dried, the peels were finely ground using a blade mill to obtain a uniform and homogeneous powder. The resulting fine particle size is essential for subsequent physicochemical and biological analyses, as it maximizes the surface area available during extraction procedures. The prepared powder was then stored under appropriate conditions and used for further investigations aimed at characterizing its medicinal potential and evaluating its possible applications in the pharmaceutical, nutraceutical, and cosmetic fields.

2.3. Physicochemical Study of Pomegranate Peel

2.3.1. pH Determination

We dissolved 1 g of pomegranate peel powder in 10 mL of distilled water. The resulting solution was placed in a stirrer for 10 minutes. The pH of this solution was measured using a pH meter. The pH value was then recorded after stabilization.

2.4. Determination of Moisture Content

To measure the water content, 5 g of pomegranate peel powder were weighed in a container and then placed in an oven at 103 ± 2 °C until a constant weight was achieved. The mass was then recorded. The moisture content of our powder is indicated by the mass loss expressed as a percentage of the initial mass : H%=(m0m0−m1)×100

Where : m0 = mass in grams of the test sample.

m1 = mass in grams of the test sample after drying.

2.5. Protein Dosage by the Bradford Method

2.5.1. Principle

The method of protein binding to Coomassie blue, also known as the Bradford method, was first introduced by Bradford in 1976[11]. To quantify a protein sample, the method involves exposing it to the dye Coomassie Brilliant Blue G-250. In its anionic state, Coomassie blue appears red and has a maximum absorption at 465 nm. However, after being mixed with proteins, the basic and aromatic side chains of the amino acids interact to form a complex. Coomassie blue then manifests in its cationic blue form, with an absorption peak at 595 nm.

The process of complex formation is rapid and typically lasts only a few minutes. It remains stable for about an hour and can be easily measured using a UV spectrophotometer at 595 nm by measuring the absorbance of its coloration. Finally, a calibration curve is established using the Beer-Lambert relationship to determine the total protein concentration of our sample [11-13]. It is important to note that this method cannot detect peptides and low molecular weight proteins (< 3000 DA).

2.6. Operating Procedure

The Bradford reagent was freshly prepared, filtered, and stored at 4 °C in the dark to ensure reagent stability for up to one month. A total of 1 g of pomegranate peel powder was dissolved in 10 mL of distilled water and gently stirred for several seconds to obtain a homogeneous extract. The resulting solution was subsequently diluted twice by the successive addition of 500 µL of distilled water. From this diluted extract, 100 µL was mixed with 100 µL of Bradford reagent.

At the same time,, a bovine serum albumin (BSA) standard solution (1 µg/mL) was prepared to establish a calibration curve using six serial dilutions, each also mixed with 100 µL of Bradford reagent. All samples and standards were incubated in the dark for 30 minutes to allow for complete color development. The absorbance of each sample was then measured spectrophotometrically at 595 nm.

Finally, a standard calibration curve was generated from the BSA solutions, and the protein concentration in the pomegranate peel extract was determined based on the linear regression equation derived from the calibration plot.

2.7. Measurement of Antioxidant Activity (by Scavenging of the DPPH Free Radical)

2.7.1. Principle

The compound DPPH (2,2-diphenyl-1-picrylhydrazyl) was one of the first free radicals used in the study of the link between phenolic compounds and their antioxidant properties [12]. Based on the degradation of the DPPH radical, this technique employs colorimetry. The synthetic DPPH radical, which is purple in color, can be stabilized by an antioxidant that transfers hydrogen to a singlet electron. This reaction causes DPPH to shift to yellow-green, and its absorbance can be measured at 515 nm.

2.8. Determination of the Percentage of Inhibition

% d‟inhibition =DO du control−DO d′échantillonDO du control x 100

Where DO = optical density.

2.9. Determination Of IC50

The IC50 (the concentration of DPPH that inhibits 50% of free radicals) was determined graphically using the formula derived from linear regression.

2.10. Animals And Housing Conditions

Twenty-four adult male Wistar rats, aged two months and weighing 140 ± 8 g, were obtained from the Pasteur Institute of Algiers (Algeria).The animals were housed in polypropylene cages under controlled temperature (24°C) and humidity (40-70%) conditions, and a 12:12 h light-dark cycle. They received unlimited tap water and a standard diet (Bouzareah, Algiers). All animal experiments began after one week of acclimatization. All procedures were carried out in compliance with current Algerian legislation on animal protection.

2.11. Irritant Effect on the Skin

The study was conducted following OECD guidelines (404 adopted on April 24, 2002). The study aimed to assess the skin irritation potential of grenadine bark from a single topical application. We used the skin irritation test method described elsewhere[15,16]. Twenty-four male Wistar rats were used for the present study, and the duration of the experiment was 14 days.

On day 0 of the test period, the rats were shaved using an electric razor on the trunk and lateral parts (approximately 10% of the body surface for application of the test substance) (EPA 712-C-98-202, 1998). The rats were housed individually and acclimatized for 24 hours prior to treatment. Various doses of pomegranate bark powder were then applied to both intact skin and skin that had been superficially abraded by gentle scarification of the stratum corneum, ensuring that no bleeding occurred [17].

2.12. Distribution Of Groups

Group 1: Six rats received no treatment (Control).
Group 2: Six Rats treated with 0.5 g of pomegranate peel powder.
Group 3: Six Rats treated with 1g of pomegranate peel powder.
Group 4: Six Rats treated with pharmaceutical healing ointment

2.13. Blood Sugar Test

Blood sugar was measured using glucometers (Accu-check active, Roche Diagnostic, Mannheim, Germany). The glucometer is a reader intended for the quantitative determination of blood glucose from fresh blood and Vital-check active test strips (MM1200).

2.14. Blood Samples

At the end of our experimental protocol, the rats in each group were weighed and then anesthetized. The blood is collected by cardiac puncture then centrifuged at 3500 rpm for 15 seconds. The serum is preserved for biochemical testing.

2.15. Biochemical Assay

Blood samples were centrifuged at 1500 rpm for 10 mins, then the plasma was separated. Selected blood parameters were performed by an automated analyzer (Architect CI 8200) in the laboratory, and included blood creatinine, urea [14].

2.16. Statistical Analysis

The results are presented as mean ± SE. The comparison of means between several groups of rats was done using the ANOVA test. Mean differences were considered significant at $p<0.05$.

3. Results

3.1. Physicochemical Quality

The table presents the results concerning the physicochemical characteristics, based on the average of six samples of pomegranate peel.

3.2. pH

The average pH value of the analyzed pomegranate peel is equal to 6.03 ± 0.009.

3.3. Water Content

The water content of the samples is equal to 26.4 ± 0.17.

Table 1.
Physicochemical Analysis of Pomegranate Peel Samples (n=6, where n refers to 6 different samples of pomegranate peel).

Parameter	**Mean**
pH	6.03±0.009
Water %	26.4±017
IC 50	125,71±0.01 µg/ml
Proteins	2.18± 0.02 µg /ml

3.4. Antioxidant Activity: Percentage of DPPH Radical Inhibition

The results of the measurement of the antioxidant power of methanolic extract of our pomegranate peel, as well as that of vitamin C, are presented in the figure below.

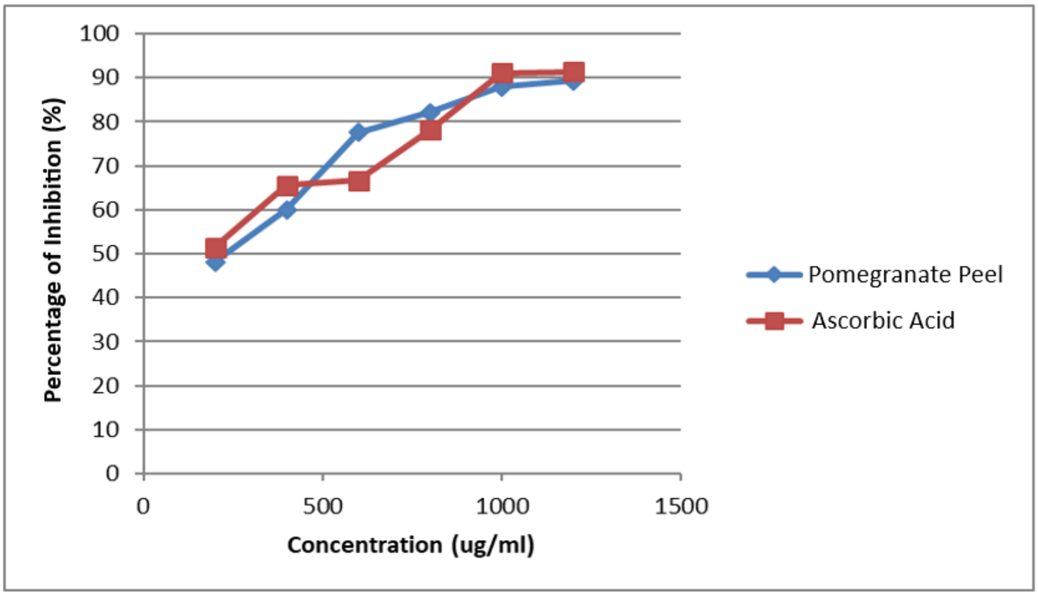
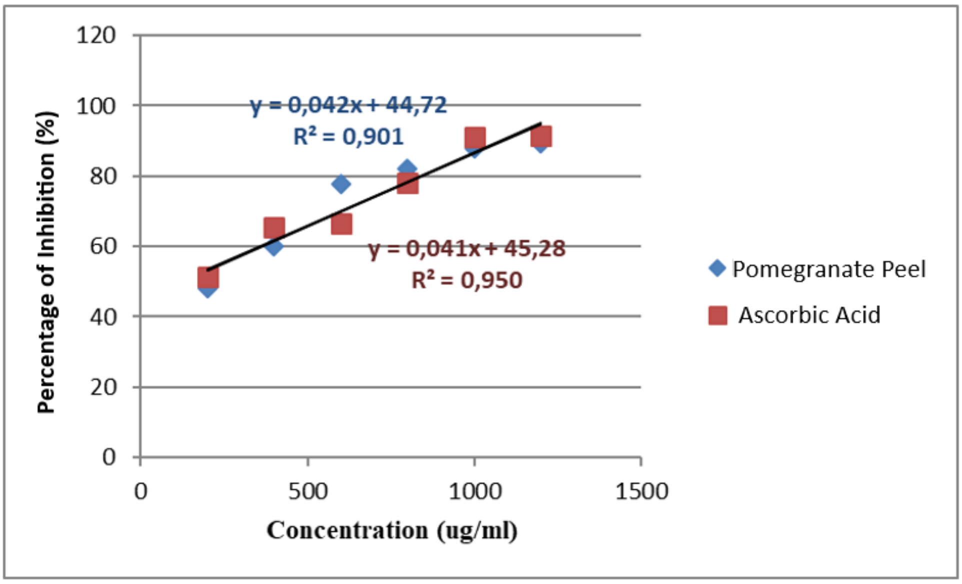


Figure 1.
Comparison of DPPH radical scavenging activity of Punica granatum L. extract and ascorbic acid.

Considering the curve above, a positive correlation is observed between the percentage of free radical inhibition and the increase in concentrations, whether for the pomegranate peel extract or for vitamin C. This reducing or inhibiting potential of the pomegranate peel extract lies in the presence of molecules with electron-donating capabilities. Our results indicate that pomegranate peel exhibits a strong reducing power, comparable to that of the standard substance (vitamin C).

3.5. Determination of IC50

The IC50 value was determined graphically through linear regression, as shown in the figure.



Figue 2.
Comparative IC-50 analysis of pomegranate peel extract and ascorbic acid.

These results indicate that the IC50 value for pomegranate peel is 125.71 µg/ml, which is very close to that of vitamin C at 115.12 µg/ml. A lower IC50 concentration suggests a higher antioxidant activity.

3.6. Determination of Protein Concentration (Bradford Method)

The results of the protein assay for pomegranate peel using the Bradford method are presented in the figure below.

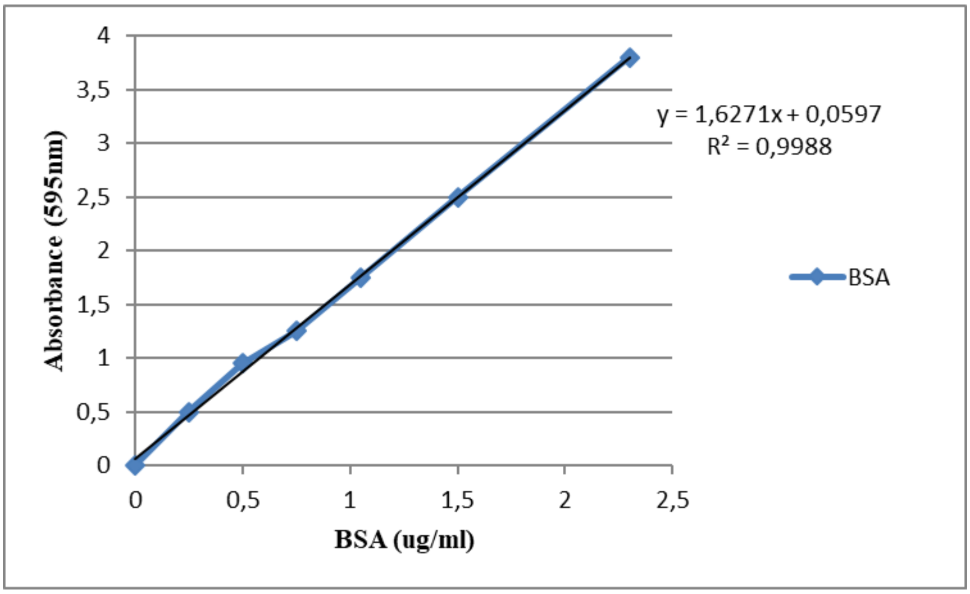


Figure 3.
Determination of Protein Concentration.

The protein concentration of our extract was determined graphically through linear regression, as illustrated in the figure. This allowed us to observe that our extract has a protein content of 2.18 µg/ml.

3.7. Blood Glucose Measurement

The analysis of blood glucose levels revealed homogeneity among the four studied groups, each exhibiting values considered normal.

Table 2.
Blood sugar values.

Blood Glucose	Values
Negative controls	1.12± 0.22
Control at 0.5g of bark powder	0.96±0.23
Control at 1 g of bark powder	1.25±0.009
Control with ointment	1.11±0.12

3.8. Biochemical Test

The statistical analysis revealed no significant differences between the two experimental groups concerning the measured parameters. These results suggest that the application of pomegranate bark in powdered form on the irritated skin, at the specified dose, does not induce disturbances in creatinine and urea levels. This indicates the safety of this intervention on renal functions.

This conclusion is essential, as it indicates that the local application of this substance does not appear to negatively affect nitrogen waste metabolism, thereby contributing to the maintenance of bodily homeostasis.

Table 3.
Biochemical test.

Parameters	Négative Controls	Controls at 0.5g of Pomegranate Bark	Controls at 1g of Pomegranate Bark	Controls with Ointment	Normal Values
Creatinine (mg/dL)	0.6±0.01	0.5±0.001	0.6±0.00	0.6±0.00	0.5-1.5
Urea (mg/dL)	06±0.00	06±0.2	06±0.03	06±0.003	06-23

3.9. General Conditions of Animals

Throughout the experimental period, no behavioral abnormalities were observed in the animals of both the treated and untreated groups, indicating stable behavior in both experimental conditions.

3.10. Evolution of Skin Condition

During the first phase of the study, we observed the following progression in wound healing. Rats treated with pomegranate bark powder showed signs of healing after just 4 days. In contrast, those treated with a healing ointment exhibited recovery between the fifth and sixth days. The control rats, however, took 8 days to heal.

These observations suggest that pomegranate bark powder promotes the healing process, with signs of recovery visible as early as the fourth day. In comparison, the wounds in the control group required between 7 and 8 days to heal, while those treated with the healing ointment began to show signs of recovery starting from the fifth day.

3.11. Quantification of Irritation State

The visual assessments of erythema and edema revealed that no edema was present in either the treated rats or the control rats. In our study, we examined the healing effect of pomegranate bark powder using an in vivo experimental model, and our results should enhance the understanding of the mechanisms involved in this process. The findings indicate that pomegranate bark aids in faster skin healing when it is cut or damaged. After 10 days of treatment with the pomegranate bark extract gel, the rats were healed, while the control rats required 16 to 18 days for complete healing.

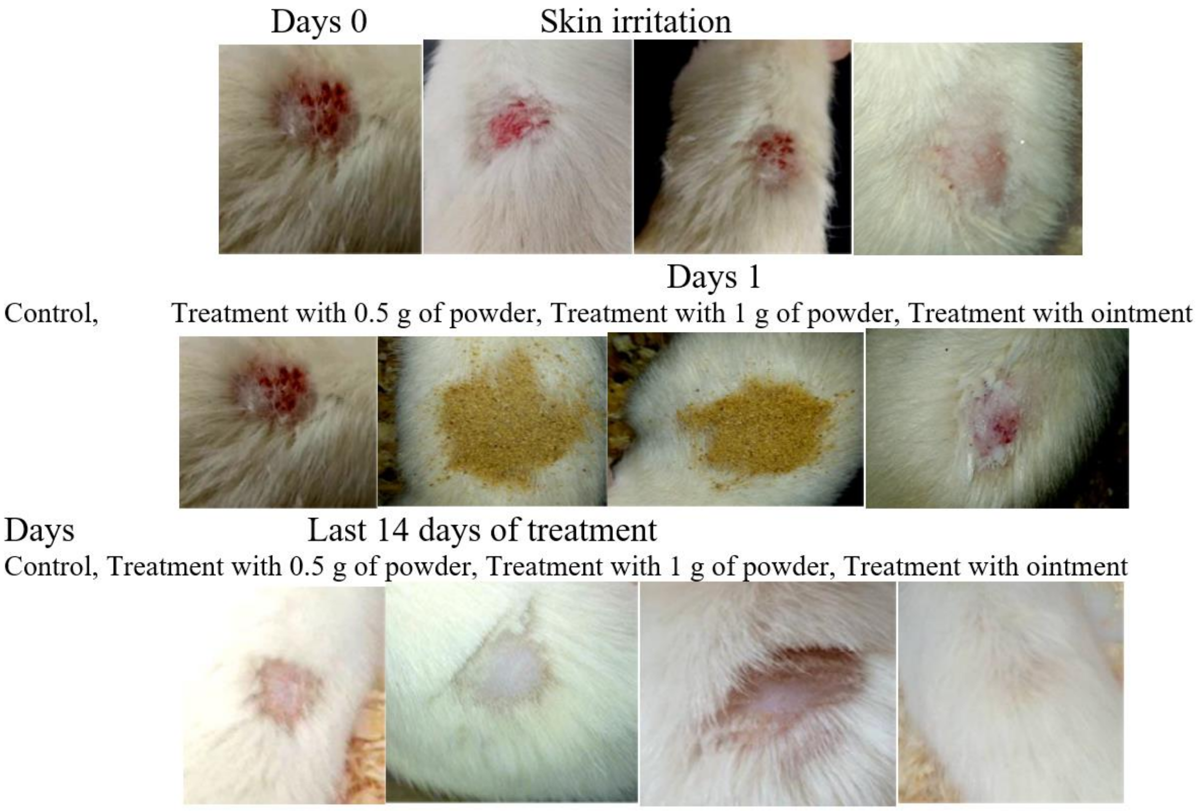


Figure 4.
Macroscopic progression of skin wound closure in rat models following various treatments.

4. Discussion

Several scientific studies have examined the pH of pomegranate peel and its implications for various applications. For instance, a study by Al-Azzawi, et al. [15] explored the physicochemical properties of pomegranate peel extract, noting that the pH varied depending on the extraction method used. Additionally, Al-Juhaimi [16] investigated how the pH of pomegranate peel extracts influences their antimicrobial activity, highlighting the importance of pH in the effectiveness of these extracts. Another study by Al-Hilal, et al. [17] focused on the incorporation of pomegranate peel extracts into dairy products, measuring changes in pH and their effects on fermentation characteristics. Furthermore, research by Hussein, et al. [18] discussed the impact of pH from pomegranate peel extracts on food stability and preservation during storage. Finally, Kalinowski, et al. [19] examined the influence of pH on the antioxidant properties of pomegranate peel extracts, suggesting that lower pH levels enhance antioxidant activity. Collectively, these studies underscore the significance of pH in determining the functional properties of pomegranate peel in various applications. Several studies have examined the water content of pomegranate peel and its implications for various applications. For instance, Al-Rawahi, et al. [20] studied the influence of different drying techniques on the water content and phenolic compounds of pomegranate peel, revealing that freeze-drying was effective in preserving volatile compounds, while sun drying resulted in the highest water loss. In another study, Al-Rawahi, et al. [20] measured and modeled the water sorption isotherm using the Brunauer-Emmett-Teller (BET) and Guggenheim-Anderson-De Boer (GAB) models. Kumar, et al. [21] also explored the effect of pomegranate peel extract on the water content of chitosan-based edible films. Additionally, Khazaei, et al. [22] evaluated the effects of spray drying conditions on the water content of pomegranate juice powder enriched with phenolic compounds from pomegranate peel, finding water content ranging from 7.84% to 11.39%. Finally, other research compared various drying methods, noting that solar drying was preferable due to its low moisture content. Collectively, these studies underscore the importance of water content in pomegranate peel and its influence on functional properties. Various studies have highlighted the antioxidant potential of pomegranate peel extracts, revealing a positive correlation between the concentrations of extracts and the inhibition of free radicals. For instance, Gil, et al. [23] demonstrated that the polyphenols present in pomegranate peel have significant electron-donor capabilities, allowing them to neutralize free radicals and stabilize reaction products. Similarly, Sahu and Padhy [24] found that pomegranate peel extract exhibited a reducing power comparable to that of vitamin C, thus underscoring its effectiveness as an antioxidant agent. Additionally, research by Sahu and Padhy [24] confirmed that pomegranate peel, rich in phenolic compounds, has inhibitory properties against free radicals, reinforcing the idea that these molecules play a crucial role in protecting cells against oxidative stress. These results support our observation of a strong reducing power in pomegranate peel extracts, similar to that of vitamin C. Previous research has also demonstrated that pomegranate peel possesses significant antioxidant potential. In addition, a study conducted by Sadeghi and Shokrollahi [25] revealed that pomegranate peel extract had an IC50 of 130 µg/ml, indicating an antioxidant activity comparable to that of vitamin C. Furthermore, a study by Afaq, et al. [26] showed that pomegranate extracts, including those from the peel, had similar IC50 values, highlighting their effectiveness as antioxidant agents. Additionally, research by Aslam and Khan [27] emphasized a strong free radical scavenging activity of pomegranate peel extracts, with an IC50 of 120 µg/ml, reinforcing the idea that these extracts can provide protection against oxidative stress. These results support our observation of an IC50 very close to that of vitamin C, thus underscoring the high antioxidant potential of pomegranate peel. Previous studies have also examined the protein concentration in various plant extracts. Kaur and Arora [28] showed that pomegranate extract contained a protein concentration of 2.5 µg/ml, thereby highlighting its nutritional potential. Additionally, Al-Bachir and Al-Shaikh [29] reported a protein content of 2.3 µg/ml in moringa leaf extracts, illustrating the protein richness of certain medicinal plants. Furthermore, a study by Kadir and Azhar [30] revealed that peach extract contained 2.1 µg/ml of protein, reinforcing the idea that plant extracts can be significant sources of protein. These results support our observation of a protein concentration of 2.18 µg/ml in our pomegranate extract, thus underscoring its nutritional relevance.

Recent studies have also examined the impact of pomegranate peel on blood sugar levels. For example, research conducted by Sadeghi and Shokrollahi [25] showed that administering pomegranate peel extracts to diabetic rats resulted in a significant reduction in blood sugar levels, suggesting a potential hypoglycemic effect. Additionally, a study by Nascimento and Costa [31] revealed that the polyphenols present in pomegranate peel could improve insulin sensitivity, thereby contributing to blood sugar regulation. Furthermore, research by Gholami and Mohammadi [32] observed that pomegranate peel extract did not significantly affect blood sugar levels in human subjects, maintaining values within the normal range. These results support our finding that all studied groups exhibit normal blood sugar levels, with no notable differences. Studies have shown that pomegranate bark (Punica granatum) possesses bioactive properties that can influence biochemical parameters such as creatinine and urea. revealed that the ethanolic extract of the bark has low toxicity, even at high doses, suggesting its safe use in experimental contexts. Karam and Al-Shaher [33] documented the safety of whole fruit extracts, with no significant effects on renal function. Vidal, et al. [34] found that while there are variations in creatinine levels, urea levels remain within normal ranges, indicating an absence of major renal disturbances. The work of Elwej, et al. [35] highlighted the nephroprotective effects of pomegranate bark, emphasizing its potential role in mitigating oxidative stress and renal inflammation. These results are corroborated by Atmani-Merabet [36] who observed no toxic effects on biochemical parameters in mice treated with bark extracts. Thus, while pomegranate bark may induce variations in certain biomarkers, it appears to be generally safe and beneficial for renal health, although further studies are needed to better understand its mechanisms of action.

Pomegranate (*Punica granatum*) is recognized for its potential in wound healing, attributed to its rich composition of polyphenols, flavonoids, and tannins, which possess antioxidant, anti-inflammatory, and antimicrobial properties. These compounds collectively aid in wound healing by reducing oxidative stress, inflammation, and preventing infections.

Several researchers have contributed to this area of study. Bassiri-Jahromi, et al. [37] identified key compounds such as ellagic acid, gallic acid, and quercetin in pomegranate bark, highlighting its antimicrobial properties. Hayouni, et al. [38] studied the wound-healing activity of *P. granatum* peel in rats. Akbari, et al. [39] examined the wound-healing potential of pomegranate in diabetic rats, noting an increase in collagen deposition and a reduction in oxidative stress.

Many plants possess antimicrobial, antioxidant, and anti-inflammatory properties, stimulating angiogenesis as well as the proliferation and migration of keratinocytes and fibroblasts, thereby influencing wound healing. Extracts from Vitis vinifera (grape) have been shown to stimulate collagen synthesis, increasing hydroxyproline content and reducing epithelialization time in treated rats [40]. Extracts from *Punica granatum* (pomegranate) are also beneficial for wound healing due to their antioxidant, anti-inflammatory, and antimicrobial properties, stimulating the proliferation of fibroblasts and collagen synthesis [41].

5. Conclusion

The present study highlights *Punica granatum* bark as a remarkably rich source of bioactive compounds, particularly proteins and antioxidants, conferring it significant pharmacological potential. Beyond its nutritive value, the bark demonstrated substantial efficacy in accelerating wound healing, underscoring its regenerative and therapeutic capabilities. These findings position pomegranate bark as a promising candidate for incorporation into both nutraceutical and biomedical applications. Harnessing its biochemical potential could pave the way for the development of innovative natural formulations aimed at enhancing human health, promoting tissue repair, and contributing to sustainable approaches in functional food and pharmaceutical industries.

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