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Phytochemical Screening and Wound Healing Activity of the Essential Oil of *Pistacia khinjuk*

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ABSTRACT

The tree or shrub *Pistacia khinjuk* is a member of the Anarcadiaceae family and is indigenous to Iraq. It is a significant medicinal plant with several pharmacological qualities used in traditional and contemporary medicine, including anti-inflammatory, antioxidant, and antihyperlipidemic effects. The current study investigated the chemical components of the essential oil of the fresh fruits of *P. khinjuk* growing in Sulaymaniah, northern Iraq, considering the body of research on the makeup of the oil that is extracted from this plant's fruits. Fresh fruits from *P. khinjuk* plants were extracted for three hours using a reflux system, and the constituents were assessed by gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC). The findings of the qualitative and quantitative examination of the essential oil are reported, together with the chemical ingredients discovered by HPLC and GC/MS. GC/MS found 21 chemicals in total, with n-hexadecanoic acid (palmitic acid) being the most abundant (46.44%). HPLC identified 5 compounds, including oleic acid as the most abundant. Furthermore, in vitro wound healing assays demonstrated that a concentration of 200 µg/mL of *P. khinjuk* fruit oil extract resulted in near-complete scratch closure of fibroblast cultures after 72 hours, significantly accelerating wound closure compared to untreated controls. This study represents the first investigation into the wound-healing properties of oil extracted from the fruits of *P. khinjuk*, highlighting its potential as a novel therapeutic agent.

Keywords: Anarcardiaceae, wound healing activity, HPLC, GC-MS, Phytochemical analysis, Pistacia khinjuk

INTRODUCTION

Since ancient times substances called phytochemicals which are derived from plants have been studied for their potential pharmacological effects. Plants have been used for medical purposes since before civilization was born [1].

Phytochemicals have a significant role in basic care and disease treatment in a number of nations. Furthermore, the use of plant-derived therapeutic products, or phytochemicals, is becoming more popular as a result of the contraindications linked to the use of conventional medications [2].

Statistics indicate that approximately 30% of medicinal drugs in the world (and their derivatives and analogs) are from natural sources, especially plants and natural products, and will continue to have a considerable impact on medical sciences [3]. Pistacia, a genus that belongs to the family Anacardiaceae, consists of twenty species, five of which are characterized as significant biological and economically important [4], and it is mainly native to the Middle East and Mediterranean areas [5].

One of the five significant species of *pistacia*, Pistacia *khinjuk* is regarded as a significant medicinal plant that contributes to the creation of many pharmaceutical goods [6]. It has numerous therapeutic applications in both traditional and modern medicine. In traditional practices, it is commonly used to treat dermatitis. Additionally, it serves as an astringent, anti-inflammatory, antipyretic, antibacterial, antiviral, pectoral, and stimulant. These properties make it effective in alleviating stomachaches, throat infections, kidney stones, and asthma [7]. Uses in modern medicine include anti-inflammatory anti-asthmatic antihyperlipidemic anticholinesterase and antidiabetic properties [8]. Additionally, its fruits are utilized as edible wild nuts to make a beverage similar to coffee, and as a source of coloring and oil [9].

The *P. khinjuk* tree is widely distributed, as it is found in many countries in the Middle East region like Turkey, Iran, Syria, Iraq, Palestine, and others [10]. Many compounds have been recognized in different parts of *P. khinjuk*, like essential oil compounds, flavonoids, phenolic compounds, tannins, monoterpenoids, and miscellaneous compounds [11].

The oil from P. khinjuk fruits has a fatty acid content of 52.12, 17.82 and 17.44, 5.73, 2.31, and 1.5% oleic palmitic linoleic palmitoleic and stearic acids respectively [12]. Additionally, fruit phenolic substances such as rutin gallic acid caffeic acid ascorbic acid synaptic acid and ferulic acid have strong antioxidant properties [9]. Essential oil from Pistacia *khinjuk* is good for hydrating skin and has applications in the cosmetics industry. Furthermore, P. *khinjuk* essential oil and several other essential oils are the best ways to cure both skin and hair [13]. Wound-healing is a dynamic phenomenon that happens following the injury to the skin, arises through a consequence of several steps, like inflammation, proliferation, and migration of numerous cells like fibroblasts [14, 15] .

The wound healing study is a popular in vitro technique for assessing two-dimensional collective cell migration [16]. Through physical exclusion or the removal of cells from the area by mechanical thermal or chemical damage, a cell-free

area is produced in a confluent monolayer in this test [17]. The cells move into the gap when exposed to the cell-free area [18]. The rate of gap closure a gauge of the speed of the cells' collective migration is the most often obtained data from the wound healing experiment [19].

Additionally, it is simple to modify the wound healing assay for medium-to-high throughput uses including small molecule screening [20] and drug discovery [21]. Ancient Iraqi folk medicine has used plant parts such as leaves fruits seeds and roots to cure a variety of illnesses [22]. Thus, the chemical makeup of the oil that is extracted from P. *khinjuk* fruits and its ability to cure wounds will be examined in this article.

Therefore, this study aimed to (1) characterize the chemical composition of the essential oil extracted from *P. khinjuk* fruits using GC-MS and HPLC, (2) investigate the *in vitro* wound healing potential of this oil, and (3) discuss the potential pharmaceutical and industrial applications of the identified compounds. While previous studies have examined the chemical composition of *P. khinjuk* oil from other regions, this is the first report on the oil from Sulaymaniyah, Iraq, and the first investigation of its wound-healing properties.

MATERIALS AND METHODS

Collection and Identification of Plant

In June and July of 2023, the new Pistacia khinjuk fruit was harvested from Sulaymaniyah, which is located in northern Iraq. Regional floras were used to identify the plant samples, which were then compared to voucher specimens from the University of Basra's College of Pharmacy.

Preparation Of P. khinjuk Fruit Extract

The following continuous heat technique was used to extract the fruit's essential oil:

According to the established protocol, the fruits were ground in a grinder, and 30 g of a powdered sample was extracted with n-hexane using a reflux extractor for three hours at 65°C. [23].

Following the extraction and filtration of the extract a rotary evaporator was used to evaporate the solvent at 45 °C under decreased pressure The sample was kept in the refrigerator at 5°C.

Gas Mass Chromatography

In accordance with published protocols, the oil extract's GC-MS analysis was performed at the Basra Oil Company Laboratory using an Agilent Technologies 7890B GC system connected to an Agilent Technologies 5977A MSD with an EI Signal detector. HP-5ms 5% phenyl, 95% methyl siloxane (30m*250um*0.25) was used, and the oven temperature was set at 40 C for five minutes, then increased to 8 C/min to 300 C for twenty minutes. The helium carrier gas flow rate was 1 ml/min, and the purge flow rate was 3 ml/min [24, 25].

High Pressurized Liquid Chromatography (HPLC) Analysis

The HPLC analysis was performed at the Department of Environment and Water, Ministry of Science and Technology, Iraq, using a previously published protocol. The HPLC system consisted of a SYKAM solvent delivery system with two pumps (German), a spectrofluorometric detector (excitation: 265 nm, emission: 315 nm), and a Shim-pack C18-ODS analytical column (250 mm x 4.6 mm). Acetonitrile water was used as the mobile phase with the following gradient conditions: A, 85:15% (0-4 min); B, 87:13% (5-8 min); and C, 97:3% (9-14 min). The mobile phase was filtered and degassed before being pumped at a flow rate of 1.5 mL/min. The column oven temperature was maintained at 50°C.

Wound Healing Assay

After studying the chemical composition of oil extracted from Pistacia Khinjuk fruit and its presence of many interesting compounds, we decided to study wound healing assay. To examine the effects of oil extract at varying doses, an in vitro scratch experiment was employed (100, 200, 300, 400, and 500µg/ml) on the migration and communication of cells at 0, 24, 48, 72, 96, and 120 hours. Some adjustments were made to the protocol. The monolayer cells should be scraped with a sterile 200 µl pipette tip. After photographing cells that had moved into the wound surface at a 10x magnification, the images were analyzed using ImageJ software which determined the gap width (mm) of the open wound area for each image [26].

Preparation Of Extract

10 mg of oil extracted was taken, and diluted in 1 ml of DMSO, then 5 concentrations as 500 µl,400 µl,300 µl,200 µl and 100 µl, with 2 preparations as negative control.

Then the preparations were added to on cells a daily basis for 5 days.

Cells Preparation

After being planted at a density of roughly 2*105 cells per milliliter into a 12-well tissue culture plate the cells were allowed to attach to the plate's surface in an incubator set at 37 °C and 5% CO2. After 24 hours of growth, the cells attained 70–80% confluence; regular medium was utilized at this point.

Photographs were taken to ensure the occurrence of confluent, and monolayer at all surfaces.
After 24 h will be aspirated media carefully without touching the bottom of the well and replaced by 1 ml of PBS for washes and rehydration and moved gently to cover the entire surface.
Using 200 Ml pipette tips scrape the cell layer in a straight line keeping the tip perpendicular to the well's bottom. Then, create a cross in each well by scratching another line perpendicular to the first line the tip must remain in contact with the well's bottom to remove the cell layer but not too much pressure should be applied.
After the scratch, the plate was gently moved to remove all loose cells that were scratched by a tip. Aspirate PBS of all wells and replace with fresh low-serum media (RPMI-1640 with 5% FBS), used low-serum media because we need to reduce nutrients to focus on migration, not on proliferation. Then plates were incubated and images were captured daily until cells migrated to meet in the middle.

RESULT AND DISCUSSION

Gass Chromatography (GC-MASS)

GC-MS analysis of *P. khinjuk* fruit oil extract identified 21 components, with n-hexadecanoic acid (palmitic acid) being the most abundant (46.44%) and cis-4-decenal the least abundant (0.48%). Other significant compounds included 2,4-decadienal (E, E) (8.78%), 1,4-benzenedicarboxylic acid, bis(2-ethylhexyl) ester (7.59%), and 2-decenal (E) (5.13%).

Other compounds identified in the GC-MS analysis included 2,4-decadienal (E, E) (8.78%), 1,4-benzenedicarboxylic acid, bis(2-ethylhexyl) ester (7.59%), 2-decenal (E) (5.13%), (Z)-3-(pentadec-8-en-1-yl) phenol (4.57%), 9-octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester (4.23%), 2,4-decadienal (E, Z) (3.66%), 2-undecenal (2.54%), 2-heptenal (E)- (2.36%), and gamma-sitosterol (1.47%).

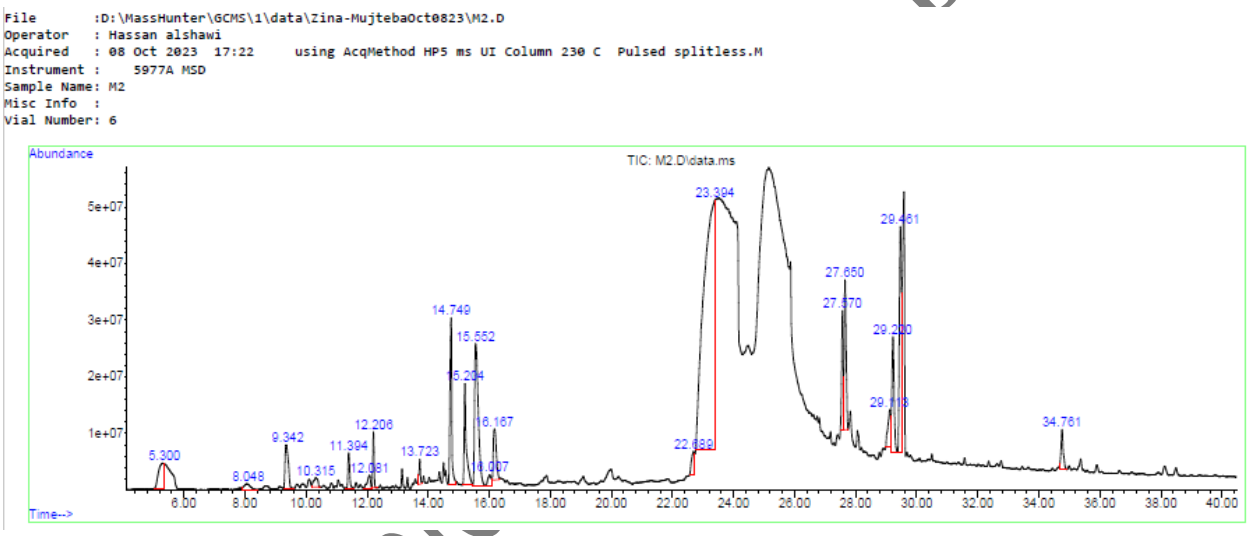


Fig. 1 GC-MS Chromatogram of *P. khinjuk* Fruit Oil Extract

Table 1 GC-MS Analysis of *P. khinjuk* Fruit Oil Extract: Compound Identification and Relative Abundance

Peak	R.T.	Area	Pct Total	Area Pct	Library/ID
1	5.3	5.1E + 07	2.077	2.0773	Octane
2	8.048	1.3E + 07	0.549	0.549	Heptanal
3	9.342	5.8E + 07	2.362	2.3623	2-Heptenal, (E)-
4	10.315	1.6E + 07	0.649	0.6486	Octanal
5	11.394	2.6E + 07	1.07	1.0696	2- Octenal, (E)-
6	12.081	1.7E + 07	0.677	0.6774	3-Ethyl-2-hexene
7	12.206	2.8E + 07	1.162	1.1615	Nonanal
8	13.723	1.2E + 07	0.477	0.4772	Cis-4-Decenal
9	14.749	1.3E + 08	5.136	5.1364	2-Decenal
10	15.204	8.9E + 07	3.66	3.6602	2,4-Decadienal, (E,Z)-
11	15.552	2.1E + 08	8.789	8.7894	2,4-Decadienal, (E,E)-
12	16.007	1.5E + 07	0.609	0.6091	Pentafluoropropionic acid, undecyl ester
13	16.167	6.2E + 07	2.549	2.5488	2-Undecenal
14	22.689	3E + 07	1.215	1.2149	6-Pentadecenoic acid, 13-methyl-, (6Z)-
15	23.394	1.1E + 09	46.438	46.4381	n-Hexadecanoic acid
16	27.57	7.5E + 07	3.089	3.0891	(Z)-3-(pentadec-8-en-1-yl) phenol
17	27.65	1.1E + 08	4.572	4.5724	(Z)-3-(pentadec-8-en-1-yl) phenol

18	29.113	3.9E + 07	1.613	1.6135	(Z)-3-(Heptadec-10-en-1-yl) phenol
19	29.22	1E + 08	4.239	4.2386	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester
20	29.461	1.9E + 08	7.595	7.5948	1,4-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester
21	34.761	3.6E + 07	1.472	1.4717	gamma.-Sitosterol

Table 1 and Figure 1 present the GC-MS analysis of P. khinjuk fruit oil extract, revealing a diverse array of compounds with biological and industrial significance. The most abundant compound identified was n-hexadecanoic acid (palmitic acid), a common saturated fatty acid found in microbes, plants, and mammals [27]. The presence of palmitic acid in P. khinjuk oil may contribute to its potential medicinal properties, as palmitic acid has demonstrated cytotoxicity against various cancer cell lines, including prostate and colon/colorectal cancer [28].

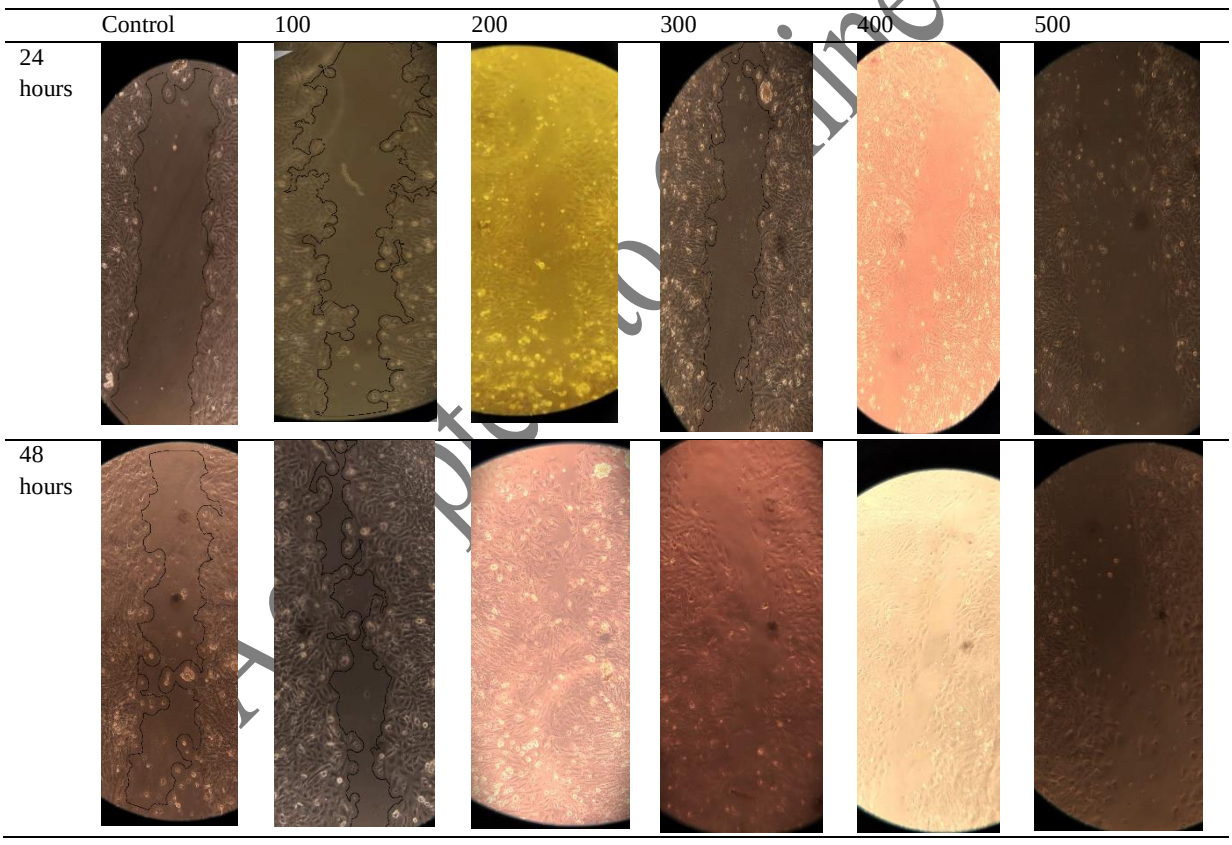
Many studies suggest that γ-Sitosterol has many potential biological activities like the anticancer effect [29], the antibacterial effect [30], hypolipidemic activity [31], the anti-hyperglycemic effect [32], and other biological activities.

Previous studies on P. khinjuk oil extracted from plants in Iran using the same method reported a different composition, with oleic acid (63.55%), palmitic acid (19.44%), and linoleic acid (13.57%) as the predominant fatty acids. Notably, gamma-sitosterol was not detected in the Iranian samples [27].

The observed compositional differences may reflect variations in environmental conditions, as the plants were collected from locations separated by over 1000 km. Although gamma-sitosterol was not detected in the Iranian samples, HPLC analysis of our *P. khinjuk* fruit oil extract confirmed the presence of oleic acid and linoleic acid, among other compounds.

Effect of *P. khinjuk* Fruit Oil Extract on Fibroblast Migration in a Scratch Assay

To assess the wound-healing potential of *P. khinjuk* fruit oil extract, we performed an *in vitro* scratch assay using confluent fibroblast cultures. Cells were treated with varying concentrations of the oil extract (100-500 µg/mL) or left untreated (control), and their migration into the denuded area was monitored over 120 hours at 37°C. The results of this assay are presented in Figures 2 and 3.



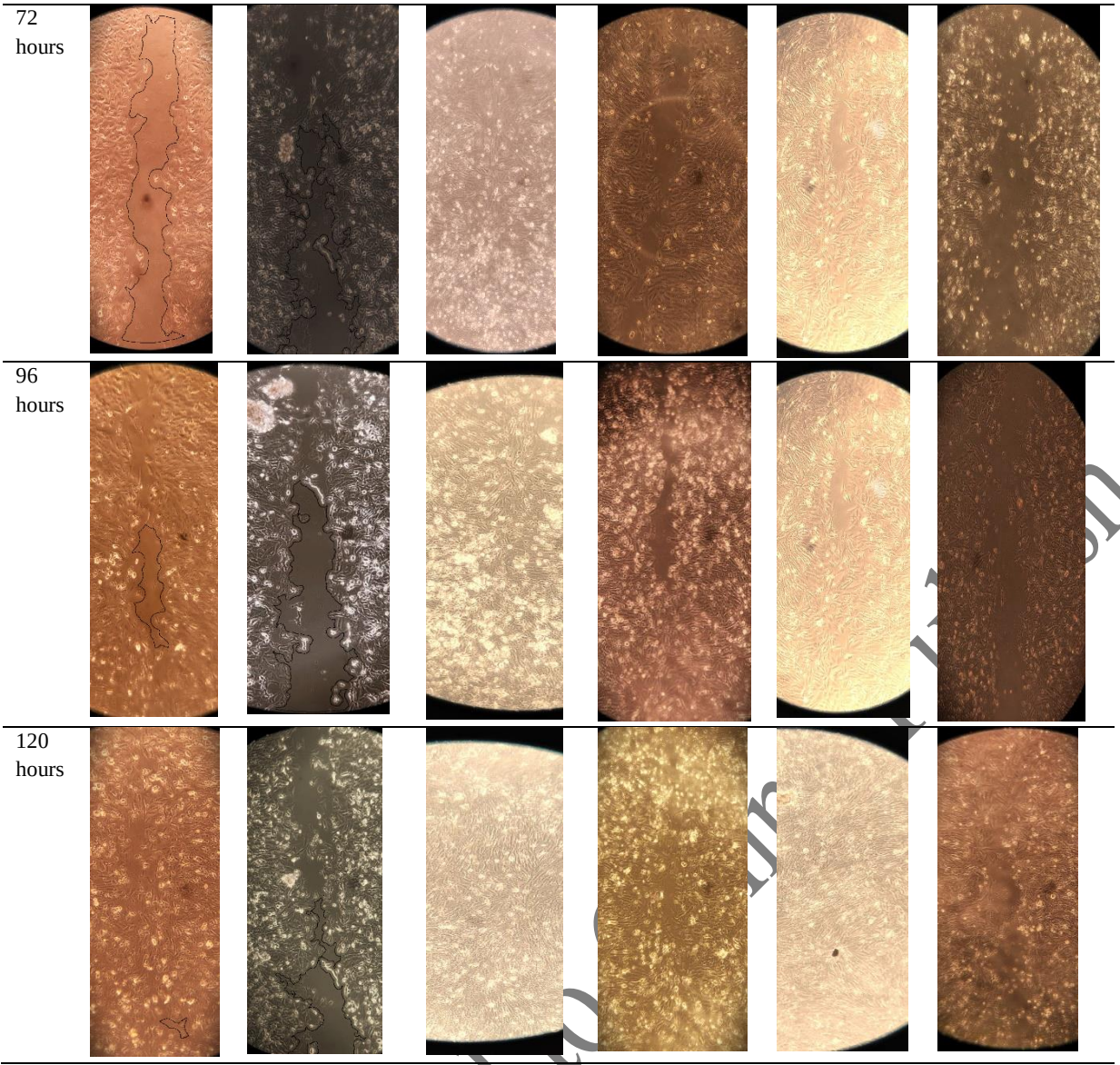


Fig. 2 Representative Images of Fibroblast Migration at Different Time Points Following Scratching

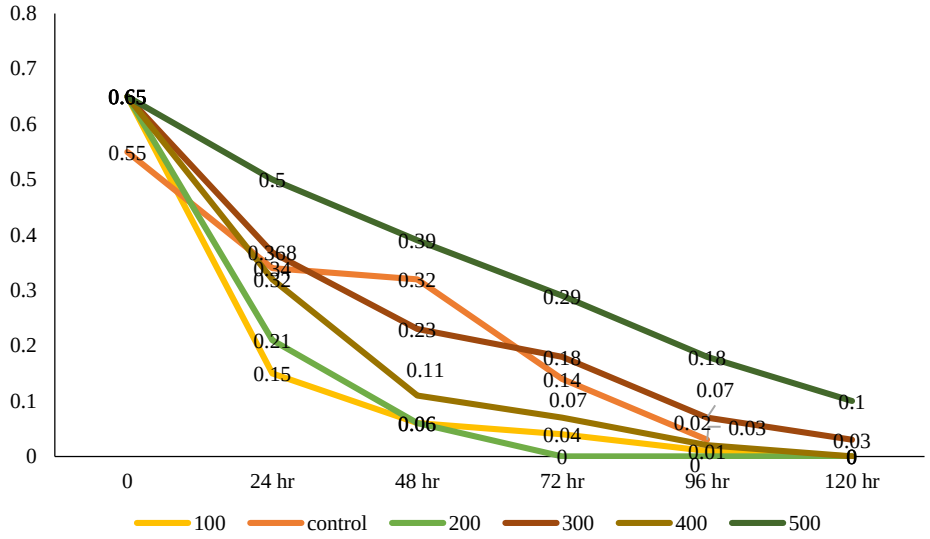


Fig. 3 Percentage of Wound Closure Over Time for Fibroblasts Treated with P. khinjuk Oil Extract

As shown in Figures 2 and 3, fibroblasts treated with 200 µg/mL of P. khinjuk fruit oil extract exhibited the most rapid migration into the denuded area, with near-complete scratch closure observed after 72 hours. In contrast, untreated control cells showed slower migration and less complete wound closure compared to all extract-treated groups.

The 200 µg/mL concentration of P. khinjuk fruit oil extract significantly accelerated wound closure compared to other concentrations tested. These findings suggest that the enhanced wound-healing activity of the oil may be attributed to its antioxidant and proliferative effects, potentially mediated by compounds such as gamma-sitosterol and palmitic acid, which have been reported to possess anti-inflammatory properties [33]. Consistent with our findings, previous studies have demonstrated that oleic acid promotes rapid wound closure and improves overall wound healing [34].

The observed wound-healing activity of *P. khinjuk* fruit oil extract may be attributed to the synergistic effects of its various chemical constituents. Palmitic acid (n-hexadecanoic acid), the most abundant compound identified by GC-MS, has been shown to possess anti-inflammatory properties and can stimulate collagen synthesis in fibroblasts [35]. While high concentrations of palmitic acid can be cytotoxic, the concentration used in our wound healing assay (200 µg/mL) likely promotes a controlled inflammatory response, which is crucial for initiating the wound healing cascade. Oleic acid, the most abundant compound identified by HPLC, is a monounsaturated fatty acid known to enhance keratinocyte migration and accelerate wound closure [36]. Furthermore, gamma-sitosterol, also identified in the oil extract, possesses antioxidant and anti-inflammatory activities, which can protect cells from oxidative stress and promote tissue regeneration [37]. These compounds, along with other minor constituents of the oil extract, likely work in concert to promote fibroblast migration, collagen deposition, and overall wound healing.

Identification and Quantification of Fatty Acids in *P. khinjuk* Fruit Oil Extract by HPLC

HPLC analysis revealed that oleic acid was the most abundant fatty acid in the *P. khinjuk* fruit oil extract (41.9%), while linolenic acid was present in the lowest quantity (0.78%). The presence of other compounds, including palmitic acid (25.49%), phenolic acid (22.9%), and stearic acid (3.15%), was also confirmed. These HPLC results are summarized in Figure 4 and Table 2.

These five fatty acids identified by HPLC possess numerous biological and industrial applications. Oleic acid (omega-9), for example, has been extensively studied for its pharmacological activities, including cytotoxic and anti-inflammatory properties [34]. Beyond its anti-inflammatory and cytotoxic effects, oleic acid has also been associated with improved cognitive performance, antioxidant activity, and antiviral properties in various studies. These diverse biological activities highlight the potential of *P. khinjuk* fruit oil as a valuable source of bioactive compounds [38].

Stearic acid finds extensive use in the pharmaceutical and biomedical industries as an emulsifier, lubricant, binder, and other additive in topical medications and enteric-coated tablets [39]. Linolenic acid also possesses biological functions, with its conjugated form demonstrating protective effects against atherosclerosis. Additionally, alpha-linolenic acid, in its conjugated form, improves lean body mass and modulates the inflammatory response [40].

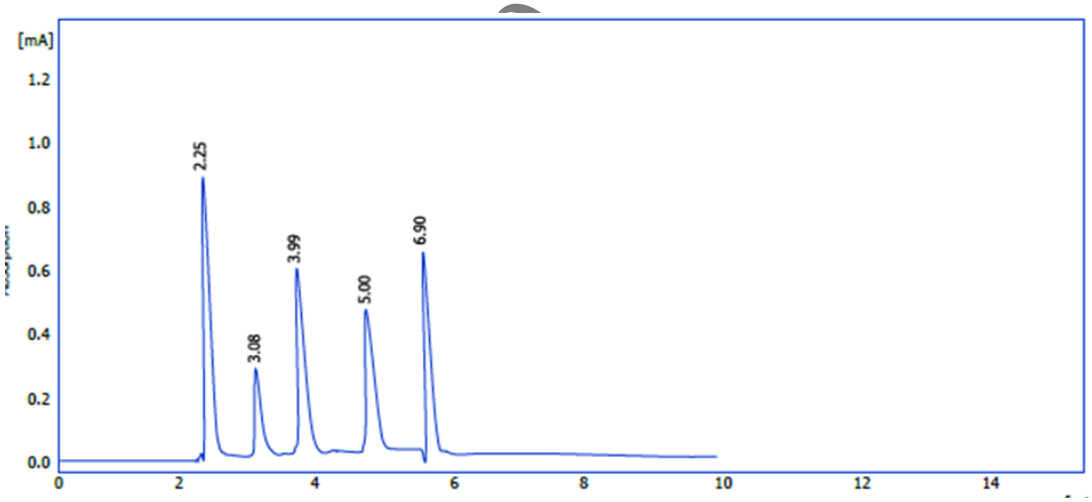


Fig. 4 Representative HPLC Chromatogram Showing Fatty Acid Composition of P. khinjuk Fruit Oil Extract

Table 2 The chromatography results of HPLC analysis

Product	Reten.Time (min)	Area (mAU.s)	Height (mAU)	Area (%)	Height (%)	W05 (min)	% w/v
Oleic acid	2.25	158745.65	900.12	30.00	30.00	0.15	41.9
linolenic acid	3.08	52314.49	320.58	15.00	15.00	0.05	0.78
Lenolic acid	3.99	123652.05	611.24	20.00	20.00	0.10	22.9
Stearic acid	5.00	112002.20	580.44	15.00	15.00	0.10	3.15
Palmitic acid	6.90	127845.64	600.32	20.00	20.00	0.10	25.49
	Total	574560.12	3012.17	100.00	100.00	0.5	

R The result of HPLC analysis shows that there are many compounds in the sample.

CONCLUSION

This study confirms that *P. khinjuk* fruit oil extract contains a diverse array of valuable fatty acids – including oleic acid (omega-9), palmitic acid, linoleic acid, α-linolenic acid (omega-3), and stearic acid, as identified by high-performance liquid chromatography (HPLC) – and sterols, particularly gamma-sitosterol, identified by gas chromatography-mass spectrometry (GC-MS). These components contribute to the oil's wide range of potential pharmaceutical and industrial applications, supported by existing research indicating cytotoxicity, antioxidant, antibacterial, and anti-inflammatory properties. Palmitic acid was the predominant oil as identified by gas chromatography, while oleic acid was the major component as identified by high-performance chromatography.

The *P. khinjuk* oil extract also demonstrated significant *in vitro* wound-healing activity, with a concentration of 200 µg/mL promoting near-complete closure of fibroblast cell scratches within 72 hours, compared to approximately 120 hours for the control. This wound-healing test is considered a novel and the first experiment that proved the oil of *P. khinjuk* effect in the treatment of wounds or scratches. Therefore, due to the high oil content in the fruits of *P. khinjuk*, which is characterized by high nutritional value, beneficial components, and wound healing activity, it can be recommended as a good oil source for industrial uses, especially in skincare and scar removal products, in addition to a good source for many beneficial compounds. Further research is warranted to fully elucidate the specific mechanisms underlying these effects and to explore the potential of *P. khinjuk* oil in various therapeutic applications.

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