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# Evaluation of the chemical content of the leaf extract of *Physalis angulate* L. extracted using GC–MS and its effect on the wound healing process

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**ABSTRACT**

*Physalis angulata*, a member of the Solanaceae family, is renowned for its therapeutic properties and medical applications. Its rich composition of potent chemical compounds render it valuable in many treatments and complementary therapies. The aforementioned statement is supported by extensive laboratory and in vivo research conducted in many locations where this plant is indigenous. This research aimed to confirm the presence of chemical compounds in the leaf extract via gas chromatography–mass spectrometry. After preparing the ethanolic extract using the hot soak method and analyzing its active chemical components, the extract was found to contain 11 compounds. The two most significant compounds were octasiloxane and phytol, which were found to be present in percentages of 15.33% and 15.28%, respectively. The MTT technique was used to assess cytotoxicity by subjecting the extract to various concentrations (1000, 500, 250, 125, 62.5, and 31.75 µg). Additionally, the impact of the leaf extract on the wound healing process was evaluated using a mouse fibroblast cell line. Results indicated that the extract did not affect the rat embryonic fibroblasts. Furthermore, the percentage of cell growth inhibition escalated as the concentration of the extract dosage increased. The scratch test demonstrated the efficacy of the treatment in wound closure. The efficacy of the leaf extract in promoting wound healing has been shown in laboratory experiments.

## Introduction

*Physalis angulata* is a member of the Solanaceae family and is referred to by several names such as gooseberry, Indian gooseberry, wild tomato, winter cherry, mullaca, and camapu. The plant is a compact and vigorous herbaceous species that consists of over 120 perennial or herbaceous varieties. It attains a length of around 50 cm. The blooms of this plant are yellow and assume a bell-shaped form as they reach maturity. The fruits of this plant are tiny and have a balloon-like shape. They dangle downwards and may be consumed, offering a flavor that can be either sour or sweet. They are shielded by a protective cover that safeguards them from diverse environmental conditions [1].

The plant is widely distributed in tropical nations, including Africa, the coastal sections of South America, and several Arab countries. Additionally, it thrives in temperate regions and mild climates [2]. It thrives on soil that is damp and rich. It thrives in full sun conditions and can even withstand partial shade. Extensive research has been conducted on the medicinal properties of this plant, which has long been used in traditional treatments. Numerous studies have demonstrated the plant extract's efficacy as an antioxidant, anti-inflammatory agent, and treatment for various diseases prevalent in tropical regions.

These characteristics are attributed to the presence of bioactive compounds such as alkaloids, flavonoids, glycosides, saponins, tannins, terpenoids, physalis, and anolides [3,4]. Additionally, *Physalis*, a component of the plant, is rich in vitamin C, beta-carotene, and vitamin K, which contribute to a robust immune system and facilitate wound healing [5]. *P. angulata* L. has also been employed in the treatment of diabetes, asthma, and malaria in Taiwan and West Africa. It is used to cure a range of bacterial and viral illnesses, and it is also regarded as a supplementary therapy for cancer,

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leukemia, skin ulcers, rashes, and ear ache. Additionally, it is consumed as a nutritious dietary ingredient [6].

Wound healing is a series of interconnected and sequential events that result in the growth, movement, and contraction of cells. It effectively repairs damaged tissues and restores their structural integrity. The process has four distinct stages: hemostasis, inflammation, proliferation, and remodeling of new tissues. Hemostasis promptly occurs upon damage to safeguard the integrity of the circulatory system. Fibroblasts are activated by many growth agents. In the proliferative phase, growth factors facilitate the formation of epithelial tissues, resulting in cell separation, proliferation, migration, and differentiation. The proliferation stage involves the development of blood vessels in newly generated cells and tissues, the creation of granulation tissue, and the accumulation of collagen due to the multiplication of fibroblasts [7].

Fibroblasts found in the dermis are the cells responsible for every step of the process. Cell migration involves the displacement of individual cells, cell sheets, or cell groups from one location to another. This process can be studied by creating a wound in a cell monolayer, allowing cells to migrate into the empty area, and observing the return and accumulation of cells in the scratched region to measure cell migration [8]. The study aimed to determine the chemical composition of the leaves, which play a crucial role in the biological effects of this process. Additionally, the study aimed to assess the cellular toxicity and potential toxic effects of the ethanolic extract of *P. angulata* on normal fetal fibroblast cells in mice. Furthermore, the study aimed to investigate the wound-healing properties of the leaf extract for potential applications in the treatment of skin infections and various wounds.

## Materials and working methods

### Plant collection & Processing

In June 2023, the foliage of the *P. angulata* plant was gathered from Khalidiya, a city located in the Anbar Governorate. The plant was categorized and assessed by the supervisor, Prof. Dr. Ashwaq Talib Hameed, based on the Iraqi Botanical Encyclopedia [9]. Subsequently, the plant was stored at the herbarium of the College of Education for Girls–Anbar University. The leaves were pulverized into a fine powder by using an electric grinder.

Plant extract preparation: The leaves were subjected to heat maceration to obtain an ethanolic extract. About 50 g of the plant powder was combined with 150 mL of concentrated ethanolic alcohol containing 70% ethanol. Prior to this step, the alcohol was diluted with 30% distilled water. The plant leaves were immersed in a solvent inside a 1000 mL glass beaker and then heated in a water bath. The flask was moved to a shaking incubator and filtered multiple times with gauze. The resulting liquid was collected, while the sediment was discarded and placed in Petri dishes inside an oven. Scraping was conducted, resulting in the formation of a powdery extract [10].

Identification of bioactive chemical components in ethanolic extracts of *P. angulata* leaves and fruits using gas chromatography–mass spectrometry (GC–MS) technology: The identification of compounds in the plant extract involved dissolving 20 g of plant powder from leaves and fruits in 200 mL of 80% ethanol. The resulting leaf and fruit samples were analyzed and compared with the chemical compounds stored in the Nist computer library, which was connected to the Nist GC-MS device. The user's text is "[11]."

Cell culture: The standard rat embryonic fibroblast (REF) cell line was grown in MEM culture media with 10% (v/v) fetal bovine serum (FBS), 100 IU penicillin, and 100 mg of streptomycin. The cells were then incubated in a humidified environment at 37 °C. DCs were extensively used for studies.

### MTT cytotoxicity assay

**-MTT assay principle:**

The MTT cytotoxicity test, which involves the use of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, is a commonly used technique for assessing cell viability and cytotoxicity. This colorimetric test is based on the capacity of live and functional cells to decrease MTT and transform yellow into purple formazan crystals by extracting a hydrogen atom from the enzyme succinate dehydrogenase, which is present in the mitochondria.

The cells were cultivated in a 96-well plate and exposed to various quantities derived from the ethanolic extract of the plant's leaves. Following the incubation period, MTT was introduced into each well and incubated for an

additional duration. Subsequently, the viable cells were examined by converting MTT into violet formazan crystals. These crystals were dissolved, and their concentration was determined by measuring the absorbance at a wavelength of 570 nm by using a spectrophotometer. The quantity of formazan generated was closely correlated with the quantity of viable cells. The cytotoxicity of *P. angulata* leaf extract was evaluated using the MTT assay. The REF cell line was placed in a 96-well microplate with 10,000 cells and cultured at 37 °C for 72 h until a single layer of cells was created. Cytotoxicity was assessed using the 3-(4,5-)bromide test. The MTT cells were subjected to various doses (1000, 500, 250, 125, 62.5, and 31.75 µg) of MTT. Following a 72-hour cell treatment, an MTT dye solution at a concentration of 2 mg/mL per 28 µL was introduced to each well.

The samples were incubated for a further 3 h. Subsequently, 100 µL of DMSO was introduced to each group and incubated for 15 min. The experiment was conducted three times, and the intensity of light was measured at a wavelength of 492 nm by using a device called a microplate reader. The cytotoxicity ratio was determined using the following equation:

The cytotoxicity percentage was calculated by subtracting the sample optical density (OD) from the control OD, dividing the result by the control OD, and multiplying by 100.

The control OD represents the mean optical density of the holes that were not treated, whereas the sample OD refers to the optical density of the holes that were treated.

The impact of *P. angulata* leaf extract on the process of wound healing:

REF cells were plated at a density of 1 × 105 cells per well on a 24-well plate. Once the cells reached 90% confluence, the plates were scratched vertically (1 mm) by using a sterile micropipette. The damaged cellular remnants were then rinsed away using MEM. The cells were exposed to a concentration of 160 µg/mL extract and cultured for 72 h to allow growth. To conduct the wound healing experiment, we assessed the distance of the gap after 24 h using Image J software and PBS solution.

The specimen was examined using a phase-contrast microscope. The advancement of migration, proliferation, and wound closure pre- and post-treatment with the investigated materials was observed by imaging via phase-contrast microscopy.

A total of 24 studies were conducted to study the wound healing process, with three replicates for each group (treated, untreated, and control groups). The scratch shrinking rate was determined using the following equation: (original width − final width) ×100 / original width.

**Statistical Analysis:**

The data acquired were analyzed using Tukey's ANOVA multiple comparisons test using GraphPad Prism 8 software. The values are reported as the mean ± standard deviation (SD) for triple measurements.

**Findings and analysis:**

The GC–MS analysis results (Table 1 and Figure 1) indicated that the ethanolic extract of *P. angulata* leaves contained several potent chemical compounds. The compound octasiloxane exhibited the highest percentage of 15.33%, whereas the compound phytol showed a percentage of approximately 15.28%. 2-Butanol had the lowest proportion at 6.23%, which was the smallest percentage among all the compounds. This finding was consistent with previous research, including the findings of GC–MS analysis in the performed study. A previous study revealed that the plant contains 24 active chemicals, one of which is cyclohexane [2]. The chemical (Z)-9,12-octadecadienoic acid (also known as (Z,Z)-9,12-octadecadienoic acid) had the maximum retention time of 27.639, whereas the compound cis-9-hexadecenal had the lowest percentage of 4.785%. Additionally, many molecules of biological significance were observed, including cis-9-hexadecenal [20], which is a natural substance with several antibacterial and anti-inflammatory effects [21].

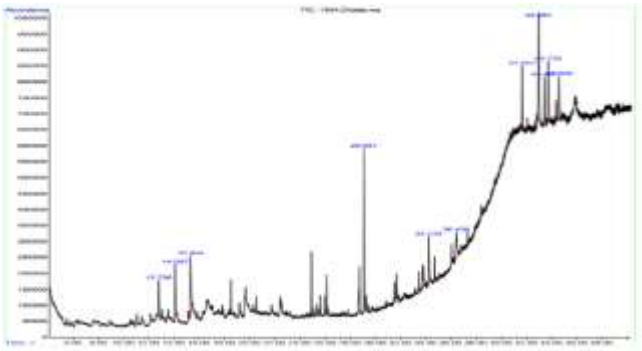
Another analysis revealed the presence of the molecule isopropyl myristate. This chemical is often used in the production of cosmetics and medicines due to its high skin absorption rate [22], as well as other substances that have a function in preventing the development of biofilms and are safe for human consumption. GC–MS technology was performed to identify various chemical components in the methanol extract of *P. angulata* leaves, including linoleic acid,

phytol, oleic acid, octadecanoic acid, and acetate. The correlation between the actions of antioxidants and antimicrobials, including Gram-positive and Gram-negative bacteria, may be attributed to the existence of these chemical components. Phytol has inherent antioxidant properties and holds significant therapeutic value. It has antibacterial and anticancer properties [24].

**Table 1 .**GC–MS analysis of *Physalis angulata* leaf extract

| Peak | RT | Area | Area% | Name |
|---|---|---|---|---|
| 1 | 12.796min | 5410594 | 6.23% | 2-Butanol |
| 2 | 14.097min | 5863750 | 6.75% | Thymol |
| 3 | 15.304min | 9939251 | 11.44% | 2-Hydroxy-3-methylbenzaldehyde |
| 4 | 29.091min | 13275318 | 15.28% | Phytol |
| 5 | 34.234min | 6661647 | 7.65% | Glycerol 1-palmitate |
| 6 | 36.436min | 5666223 | 6.52% | Octasiloxane,1,1,3,3,5,5,7,7,9... |
| 7 | 41.652min | 6406472 | 7.37% | Vitamin E |
| 8 | 42.957min | 13321718 | 15.33% | Octasiloxane,1,1,3,3,5,5,7,7,9… |
| 9 | 43.733min | 5811081 | 6.69% | Heptasiloxane,1,1,3,3,5,5,7,7,9.. |
| 10 | 43.427min | 7727969 | 8.89% | Heptasiloxane,1,1,3,3,5,5,7,7,9.. |
| 11 | 44.569min | 6821871 | 7.85% | 1.2-Bis(trimethylsilyl) benzene |

**Picture 1. GC–MS analysis of *Physalis angulata* leaf extract**

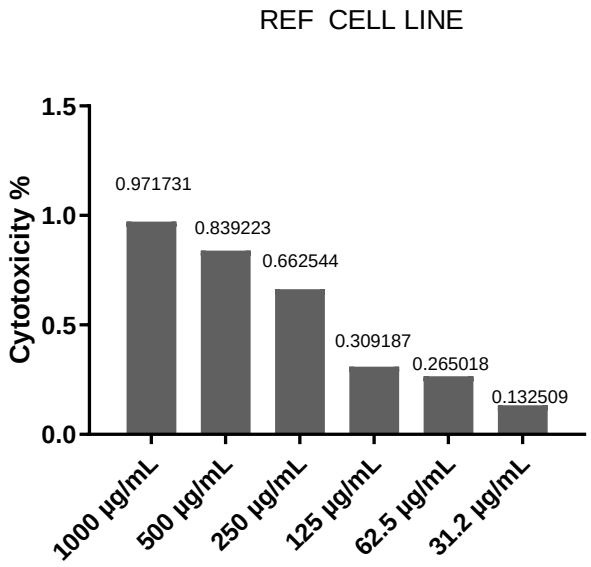


**Cytotoxicity test of Physalis angulata leaf extract**

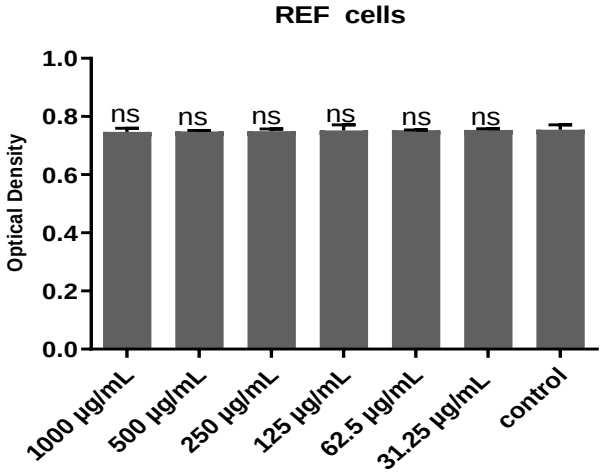
A series of REF cells were exposed to leaf extract at various doses (1000, 500, 250, 125, 62.5, 31.75 micrograms). The impact of the extract on the normal fibroblast cell line of mice was then monitored over a period of 72 hours. The cell inhibition percentage was determined by comparing it to the control sample after measuring the absorbance. The study revealed a positive correlation between the concentration of the extract and the percentage of cell growth inhibition. The maximum level of inhibition, reaching0.97%, was seen at a concentration of 1000 micrograms, as shown in Figure2.

The statistical analysis findings indicate that there were no significant changes, suggesting that the extract had no impact on the natural fibroblast cells employed. Figure 3 demonstrates that the ethanolic extract of P. angulata leaves has little to no impact on the cells, indicating its safety. The safety of the extract may be linked to the presence of chemical compounds that possess diverse biological actions, as well as the low concentration or lack of harmful chemical components. The safety of these plant extracts enables us to validate the biological efficacy of their chemical constituents used as therapeutic agents against various ailments. The findings are in line with prior research, which determined by photochemical analysis that the methanol extract derived from the leaves of the P. angulata plant contains alkaloids, steroids, and flavonoids. The methanol extract derived from the leaves of the P. angulata plant did not result in any deaths when administered at a dosage of 2000 mg/kg. Therefore, the extracts may be deemed safe and non-toxic for in vivo investigations. Additionally, the extract exhibited varying degrees of inhibitory efficacy against the quantities used. The values are 100% (1 µg), 133% (2 µg), and 126% (4 µg).

The strong correlation between the amount of polyphenols and the IC50 values in fibroblasts indicates that the plant extracts are cytotoxic. However, despite having a high percentage of polyphenols and antioxidant activity, the extracts do not have a significant toxic effect on fibroblast cells. This suggests that the antioxidant activity of the extracts may help protect against their cytotoxic effects.



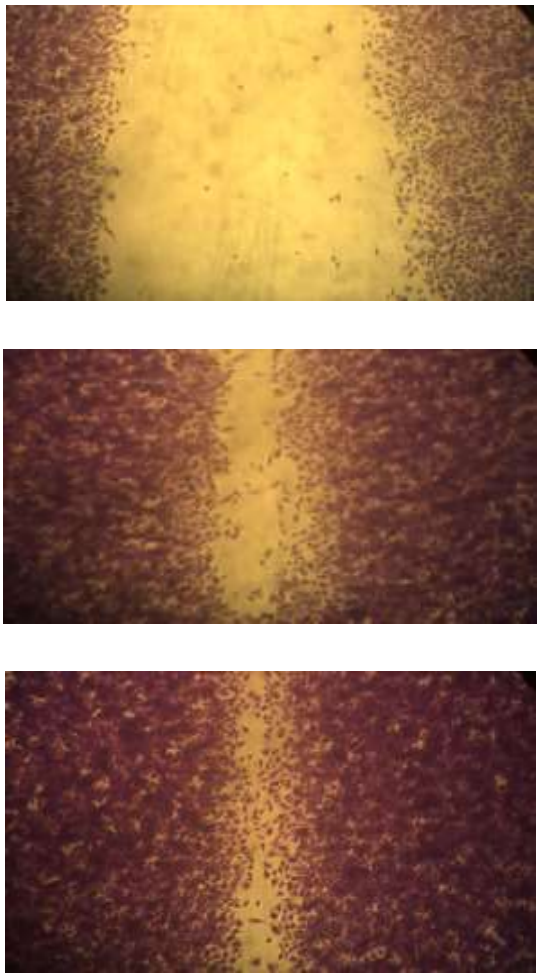

**Picture 2**: Inhibition curve showing the effect of P. angulata leaf extract on REF cell viability.




**Picture 3:** Optical density (OD) reading curve over time for REF cells treated with the ethanolic extract of *P. angulata* leaves.

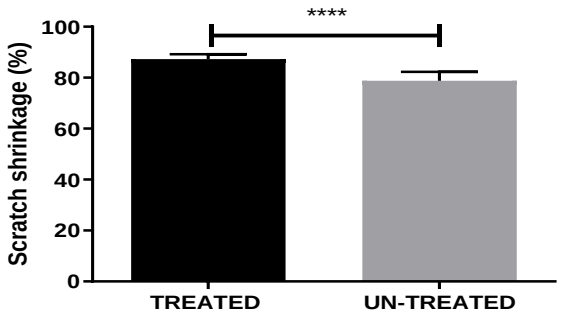
**Experiment conducted to evaluate the efficacy of P. angulata leaf extract in promoting wound healing.**

The use of plant extracts for the treatment of different types of wounds has seen a surge in interest, and it is widely regarded as one of the most important approaches to wound care. Topical antimicrobial therapy effectively halts bleeding and expedites the process of wound healing. The actions of the components found in the plant extract, either alone or in combination, are responsible for this phenomenon. These compounds serve as antioxidants and antimicrobials. The plant extract stimulates the mitotic activity of fibroblast cells, causing them to travel from the margins of the wound region to the source of the wound infection. Statistical analysis demonstrated the efficacy of the plant extract in promoting wound healing in a laboratory setting. The scrape was analyzed using a biological concentration of 160 µg/mL. Fibroblast migration was observed at the site of the scratch, as shown in Figure 4. Following 72 h of treatment with the leaf extract of the *P. angulata* plant, we observed that wound closure was much greater compared with the absence of extract therapy. After 72 h of treatment with the plant extract, the scratch shrinking rate was 89% greater compared with the control sample, which had a shrinkage rate of 78% (Figure 5).



A: 0 time B: Untreated C: Treated

**Picture** :**4** Distance-closing scratch assay for the treated and control REF cell lines from each of the experimental cell groups over 72 h per image.




**Picture 5:** Scratch shrinkage rate of the treated and control REF cell lines from each of the experimental cell groups at 72 h after treatment.

The healing process has been shown to be influenced by the active components found in plant extracts. These components contribute to antibacterial activity and function as free radical scavengers. Additionally, they play a crucial function in promoting cell proliferation by facilitating angiogenesis and enhancing collagen synthesis. This phenomenon takes place when the active compounds included in the plant extracts interact with different phases of the wound healing process, thereby diminishing the components that impede the successful completion of the healing process. Terpenes, polyphenols, and glycosides are the key compounds that can attach to cellular receptors at the wound site, initiating the healing process. The plant has been shown to contain phytochemicals such as tannins, flavonoids, and polyphenols. These compounds have strong antioxidant properties and contribute to the healing of wounds. As a result, the plant is often used in traditional medicine in Ghana to help in wound healing. In a separate investigation, creams containing an extract derived from the *P. angulata* plant were used. These creams showed notable efficacy in promoting wound healing ($P<0.001$). Furthermore, the size of the wound decreased over time after its development. Various plant extracts have been used in laboratory settings to assess the wound healing process. These extracts include chemical components that facilitate the migration and proliferation of fibroblast cells, which are employed in the scratch test. The polarity of solvents also influences the productivity of these substances and their capacity to activate cells by lowering variables that impede the effectiveness of the wound healing process, such as cytokines that induce inflammation and oxidative stress. In the laboratory, 29 to 30 wound-resistant medicinal groups were tested, and three plants were found to have the strongest activity as steroidal glycosides: licorice (*Glycyrrhiza glabra* L.), black cumin (*Nigella sativa* L.), and mangosteen (*Garcinia mangostana* L.). These plants have the ability to stimulate the proliferation of fibroblast cells, which helps in the healing process by promoting the removal of the wound.

## Conclusions and recommendations

This investigation showed that the ethanolic extract derived from the leaves of the *P. angulata* plant includes many potent chemical components that possess the ability to eliminate free radicals and function as antioxidants and antimicrobials. Furthermore, the outcomes of the toxicity test conducted using the MTT assay indicated that the extract exhibited no toxicity toward the fibroblast cells compared with the control sample. The methanol leaf extract of *P. angulata* exhibits preventative and therapeutic anti-inflammatory activities. Additionally, it promotes wound healing by facilitating cell migration, angiogenesis, and collagen production at the site of injury. The presence of secondary metabolic chemicals, such as flavonoids, glycosides, and polyphenols, in the plant extract may account for all of these features and biological activities. Additional research is necessary to further investigate the efficacy and mechanisms of action of the plant extract.

**Conflicts of Interest:**

The authors declare no conflict of interest.

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# تقييم المحتوى الكيميائي لمستخلص اوراق نبات Physalis angulate L. المستخرج بتقنية ال GC-MASS وتاثيره على عملية التئام الجروح

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**الخلاصة:**

عرف نبات الحرنكش التابع للعائلة الباذنجانية بفوائده واستخداماته الطبية نظرا لاحتوائه على المركبات الكيميائية الفعالة التي جعلته يستخدم في الكثير من العلاجات والأدوية التكميلية ، هذا ما أثبتته معظم الأبحاث والدراسات المختبرية وفي الجسم الحي لهذا النبات في مختلف الدول المتواجد فيها ، كان الهدف من البحث هو التحقق من المركبات الكيميائية المستخرجة من مستخلص الأوراق بتقنية طيف الكتلة كروموتوغرافيا الغاز GC-MASS بعد تجهيز المستخلص الايثانولي بطريقة النقع الحار وتحليل المكونات الكيميائية النشطة للمستخلص تبين احتوائه على 11 مركب أهمها مركب Octasiloxane ومركب Phytol ظهرت بنسب 15.33% ، 15.28% على التوالي. كذلك تم اختبارالسمية الخلوية بطريقة ال MTT باستخدام مجموعة من التراكيز (1000 ، 500 ، 250 ، 125 ، 62.5 ، 31.75 ميكروغرام) من المستخلص إضافة الى تقييم تاثير مستخلص الأوراق على عملية التئام الجروح باستخدام خط الخلايا الليفية للفئران ، أظهرت النتائج انه لم يكن للمستخلص تاثير سام للخلايا الليفية الجنينية للفئران REF وأن النسبة المئوية لتثبيط نمو الخلايا تزداد مع زيادة تركيز جرعة المستخلص ، وكان له فعالية في اغلاق الجرح في مقايسة الخدش ، تم اثبات فعالية مستخلص الأوراق على عملية التئام الجرح في المختبر.

**الكلمات المفتاحية:** نبات الحرنكش Physalis angulata ، تقنية كروموتوغرافيا الغاز ، الخلايا الليفية الجنينية للفئران ، اختبار MTT، اختبار الخدش.