HISTOLOGICAL EVALUATION OF THE WOUND HEALING ACTIVITY OF ALLIUM AMPELOPRASUM METHANOL EXTRACT

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Abstract. Allium ampeloprasum extract has the potential to facilitate wound healing due to its anti-inflammatory, antioxidant, and antibacterial properties. In this study, we aimed to investigate the potential wound-healing activity of the methanol extract derived from Allium ampeloprasum. Using 30 healthy male Wester rats; control group: 15 rats; normal saline applied locally instead of plant-extract. Experimental group: 15 rats received a daily local application of 200 mg/kg of the plant extract. Then, for each healing period of 0, 5, or 10 days, each group was divided into three-subgroups: A, B, and C, each with five rats. There was a significant difference in the percentage of wound contraction between the control and experimental groups (p 0.02). Moreover, at the 5 and 10 days healing periods, there was a significant difference in the inflammatory cell count, the experiment group showed a greater reduction in the inflammatory cells compared to the control group. Measuring the epithelial thickness revealed that the experiment group had considerably thicker epithelial layers (p 0.04). Allium ampeloprasum extracts have shown promising activity as wound-healing promoters. These extracts have many benefits in the management of acute wounds due to their capacity to increase wound contraction, minimise inflammatory cell infiltration, and boost epithelization

Keywords: Histology, wound healing, plant extract, leek

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Introduction

For thousands of years, plants have been used for medicinal purposes, and many traditional healing practices maintain herbal-based therapy as a replacement therapy. Because of its perceived effectiveness, safety, and natural origin, many individuals prefer to use plant extracts to facilitate wound healing. Many plants have a wide variety of active chemicals that can aid in wound healing by decreasing inflammation, increasing blood flow, and promoting tissue repair.

The *Allium ampeloprasum* "leek" is a vegetable that is often known as the broadleaf wild leek. The edible portion of the plant is a bundle of leaf sheaths, which is sometimes incorrectly referred to as a stem or stalk. The onion, garlic, shallot, scallion, chives and Chinese onion are all members of the genus Allium [1-2].

Leeks have high nutritional value and are rich in biologically active compounds and phytochemicals. Leeks are high in dietary fibers, fructans, polyunsaturated fatty acids (PUFA), primarily linoleic acid, different amino acids, primarily glutamine, glutamic acid, aspartic acid, arginine, and alanine, and organic acids such as ascorbic acid and malic acid [3-4].

Moreover, the Allium genus is rich in organic sulphur compounds, quercetin, flavonoids, and other chemicals. These ingredients provide several health benefits, including the prevention of cancer, cardiovascular disease, and heart-related disorders. Anti-inflammatory, anti-obesity, anti-diabetic, antioxidant, antibacterial, neuroprotective, and immunological activities are also present [5-6]. Wound management plays a crucial role in medical and societal situations by promoting healing, preventing infections, reducing pain, minimizing scarring, enhancing mobility, improving socioeconomic outcomes, and maintaining psychosocial well-being [7].

Wound healing is a complicated process involving the interaction of several cell types, growth factors, hormones, and extracellular matrix proteins. The process is divided into four stages: the Hemostasis phase (formation of the blood clot), inflammatory (immune cells relocate to the site of damage and produce cytokines and growth factors), proliferative (collagen and extracellular matrix collaborate to rebuild a new tissue), and finally the maturation phase (the collagen is remodelled and the wound fully closed) [8-9].

The methanol extraction was chosen for the *Allium ampeloprasum*, due to its ability to efficiently extract a wide range of bioactive compounds from the plant material, its availability and cost-effectiveness, as well as its compatibility with subsequent analysis techniques [10].

Allium ampeloprasum methanol extract has the potential to facilitate wound healing due to its anti-inflammatory, antioxidant and antibacterial properties. In particular, its high concentration of bioactive compounds such as flavonoids, which play a key role in wound healing activity due to their radical scavenging properties which enhance antioxidative enzyme activity [11-12].

Materials and Methods

Plant Collection and Preparation

The first step was to choose healthy *Allium Ampeloprasum* leaves " Aerial part" and remove any undesired debris. After that, the leaves were spread out on a clean, dry surface and allowed to dry at room temperature 22 ºC. Drying the leaves removes any moisture that may interfere with the extraction process and also aids in the concentration of the desired chemicals [13].

Preparation of Methanolic Extract

After drying, the leaves were ground using a blender until reached a fine powder, then put in a clean, dry container. After that, we used the maceration technique to prepare the extract by adding 750 ml of the solvent "methanol" to 250 g of dry plant powder. After sealing the container, the solvent was allowed to penetrate the plant material and extract the necessary chemicals. Following the completion of the extraction phase, the plant material was filtered out of the solvent using filter paper. The resultant solution was evaporated to remove the solvent and concentrate the extract, which contained the required chemicals. A rotary evaporator is used in this step [14].

Plant Extract Dose Calculation

To calculate the concentration of the extract, we divide the weight of the collected plant extract by the volume of the solvent applied. Thus, as we obtained a net weight of 75 g of *Allium Ampeloprasum* extract from 750 ml of solvent, the concentration of the extract would be:

Concentration = 75 g/750 ml = 0.1 g/ml

Therefore, as we needed to use a concentration of 20% plant extract and apply it to the rat's skin wound, the correct dosage for allium extracts applied to the wound was decided to be 200 mg/kg [15-16].

Animal Preparation

All experiment procedures followed Baghdad University's College of Dentistry's experimental animal ethics standards, ethical approval No. 422721. Thirty healthy male albinos Wistar rats (Rattus norvegicus) weighing 300 ± 10 g were used in the study. The laboratory rats were split into two groups at random: A control (15 rats) and B experimental (15 rats). Furthermore, we separated the animals in each group into three healing periods: day 0, day 5, and finally day 10. They were split into different cages, and a red marker was used to assign sequence numbers to each rat so that specific individuals involved in the study could be distinguished.

Following the administration of general anesthesia, 1.5 cm surgical incisional wounds with full-thickness skin were created in the skin of each rat, including both the epidermis and the dermis layer. All of the animals in Group A got daily topically administered Allium extract doses of 200 mg/kg, whereas the animals in Group B received normal saline.

Statistical Analysis

The statistical analysis was conducted using the SPSS "Statistical Package for the Social Sciences" software, version 28. The independent sample t-test is used to find the correlation between the two groups; in addition, the dependent sample t-test is used to find the correlation between individuals in the same group at different healing periods.

Preliminary Qualitative Phytochemical Analysis

The ethanolic extracts were analysed using conventional methods to determine the active components via chemical testing.

a) Test for Alkaloids

The alcoholic extract (10 ml) was heated in a steam bath with 1% HCL (5 ml). Mayer's reagent (consisting of 1.35 gm mercuric chloride in 60 ml water and 5 gm potassium iodide in 10 ml water) produced a white precipitate, whereas Wagner's reagent (consisting of 1.27 g iodine and 2 g potassium iodide in 100 ml water) produced a reddish-brown precipitate. The presence of alkaloids is indicated by these colour changes.

b) Test for Flavonoid

Lead acetate test: To 5 ml of the alcoholic extract, a 10% lead acetate solution (1ml) was added. The presence of a yellowish-white precipitate suggested that flavonoids were present. NaOH test: The extract (5 ml) was treated with aqueous NaOH and HCl, and the production of a yellow-orange colour was detected as an indicator of flavonoids' presence.

c) Test for Steroids

Liebermann-Burchard test: The extract (3 ml) was diluted with chloroform and acetic anhydride, and then drops of sulphuric acid were added. Steroids were detected by the presence of a dark pink or red colour formation. H_2SO_4 test: The emergence of a greenish colour after treating the organic extract (2 ml) with sulphuric and acetic acids was taken as evidence for the presence of steroids.

High-performance Thin Layer Chromatography (HPTLC) Examination of Methanol Fraction

In order to prepare the standards (1 mg) and samples (small quantities, measured in milligrammes) for HPTLC, they were dissolved separately in 1 ml of pure methanol. The samples were developed in a solvent system of 100:11:11:27 ethyl acetate, formic acid, acetic acid, and water. Following that the compounds were detected using UV light at a wavelength of 254 nanometers [17].

Slide Preparation

For preparing slides for H&E staining: we fix tissue in formalin (10% neutral buffered formalin) for 24–48 hours. Then dehydrate the tissue using a series of alcohol washes (70%,

80%, 95%, and 100% ethanol). We are followed by clear tissue using xylene (or xylene substitutes) to dissolve alcohol. Infiltrate and embed tissue in melted paraffin wax. Then we cut thin sections (4-6 micrometres) using a microtome and placed them on glass slides. Dry slides overnight and remove wax through deparaffinization using xylene—Rehydrate sections with graded alcohols in reverse order. Finally, we performed H&E staining using hematoxylin to stain nuclei and eosin for cytoplasmic staining [18].

Measuring the Percentage of Wound Contraction

Equation 1 was used to calculate the percentage of wound contraction:

$$\text{Wound healing \%} = [(WD0-WD10)/WD0] \times 100 \quad (1)$$

where the WD0 is equivalent to the incisional wound on day 0 and the WD10 is the incisional wound on day 10 of the healing period [19].

Inflammatory Cell Count

Counting the inflammatory cells for the studied groups at the day 0, day 5, and day 10 healing periods, we captured photos of the field of vision, use microscopic power x40, and, with the aid of ImageJ software, automated cell counting.

Measure the Epithelial Thickness

To determine the degree of epithelization, we measured the epithelium thickness at ten-day wound healing periods for both the control and experiment groups. using ImageJ software and follow these steps. First, choose "File" and then "Open" to import the wound image into ImageJ. Second, draw a straight line across the wound epithelium with the "Rectangle" tool from the toolbar. Check that the line is perpendicular to the surface. Third, go to the "Analyse" menu and select "Set Scale." Enter the known distance in pixels and the matching measurement in micrometres or any other preferred unit. To measure the length of the line created across the wound epithelium, use the "Straight" line selection tool from the toolbar. Make a note of this measurement. Select "Measure" from the "Analyse" menu. A window with numerous measurements, including the length of the line drawn across the wound epithelium, will emerge. Calculate the actual thickness by multiplying the measured length by the scale factor determined in step 3; Figure 1, Epithelial thickness measurement by ImageJ software.

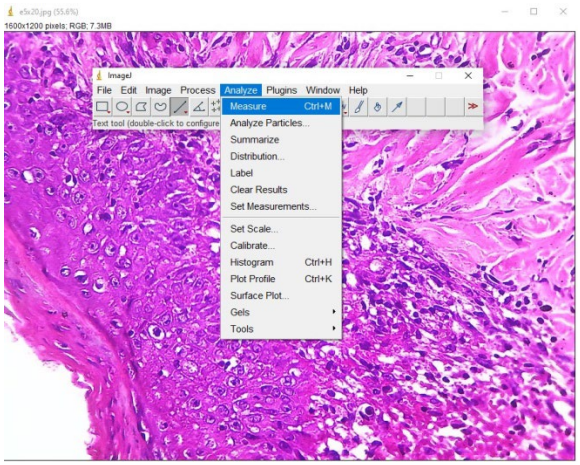

Figure 1: Epithelial thickness measurement by ImageJ software

Results and Discussion

Phytochemical screening of *Allium ampeloprasum* leaf extracts revealed a crude positive for the flavonoid and a negative for both alkaloids and steroids. Table 1 shows the phytochemical screening of *Allium ampeloprasum* leaf extracts.

Table 1: Phytochemical screening of *Allium ampeloprasum* leaf extracts

Active constituents	Results
Alkaloids	-
Flavonoids	+
Steroids	-

The comparison of the Max Rf values of the sample of Table 2 (HPTLC of Allium ampeloprasum methanol extract) and the standards of Table 3 (HPTLC standards), revealed that the methanol fraction of *Allium ampeloprasum* contains rutin, isoquercetin, quercetin, astragalin, and catechin. Additionally, two unidentified compounds were detected, but their identification was hindered by the lack of compatible standards.

Table 2: HPTLC of *Allium ampeloprasum* methanol extract

Peak		Start Rf	Start height	Max Rf	Max Height	Max %	End Rf	End Height
1.	Rutin	0.01	3.2	0.02	39.1	13.35	0.06	1.1
2.	Isoquercetin	0.01	0.5	0.05	89.8	87.74	0.06	5.2
3.	Quercetin	0.01	3.2	0.31	258.8	86.65	0.45	0.2
4.	Astragalin	0.01	2.0	0.42	98.2	12.12	0.03	2.3
5.	Catechin	0.01	0.2	0.91	19.1	46.59	0.05	5.0

Table 3: HPTLC standards

Peak	Start Rf	Start height	Max Rf	Max Height	Max %	End Rf	End Height
1.	0.01	6.5	0.02	84.1	4.94	0.03	26.9
2.	0.03	26.9	0.05	47.1	2.76	0.08	5.8
3.	0.18	3.9	0.24	179.9	10.56	0.28	15.7
4.	0.28	15.7	0.33	63.5	3.73	0.36	6.4
5.	0.40	1.2	0.42	99.8	5.86	0.47	4.1
6.	0.56	6.2	0.67	655.3	38.47	0.73	0.8
7.	0.85	0.1	0.91	573.8	33.68	0.95	5.0

Phytochemicals such as rutin, isoquercetin, quercetin, astragalin and catechin in the extracts have antioxidative, antibacterial, anti-inflammatory, and proangiogenic effects, which lead to positive effects on wound healing. *Allium ampeloprasum* methanol extracts are rich in antioxidant compounds, which can scavenge free radicals, decreasing oxidative stress and minimizing cellular damage. The antibacterial qualities restrict pathogen growth, lowering the risk of wound infection. Furthermore, these extracts modify the inflammatory response by decreasing pro-inflammatory mediators and increasing the production of anti-inflammatory cytokines [20-21].

Wound healing is a complex biological process involving several stages, hemostasis, inflammation, proliferation, and remodeling. One characteristic feature of plant extracts used to promote wound healing is their ability to accelerate the regeneration of damaged tissues and promote the formation of new blood vessels, a process known as angiogenesis. Additionally, plant extracts often possess antimicrobial properties that help prevent infections in wounds, and they can also reduce inflammation and promote the production of collagen, which is crucial for wound closure and tissue repair [22-23].

When examining histological slides under the light microscope, we can observe several key features related to wound healing. Figure 2 shows histological changes during the wound-healing process.

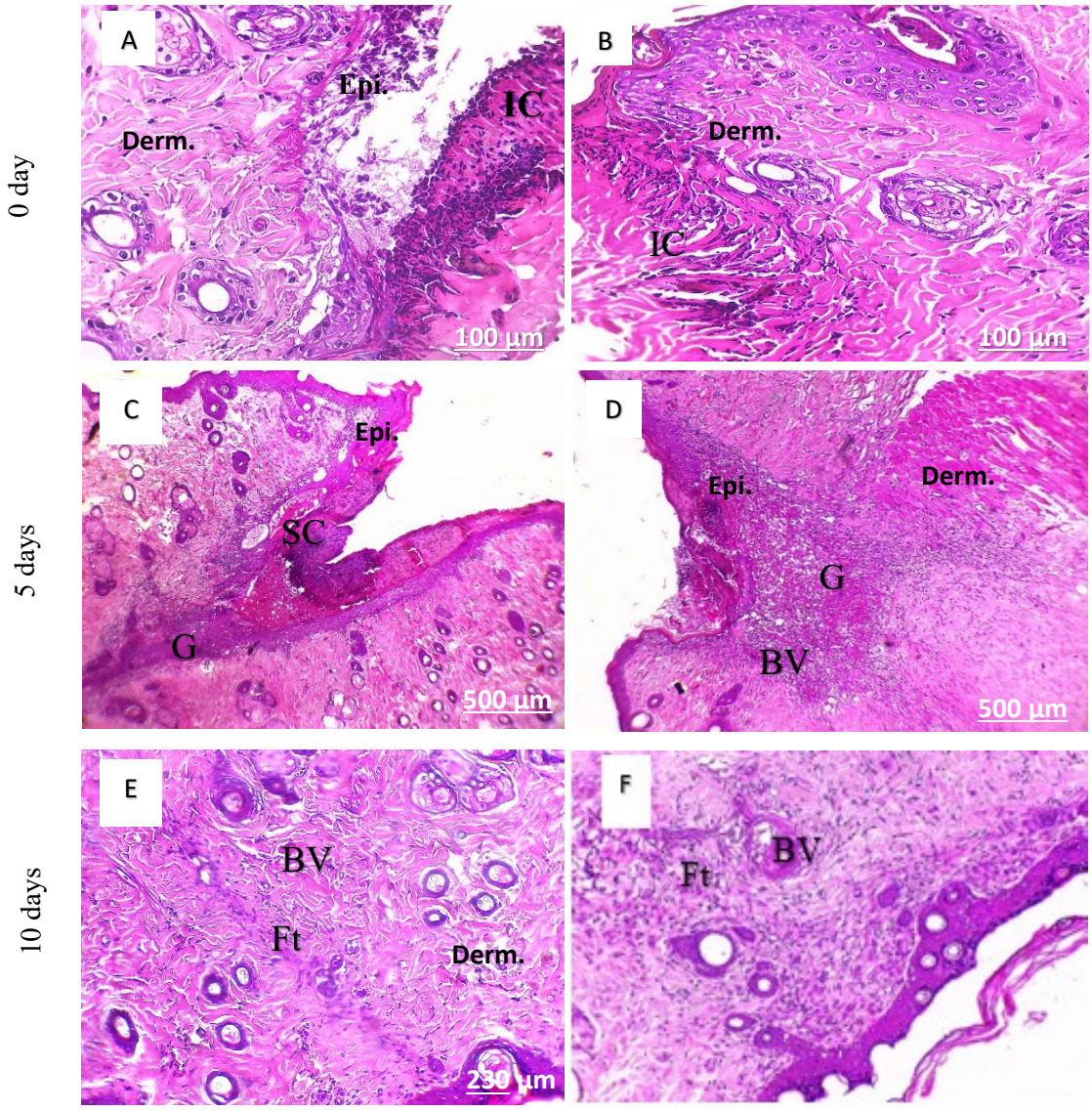

Figure 2: Histological changes during the wound-healing process. Histology of the wound tissue of experimental (B,D,F), and control (A,C,E) groups at day 0 (A,B), day 5 (B,C) and day 10 (E,F) after incision. (Epi.) Epidermis layer, (Derm.) dermis layer, (SC) scab, (IC) inflammatory cells, (G) granulation, (BV) blood vessels and (Ft) fibrous tissue (collagen)

At day 0, immediately after the wound is created, the histological analysis may reveal disrupted tissue architecture, damaged blood vessels, and inflammatory cells infiltrating the wound site (Figure 2(A) and (B)). Five days later, signs of early wound healing become more visible. Granulation tissue can be seen, which is characterized by an increased number of fibroblasts and newly created blood vessels (angiogenesis). The presence of these characteristics indicates that the wound is entering the proliferative phase of healing. Furthermore, when compared to the day 0 time point, the degree of inflammation normally begins to reduce (Figure 2(C) and (D)). By ten days, the histological examination shows further maturation of the wound. Collagen fibers become more organized, and fibroblasts continue to deposit extracellular matrix components. Blood vessels within the wound may become more established and functional. The inflammatory response further diminished, and the tissue architecture started to resemble normal tissue, even though with some remodelling and scarring (Figure 2(E) and (F)).

Wound Contraction

A significant difference in the percentage of wound contraction between the control and experimental groups was detected in a comparison analysis. The wound contraction was lower in the control group, which got no intervention. The experimental group, on the other hand, revealed a considerably higher rate of wound contraction. Figure 3 shows the percentage of wound contraction in the control and experimental groups. T-test revealed a significant difference in the percentage of wound contraction between the control and experimental groups (Table 4).

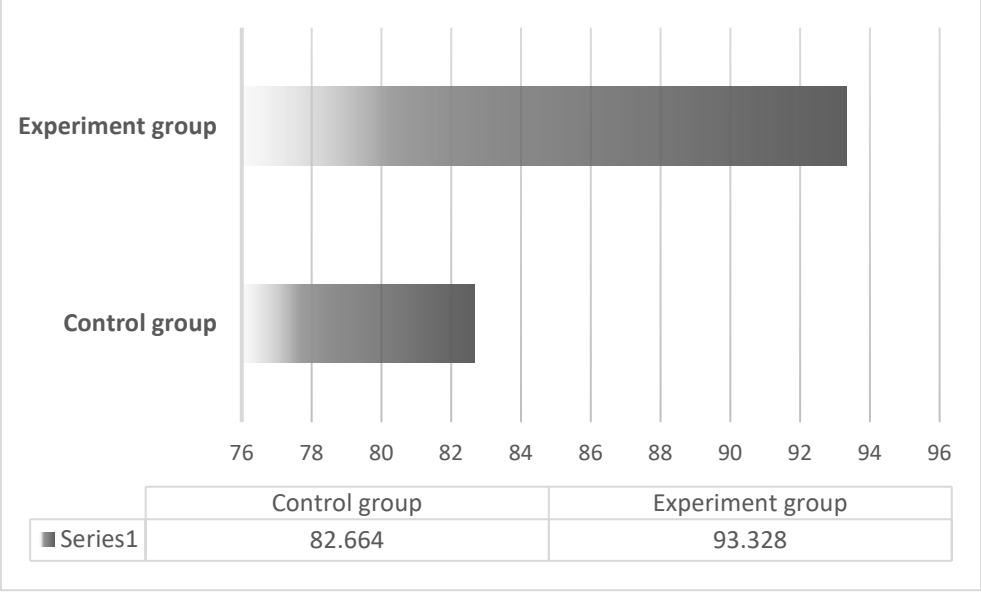

Figure 3: Percentage of wound contraction in the control and experimental groups

Table 4: T-test revealed a significant difference in the percentage of wound contraction between the control and experimental groups

	Mean	Std. Deviation	T-Test	P value
Control group	82.664	3.647	-4.355	.002*
Experiment group	93.328	4.082		
Correlation is high significant at the 0.01 level (2-tailed).				

The experimental group treated with methanol extract showed a substantial improvement in wound contraction "the process by which the wound edges gradually close, reducing the wound size", and this agrees with the study by Nițulescu, et al, apply *Allium ampeloprasum* on haemorrhoid induced wound contraction [24]. The application of the *Allium ampeloprasum* extract to the wound area appeared to accelerate wound closure. This is attributed to the presence of flavonoid constituents, like quercetin and rutin which are abundant in plants such as onions, garlic, citrus fruits and green tea. These substances have been shown to increase fibroblast proliferation and collagen synthesis, as previous studies by, Abd and Ali, and research by Kozłowska and Szostak-Węgierek, flavonoids proved to have antioxidant activity which plays an important role in improving wound healing [25-26].

Inflammatory Cells Count

When comparing the control and experimental groups at each period, at day 0 healing periods, there was no significant difference between the two groups. During the fifth and tenth periods, there is a significant difference, with a reduction in the inflammatory cell count in the experiment group more than in the control group. Figure 4 shows the inflammatory cell count. The T-test revealed a significant difference in the inflammatory cell count at five and ten days between the control and experimental groups.

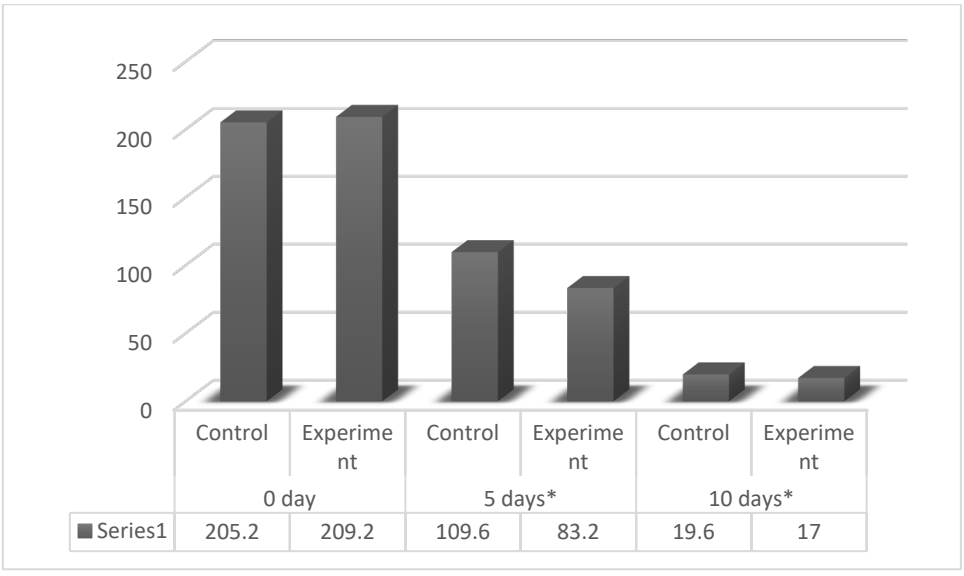

Figure 4: Inflammatory cell counts during the 5 and 10 periods

The *Allium ampeloprasum* methanol extract has been shown to have anti-inflammatory activity. A study by Lee et al., on rat dorsal wounds revealed that using a rutin-conjugated hydrogel significantly facilitated the healing process [27]. Subsequently, this was proved by the finding of this study, when *Allium ampeloprasum* extract was applied to wounds, there was a significant decrease in the number of inflammatory cells compared to the control group. Along with this, the reduction in inflammatory cell count provides a premium environment for wound healing by reducing excessive inflammation and limiting tissue damage. This agrees with the finding of Landén et al, the transition from inflammation to proliferation is critical for wound healing to be effective. A balanced transition to the proliferative phase has been linked to improved wound healing outcomes [28].

Measure the Epithelial Thickness

The results revealed significant variations in epithelial thickness between the experimental and control groups. The experiment group had considerably thicker epithelial layers. Table 5, Measure the epithelial thickness T-test revealed a significant difference between the control and experimental groups.

Table 5: Measure the epithelial thickness T-test revealed a significant difference between the control and experimental groups

	Mean	**Std. Deviation**	**T-test**	**P value**
Control group	359.3	32.89	2.335	0.04*
Experiment group	437.4	67.03		

Correlation is significant at the 0.05 level (2-tailed).

Likewise, another significant difference between the experiment and the control group is the degree of epithelization. Epithelization is a critical stage of wound healing in which epithelial cells migrate and proliferate to produce a new protective layer over the affected area. The *Allium ampeloprasum* methanol extract was found to increase epithelial cell proliferation and migration. It accelerated the re-epithelization process, resulting in a faster wound closure. This finding is supported by the Kuhlmann et al, study, which concludes that wound healing is improved by earlier re-epithelization, and faster wound closure, and eventually will lead to a better cosmetic outcome [29].

In addition, the crude extract of *Allium ampeloprasum* contains an interesting compound, catechin, the molecule with the most potent effect on cell proliferation and migration. Catechin is found in a range of foods and herbs and has been shown to have antioxidant, anti-inflammatory, and anti-cancer characteristics [29]. Besides, the findings of a recent study demonstrated that the active component responsible for wound healing activity, catechin, has a great potential for wound healing by increasing human fibroblast cell proliferation and migration, which agrees with Chaniad et al., findings [30].

This study concentrates on the potential therapeutic properties of *Allium ampeloprasum* methanol extract and its capacity to promote wound closure and tissue regeneration. However,

it is important to note the limitations of the study, including the focus on histological evaluation alone and the use of animal models. These findings serve as a base for further research, including clinical trials, to fully explore the therapeutic applicability of *Allium ampeloprasum* methanol extract. More research is needed, however, to optimise extraction procedures, and understand the underlying molecular mechanisms of action.

Conclusions

Allium ampeloprasum extracts have shown promising activity as wound-healing promoters. These extracts have many benefits in the management of acute and chronic wounds due to their capacity to increase wound contraction, minimise inflammatory cell infiltration, and boost epithelization. Plant methanol extracts, on the other hand, present an interesting option for the development of innovative wound-healing medicines that link the power of nature's pharmacopoeia.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with animal ethics standards, ethical approval No. 422721 from Baghdad University's College of Dentistry.

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