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Quality Standardisation and Comparative Study of Leaf and Root of *Kalanchoe pinnata* in the Treatment of Wound Healing on Albino Rats

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ABSTRACT

A wound is a disruption of the cellular and anatomic continuity of a tissue, with or without microbial infection. The perennial plant *Kalanchoe pinnata* (Bryophyllum) is a member of the Crassulaceae family. The present study was based on the quality standardisation and comparative study of leaf and root of *Kalanchoe pinnata* in the treatment of wound healing on albino rats. The plant was gathered in Uttarakhand, Dehradun, India. The plant leaves were used after being thoroughly rinsed in water. Additionally, the root was scrubbed and allowed to dry at room temperature in the shade and/or away from the sun. The plant was identified and authenticated by Dr Sunita Garg (Reference No. NIScPR/RHMD/Consult/2023/4486-87). The leaves and roots of *Kalanchoe pinnata* were extracted using methanol solvent. Physicochemical and phytochemical screening was done for both the extracts. The Methanolic extract of *Kalanchoe pinnata* root and leaf (5% Ointment Topical Application for 15 days) was assessed and compared for its wound healing activity on excisional wound healing animal using Albino rats. GC-MS analysis of the MEKP spectral spectrum showed, the presence of eight essential ingredients was shown as flavone with a retention time of 16.08 and a peak area of 11.9%, palmitic acid with a retention time of 16.73 and a peak area of 8.4%, and phytol with a retention time of 17.82 and a peak area of 39.8%. etc. It was found that MEKP Leaf (5% Ointment) showed superior contraction of wound and faster epithelialization compared to MEKP Root (5% Ointment). Our findings support the possibility of using this plant therapeutically in routine medical care. By following these recommendations, we can drive the development of safe, effective, and accessible phytoconstituent-based wound healing agents.

Keywords: *Kalanchoe pinnata*, herbal extract, phytochemicals, wound healing, excision model.

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INTRODUCTION

A wound is a disruption of the cellular and anatomic continuity of a tissue, with or without microbial infection. It could result from the tissues being subjected to chemical, thermal, physical, microbiological, or immunological stress [1]. The aetiology, location, forms of injury, and clinical appearance of wounds are some of the factors used to categorise wounds [2]. Wound healing is the process by which tissue is repaired. It is made up of a continuous sequence of inflammation and repair in which epithelial, endothelial, inflammatory cells, platelets, and fibroblasts briefly come together outside of their normal domains and interact to restore resemblances of their used discipline before returning to their normal function [3][4]. More than 25% of our contemporary medical pharmacopoeia is derived from herbs, according to the World Health Organisation, while around 80% of inhabitants in underdeveloped countries rely on traditional herbal treatment [5][6]. More than 25% of our contemporary medical pharmacopoeia is derived from herbs, according to the WHO, while around 80% of inhabitants in underdeveloped countries rely on traditional herbal treatment [7]. About 60% of the world's population relies almost exclusively on plants for treatment since they have less side effects than synthetic pharmaceuticals, and natural products have long been acknowledged as a significant source of therapeutically effective medicines.

Plant profile

The perennial plant *Kalanchoe pinnata* (Bryophyllum) is a member of the Crassulaceae family. It has been used for a very long time and is now a vital component of many medicines [8]. Asia, Africa, North America, Central America, and the Caribbean make up the majority of the region where there is ambiguity on the actual number of species within the genus. It can also be found in Europe and South America [9]. Morphology, growth habits, flower colour, leaves, stems, and chemical makeup all exhibit a great deal of variation. Pollination of KP is fairly simple. Depending on where in the world you are, KP has several names. It is frequently referred to in English as "floppers," "good luck leaf," "air plant," "life plant," "Hawaiian air plant," and "American life plant."

Taxonomy [10]
Kingdom- Plantae
Subkingdom-Tracheobionta
Super division- Spermatophyta
Division- Magnoliophyta
Class- Magnoliopsida
Subclass- Rosidae
Order- Saxifragales
Family- Crassulaceae
Genus- *Kalanchoe*
Species- *pinnata*

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Synonyms- *B. germinans, Verea pinnata, C. rhizophilla, Crassuvia floripendia, Cotyledon calycina Bryophyllum calycinum, B. pinnatum, C. calyculata, C. pinnata, Crassula pinnata, Sedum madagascariense,*

It is a glabrous herb that grows between 0.3 and 1.2 metres tall. The younger stems are reddish with white specks, while the elder ones have four sharp angles. Variable decussate leaves are normally simple or sometimes compound, 8-12 cm long and 6-8 cm wide, while the top leaves are typically 3-5 cm long and pointed with long petioles that are connected by a ridge around the stem. Oval or elliptic, crenate or serrated leaflets. The lateral nerves at the ends of the leaves frequently develop into buds that include roots, stems, and leaves. These buds then fall off and immediately sprout new plants [11]. Reddish-purple flowers with a slender pedicle are suspended in broad, spreading panicles. Calyx has triangular teeth, is striated, and is red and green at the base.

a. Leaves **b. Flowers** **c. Roots**

Fig. 1 Depiction of *Kalanchoe pinnata* shrub

Fruits- Fruits are enclosed in the persistent papery calyx and corolla.

Seed- Small, barely striate, smooth, oblong-ellipsoid seeds. The leaves frequently develop buds with roots, stems, and leaves at the lateral nerves' extremities, which fall off and instantly sprout new plants.

Root- The root is odourless, bitter to the taste, and varies in size. It is dark brown in appearance.

Major phytoconstituents in different parts of plant are arachidic acid, bryophyllin, bryophyllin-A-C, astragalin, behenic acid, β-bryophollone, benzenoids, β-sitosterol, bryophollenone, bryophyllol, bryotoxin-C, clerosterol, coumaric acid, epigallocatechin, ferulic acid, flavonoids, caffeic acid, campesterol, cardenolides, cinnamic acid, isofucosterol, kaempferol, oxalic acid, oxaloacetate, palmitic acid, friedelin, glutinol, hentriacontane, patuletin, pseudotaraxasterol, pyruvate, quercetin, steroids, peposterol, phosphoenolpyruvate, protocatechuic acid, stigmasterol, succinic acid, syringic acid, taraxerol and triacontane. Plant also contains some essential fatty acids include behenic acid, stearic acid arachidic acid, and palmitic acid.

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MATERIALS AND METHODS

Drugs and equipment

- Xylazine inj
- Ketamine inj
- Povidine Iodine (5%) Cream
- UV Visible Spectrophotometer system-Systronics-117
- Centrifuge Machine-Labnet
- Rotary evaporator
- Electronic balance
- Rat oral feeding needle
- Rat holder

Collection and Authentication of plant

The plant was gathered in Uttarakhand, Dehradun, India. The plant leaves were used after being thoroughly rinsed in water. Additionally, the root was scrubbed and allowed to dry at room temperature in the shade and/or away from the sun. The plant was identified and authenticated by Dr Sunita Garg (Reference No. NIScPR/RHMD/Consult/2023/4486-87).

Extraction Process

Preparation of Methanolic extract of KP (MEKP) Leaves

A conical flask was filled with freshly gathered KP leaves that had been rinsed under running water and manually cut into tiny pieces. The conical flask was filled with 100 cc of methanol and macerated for 7 days to enhance the extraction [12]. It was created as a methanolic stock solution after 7 days, filtered using Whatmann filter paper, and kept in an appropriate container. The methanolic extract was pipetted into an Eppendorf tube with 1.5 ml, allowing for the GC-MS analysis of different components.

Preparation of MEKP Roots

A grinder was used to grind the KP root after it had been collected and get dried in the shade. The substance's powdered form was used for the extraction. Material was passed through 120 meshes to eliminate fine powders, then extraction was carried out using coarse powder. A method from Mukherjee was used to extract plant powder. For extraction, methanol was used [13].

Physicochemical Evaluation [14]

Moisture content/ Loss on drying Determination

In order to calculate the moisture content, 10 gm of leaf and root powder were dried at 105°C in a hot air oven. Utilising the initial leaf and root powder weight, the % was calculated.

Extractive values Determination

Using the following methods, the extractive values of KP leaves were calculated:

Water soluble

4 gm of powdered dried leaf and root was combined with 100 ml of water, and the mixture was completely mixed after an hour. On a water bath, the mixture was brought to a boil (100 ^{0}C), and then filtered. A pre-weighed porcelain plate was used to evaporate and dry the filtrate at 105 ^{o}C. Water's extractive value was calculated.

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Alcohol soluble

100 millilitres of alcohol were shaken with 4 gm of powdered material, which was then macerated for 16 hours before being filtered. The filtrate was next dried at 105°C in a porcelain plate that had been pre-weighed. Alcohol's extractive value was calculated.

Ash values Determination

The total ash, acid insoluble ash, and water-soluble ash values of the leaf and root of KP were ascertained using the following techniques:

Total ash Determination

A pre-weighed silica crucible containing 2gm of leaf and root powder was burned at 500 to 600 °C until carbon-free ash was formed. The starting weight of the dry powder was utilised as a guideline when calculating the percentage of ash.

Acid insoluble ash Determination

The ash created from full ash was boiled for 5 minutes with 25 cc of 1 N HCl to remove insoluble particles. The residue was then collected using the ashless filter paper. The filter paper was put into an already weighted silica crucible and heated to 650ºC in a muffle furnace until it was carbon-free. Using dry powder to determine the amount of acid-insoluble ash.

Water soluble ash value Determination

The entire ash quantity was subjected to a 5-minute heating process with 25 cc of water. The dissolved components were gathered using filter paper that leaves no residue. This filter paper was then put into a silica crucible of known weight and heated to 450°C in a specialized furnace. The percentage of water-soluble ash was determined in relation to the weight of the dried powder.

GC-MS Analysis

The analysis was performed using an Agilent 6890 N gas chromatograph equipped with a front injector type 1079 and a mass selective detector. A DB 5 MS capillary column (30m x 0.25 mm, film thickness 0.25 m) was installed in the chromatograph. The oven temperature was initially set at 45 °C and then ramped up to 300 °C at a rate of 10 °C/min, followed by a 5-minute hold at 200 °C. The injector temperature was held constant at 280 °C, and helium was used as the carrier gas at a flow rate of 1.0 mL/min. A 1:10 dilution of the sample with acetone was prepared, and one microlitre of the diluted sample was injected in the split mode at a 1:100 ratio. The GC peak area was utilized to calculate the sample percentage. The material was subjected to analysis using both Agilent gas chromatography and a JEOL GC MATE-II HR mass spectrometer with gas chromatography-mass spectrometry (GC-MS). The same GC parameters and column as previously described were used for the GC analysis. The mass spectrometer operated in electron impact mode at 70 eV, with the ion source and transfer line maintained at a constant temperature of 250°C. A centroid scanner was employed to scan the mass range from 40 to 1000 amu to obtain the mass spectra. The entire MS analysis took 35 minutes to complete. The sample percentage was determined based on the GC peak area [15][16].

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Qualitative Analysis of Phytochemicals [17]

The qualitative phytochemical screening was performed as below-

- **Tannins (Ferric chloride test):** Boil approximately 0.5g of the extract in 20 ml of water, filter, and add a few drops of 0.1% ferric chloride. Observe for the presence of blue-black or brownish-green color.
- **Saponins (Frothing test):** Combine 5 mg of the extract with 20 ml of distilled water, agitate for 15 minutes, and check for the formation of foam, indicating the presence of saponins.
- **Flavonoids (Lead acetate test):** After consuming 10 mg of the extract, add a few drops of 10% lead acetate solution. A yellow precipitate indicates the presence of flavonoids.
- **Terpenoids (Salkowski's test):** Mix 5 mg of the extract with 2 ml of chloroform, then carefully add 3 ml of concentrated H2SO4. Observe a reddish-brown interface to confirm the presence of terpenoids.
- **Alkaloids (Wagner's Test):** Acidify 2 mg of the extract with 1.5% v/v hydrochloric acid, filter, and add Wagner's reagent. The formation of a brown or reddish precipitate suggests the presence of alkaloids.
- **Steroid (Salkowski's test):** Dissolve 5 mg of the extract in a mixture of 2 ml chloroform and 2 ml strong sulfuric acid. The appearance of a red color indicates the presence of steroids.
- **Glycosides (sodium hydroxide test):** Dissolve the extract (about 5 mg) in 1 ml of water and add 5–6 drops of 10% NaOH. The development of a yellow color signifies the presence of glycosides.

Experimental Animals

Male Albino rats, purchased from a facility that has been approved by CPCSEA, were used in the experiment. They were between 6 and 8 weeks old and weighed between 120 and 150 g at birth. Animals were separately housed in polypropylene cages with stainless steel top grills for a six-day acclimatisation phase. Rat pellets, a common laboratory meal, were fed to the rats. Ad libitum access to purified drinking water was maintained under laboratory conditions of 22 °C (3 °C) temperature, 36–64 % relative humidity, and a 12-hour cycle of light and darkness. The animals were given bedding made of sterilised paddy husk.

Preparation Of Ointments for Topical Administration

According to Mahmood *et al.*, 2005 [18] concentrations of both MEKP leaves and roots were obtained to make ointments using simple ointment as the foundation. These concentrations were 1% (1g extract/100g Simple ointment, w/w) and 5% (5g extract/100g Simple ointment, w/w). The prepared ointments were stored in the freezer (40ºC) until they were used. Composition of 100 gm of Simple Ointment:

Bees wax or Wool fat: 15g

White or Yellow paraffin: 85g.

Acute Dermal Toxicity

Acute dermal toxicity was conducted According to the OECD guideline 402 (acute dermal toxicity 1987), for this topical application of the respective plant extracts for acute dermal toxicity was conducted. Because the plant extracts showed no visible signs of skin irritation, inflammation, swelling, or any other changes on the skin, topical application of the plant extracts was deemed safe for the present study.

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Group design

There were six animal groups of 6 animals each and treatment was done for 15 Days as follows:

- Group-I: Vehicle Control (Excision Wound) + Simple Ointment Base daily for 15 days
- Group-II: Standard (Excision Wound) + Povidone Iodine (5%) ointment daily 15 days
- Group-III: (Excision) + MEKP LEAVES (1%) ointment daily for 15 days.
- Group-IV: (Excision) + MEKP LEAVES (5%) ointment daily for 15 days.
- Group-V: (Excision) + MEKP ROOT (1%) ointment daily for 15 days.
- Group-VI: (Excision) + MEKP ROOT (5%) ointment daily for 15 days.
- Total number of animals- 36

PROTOCOL

Excision Wound Model

There were six groups of animal total, with six creatures in each group. Animals in Group I received a basic ointment base and the vehicle control as a topically administered treatment. Standard treatment for group II animals involved topically applying a povidone-iodine (5%) ointment. Group III, IV, V, and VI received treatments with 1% and 5% w/w MEKP of ointments made from KP plant leaves and roots.

Xylazine and ketamin were administered intraperitoneally (i/p) to anaesthetize the animals at dose rates of 13 mg/kg body weight and 87 mg/kg body weight, respectively. Without removing the water supplement, the animals were fasted for the entire night [19]. The fur on the animals' backs was shaved off, and the designated wound site was marked. A sterile full-thickness excision wound, measuring 500 mm^2 in a circular shape and 0.2 cm deep, was created using toothed forceps, a surgical blade, and pointed scissors. The entire wound was left uncovered for examination [20]. A total of 15 days were spent conducting the experiment. In this model, the standard metrics used to assess each plant's capacity for healing were wound closure rate and epithelization times. The number of days needed for an epithelization period to occur without a scar or a still-open wound was noted. The results were expressed as Mean ± SEM and the wound area (mm2) post-wounding day and the duration of epithelialization in day(s) were recorded.

RESULTS AND DISCUSSION

Percentage Yield of Extract

Higher % yield obtained from leaf (8%) compared to root (7.2%) of MEKP.

Table 1: Percentage Yield of MEKP Leaf and Root

Part	% Yield
Leaf	8
Root	7.2

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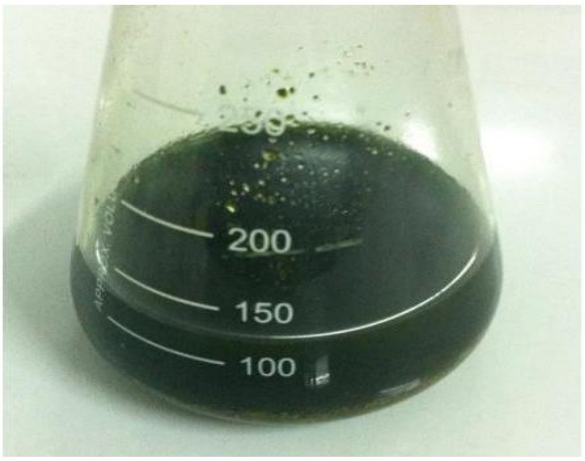

Fig 2. MEKP Leaves

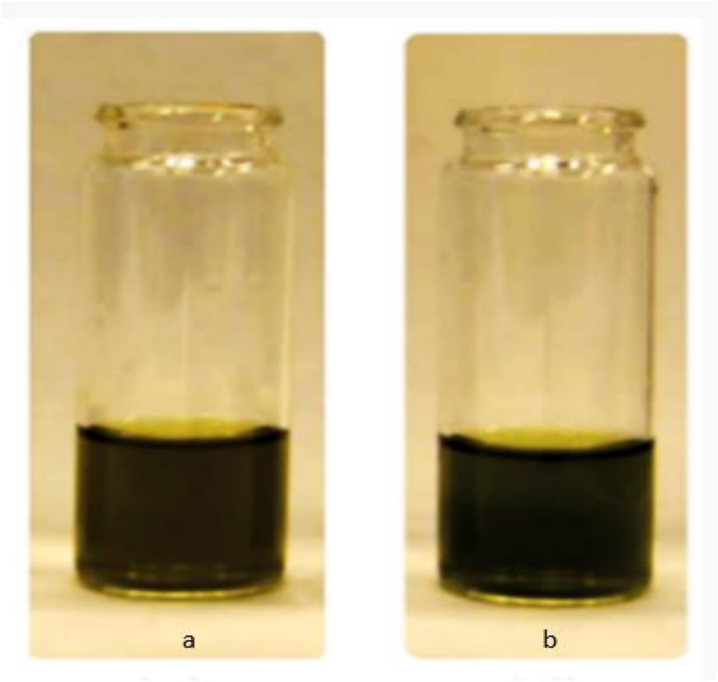

Fig 3. MEKP extract a) Leaves b) Roots

Table 2. KP Leaf Powder: Physicochemical Properties

Parameters	Values (%w/w)
Loss on drying	1.31
Water- soluble-ash	2.25
Total-ash	9.50
Sulphated-ash	17.0
Acid insoluble-ash	0.50

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Table 3. Morphology of KP roots

Character	Observation
Colour	Dark Brown
Size	Varying in size
Taste	Bitter
Odor	Odorless

Table 4. KP Roots: Physicochemical Properties

Parameters	Values (%w/w)
Loss on drying	11.0
Foreign inorganic matter	0.1
Total ash	11.8
Water- soluble-ash	4.5
Acid insoluble-ash	2.7
Sulphated-ash	17.0

Table 5. Outcome of Phytochemical Screening

Test	Phytochemical Category	Leaf	Root
Wagner's Test	Alkaloid	+	+
Lead acetate Test	Flavonoids	+	+
Salkowski Test	Terpenoids	+	+
Ferric Chloride Test	Tannin	+	+
Frothing Test	Saponins	+	+
Sodium Hydroxide Test	Glycosides	+	+
Salkowski Test	Steroids	+	+

GC-MS Leave

The chromatogram of significant MEKP peaks found during GC-MS analysis. Eight major components and relative concentrations of several compounds are eluted as a function of retention time. The peak heights represent the relative concentrations of the substances present in KP. Phytol, flavonoids, and fatty acids (palmitic acid, octadecanoic acid, methyl ester, and 13-Docosenic acid) are some phytocomponents that may have antibacterial activities. These compounds may be responsible for the antibacterial and antioxidant effects of KP. GC-MS analysis of the MEKP spectral spectrum showed the presence of eight essential ingredients was shown as flavone with a retention time of 16.08 and a peak area of 11.9%, palmitic acid with a retention time of 16.73 and a peak area of 8.4%, and phytol with a retention time of 17.82 and a peak area of 39.8%. Oleic acid, with a retention time of 18.47 and a peak area of 14.3%, and methyl ester, with a retention time of 18.03 and a peak area of 10.7% Benzene1-ethoxy-4 [(4-pentyl phenyl)] ethnyl with retention time 19.65 and peak area 3.2%, 13-Docosenic acid with retention time 20.65 and peak area 7.8%, and 3,4-Dihydroxy

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1,6 bis (3-methoxy phenyl) hexa 2,4 diene 1,6 dione with retention time 21.40 and peak area 3.9%, are the three compounds that follow.

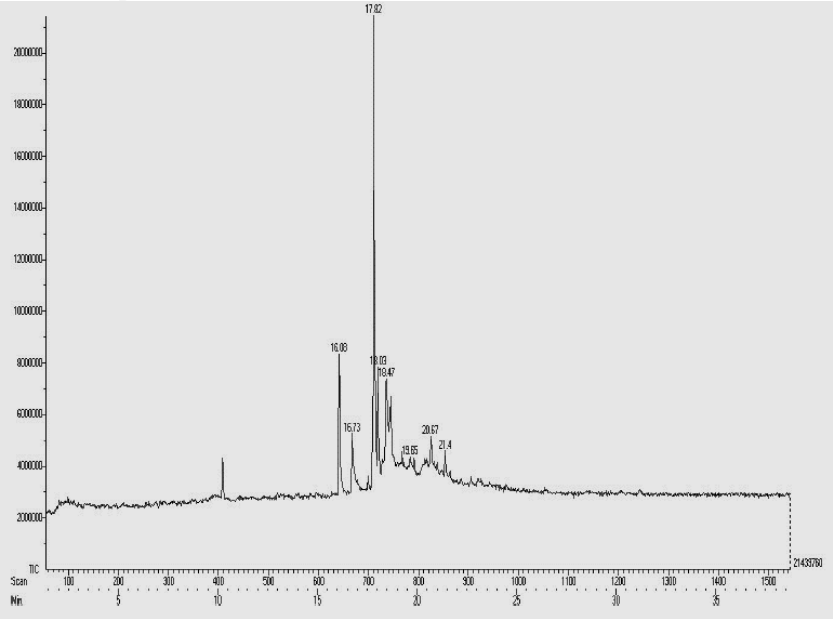

Fig 4. GC-MS Graph of MEKP Leaves

Table 6. Identified Compounds in the MEKP Leaf in GC-MS

RT	Peak area %	Compound	Molecular Formula	Mol. Wt. g/mmol
11.48	13.6	5-Oxotetrahydrofuran-2,3-dicarboxylic acid dimethyl ester	C8H10O6	641
16.08	11.9	Flavone	C15H10O2	222.24
16.73	8.4	Palmitic acid	C16H32O2	256.43
17.82	39.8	Phytol	C20H40O	296.54
18.03	10.7	Octadecanoic acid. Methyl ester	C19H38O2	298.51
18.47	14.3	Oleic acid	C18H34O2	282.47
19.65	3.2	Benzene 1-ethoxy-4[(4 pentylphenyl)]ethnyl	C21H24O	292.42
20.65	7.8	13-Docosenic acid	C22H42O2	338.58
21.40	3.9	3,4-Dihydroxy 1,6 bis (3-methoxy phenyl) hexa 2,4 diene 1,6 dione	C20H18O6	354.36
29.08	2.1	Squalene	C30H50	410

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GCMS-Root

This analysis revealed the presence of 34 different chemicals, known as phytochemical components, which are likely responsible for the plant's therapeutic properties. The table in GC-MS Table 7 provides information on these phytochemical components, including their Retention Time (RT), Name, and Percentage Peak Area.

The first identified molecule, 2-Furancarboxaldehyde,5-methyl, had a relatively short retention time of 5.08 minutes, while the last compound, 2,6,6,9,2',6',6',9'-Octamethyl-[8,8']bi[tricycle[5.4.0.0(2,9)undecyl], had the longest retention time at 27.448 minutes. These identified chemicals exhibit a wide range of biological characteristics, including antioxidant, hypocholesterolemic, nematicide, insecticide, lubricant, and hemolytic activity properties. For instance, n-Hexadecanoic acid (R/T 17.886) has antioxidant and hypocholesterolemic properties. Lanosta-8,24-dien-3-ol acetate, 3.beta. (R/T 21.290), can serve as an analgesic, anti-inflammatory, and anti-cancer agent. Squalene (triterpene) (R/T 25.533) is a phenolic substance commonly found in the latex and resins of various plants, where it acts as a chemical defense against specific pathogens causing diseases in humans and animals. Squalene also functions as a natural moisturizer in cosmetics. Additionally, 5-Hydroxymethylfurfural (R/T 9.102) is utilized as an anti-inflammatory and antioxidant agent. These findings align with the results obtained in this investigation [21][22].

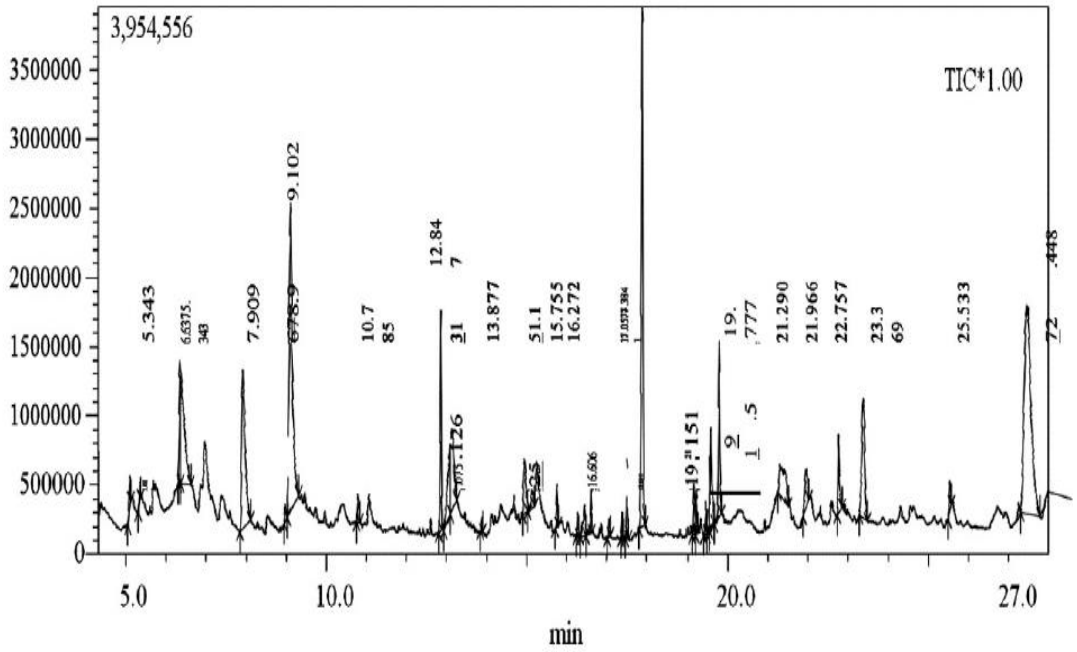

Fig 5. GC-MS Graph of MEKP roots

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Table 7: Identified Compounds in the MEKP Roots in GC-MS

R. Time	Area%	Name
5.088	0.69	2-Furancarboxaldehyde, 5-Methyl-
5.343	0.37	1-Octen-3-ol
6.343	2.77	5*H*-1,4-Dioxepin, 2,3-dihydro-2,5-dimethyl-
6.375	5.15	1,2,3-Propanetriol
7.909	8.02	2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one
8.967	0.36	2,3-Dihydro-benzofuran
9.102	13.84	5-Hydroxymethylfurfural
10.785	0.60	Phenol, 2,6-dimethoxy-
12.847	2.89	Phenol, 2,4-bis(1,1-dimethylethyl)-
13.075	3.31	benzoic acid, 4-ethoxy-, ethyl ester
13.126	3.24	D-Allose
		2-[1,1-Dimethyl-2-pentenyl]-1,1-
13.877	0.17	dimethylcyclopropane-D
14.939	1.91	Hydrazinecarboxamide, 2-(2-methylcyclohexylidene)-
15.125	0.71	Dodecane, 4,6-dimethyl-
15.755	0.62	Tetradecanoic acid
16.272	0.31	3-Heptadecanol
16.447	0.94	Isopropyl myristate
16.606	0.69	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*- (E)]]-
17.057	0.25	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
17.384	0.35	Tricosane
17.503	1.01	Hexadecanoic acid, methyl ester
17.886	10.29	Hexadecanoic acid
19.151	0.60	Methyl octadeca-9,12-dienoate
19.210	0.56	8,11,14-Docosatrienoic acid, methyl ester
19.442	0.49	Tetracosanoic acid, methyl ester
19.514	1.88	11,14-Eicosadienoic acid, methyl ester
19.570	3.02	7-Tetradecenal, (Z)-
19.777	2.76	Octadecanoic acid
21.290	2.82	Lanosta-8,24-dien-3-ol, acetate, (3.beta.)-
21.966	1.61	Heneicosane
22.757	1.47	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
23.369	4.98	9,19-Cyclolanost-23-ene-3,25-diol, 3-acetate, (3.beta.,23E)-
25.533	0.63	Squalene
27.448	20.70	2,6,6,9,2',6',6',9'-Octamethyl-[8,8']bi[tricyclo[5.4.0.0(2,9)]undecyl]

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Wound Healing Activity of MEKP Leaf and Root

It was found that MEKP Leaf (5% Ointment) showed superior contraction of wound and faster epithelialization compared to MEKP Root (5% Ointment). These finding of MEKP Leave (5% Ointment) were similar at some extent to that of standard Povidone Iodine 5% ointment.

Table 8. Wound Healing Activity of MEKP Leaf and Root

Animal Group	CONTRACTED WOUND AREA (mm2)				EPITHELIALIZATION PERIODS (days)
	Day-0	Day-5	Day-10	Day-15	
Group I- Simple Ointment Base	299.70 ± 2.53	270.52± 1.42	239.90±1.57	210.50±1.68	36.52±1.53
Group II- Povidone Iodine (5%)	301.50 ± 1.51	250.50± 1.50	155.25± 1.25	90.60±2.42	16.25±1.25
Group III- MEKP LEAVES (1%) ointment	293.10 ± 2.26	260.72±2.54	180.00±2.50	132.50±1.50	20.65±1.50
Group IV- MEKP LEAVES (5%) ointment	298.10 ± 2.45	257.50±2.50	150.30±2.04	98.50±3.56	17.50±1.23
Group V- MEKP ROOT (1%) ointment	299.20 ± 3.50	265.50±3.50	200.50±2.05	150.23±2.25	22.08±2.50
Group VI- MEKP ROOT (5%) ointment	293.10±3.96	260.50±1.90	156.50±1.35	118.80±1.45	19.10±2.50

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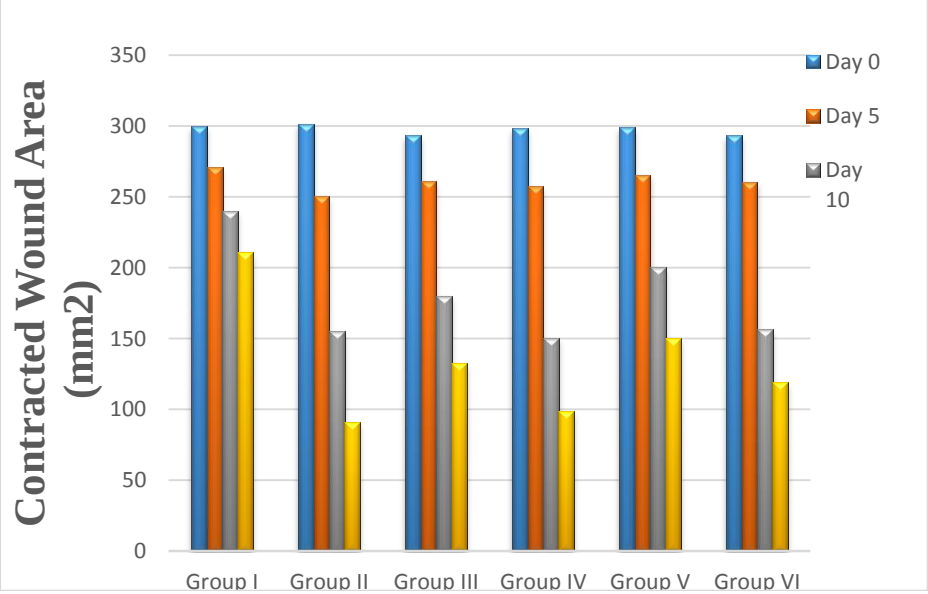

Fig 6. Effect of MEKP leaves and root on wound closure rate and epithelialization

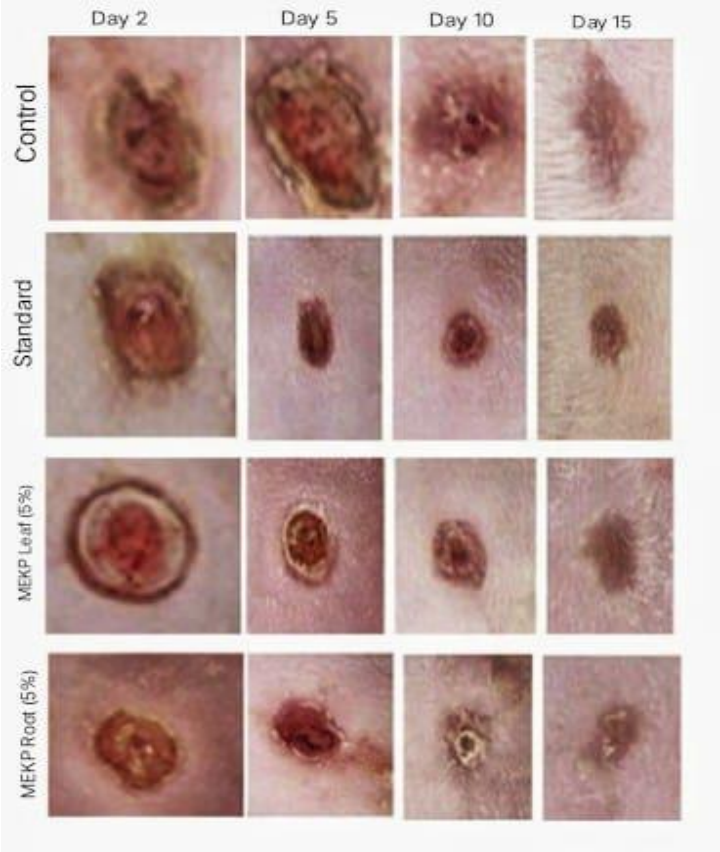

Fig 7. Wound contracted area and scar in response to MEKP Leaf and Root

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In this study, both acid-insoluble ash and water-soluble ash were found, with a higher prevalence of water-soluble ash. Notably, the total ash content was recorded at 9.50%. The preference for water as a solvent, considering that leaves are typically consumed with water, resulted in a higher extractive value for water compared to alcohol. Moisture content, evaluated using the LOD parameter, showed that the dried leaf powder had a negligible moisture content of only 1.31%, which can be disregarded.

The assessment of primary and secondary plant components, including their presence and quantities, can be achieved through phytochemical analysis, covering both qualitative and quantitative aspects [23]. The effects of the extraction conditions were evaluated using the percentage yield (mass of extract/mass of dry matter).

The root is odourless, bitter to the taste, and varies in size. It is dark brown in appearance. The transverse section reveals the epidermis, endodermis, cortex, and other structures. Powder properties include fibre, xylem vessels, cork cells, calcium oxalate crystals, etc. While the findings of the physical parameter evaluation are noteworthy, the water soluble extractive value is higher than the others. According to preliminary phytochemical research, the petroleum ether extract contains steroids, the chloroform extract contains alkaloids and steroids, the MEKP contains alkaloids, glycosides, steroids, saponins, flavonoids, carbohydrates, proteins, tannins, and amino acids, and the aqueous extract contains all of the aforementioned compounds.

Antioxidant, hypocholesterolemic, nematicide, insecticide, lubricant, and hemolytic activity agents can all be found in n-Hexadecanoic acid (R/T 17.886). Lanosta-8,24-dien-3-ol acetate, 3.beta.-(R/T 21.290), can be used as an analgesic, an anti-inflammatory, and an anti-cancer agent. Squalene (triterpine) (R/T 25.533) is a phenolic substance, and terpines are present in the latex and resins of various plants. It is generally accepted that the physiological role of these compounds is to act as a chemical defence against specific pathogens that cause diseases in humans and animals. Squalene serves as a natural moisturiser in cosmetics.

Plants are the source of a variety of pharmacologically strong substances, and plant products are possible wound healing agents. These products are frequently chosen due to their vast availability, lack of toxicity, lack of undesirable side effects, and potency as unprocessed ingredients. The fact that the drug is more effective the faster the wound contracts can be used to support this. The wound will close more quickly if the medication is more effective [24].

The cream's capacity to hasten the healing of rat excision wounds was examined by topical application of the MEKP leaves and roots cream for 15 continuous days. Animals treated with the MEKP leaves cream (5%) shown better reduction in the wound area on day 15 when compared to those treated with MEKP roots and common cream base. When compared to control groups, both MEKP creams were able to considerably accelerate wound contraction and speed up re-epithelialization.

On the fifteenth day, macroscopic examination revealed that the groups treated with MEKP leaves creams (5%) had cleaner wounds and had not yet displayed any signs of inflammation. This was nearly identical to the conventional therapy of Povidone Iodine (5%) cream.

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Additionally, the therapy with KP creams and the positive control promoted the regeneration of the skin's appendages, including the sebaceous and hair follicles. Aqueous and organic leaf extracts of KP have already been tested in wound models when administered orally and topically [25]. Flavonol glycosides have been shown to promote the healing of wounds.
In a study using an excisional wound model on rats, ethanol extracts of KP leaves reduced the size of the lesions by 86% in 11 days. In addition, a similar study using an aqueous KP leaf extract revealed 92% healing at day 12. This figure is a little lower than what we discovered in the current investigation, when the extract was added to a cream in contrast to their technique. These investigations failed to pinpoint the compound(s) that enhance healing. Inflammation, cellular proliferation, and/or wound remodelling are all stages of wound healing that wound healing agents can affect [26]. The release of prostaglandins, leukotrienes, and reactive oxygen and nitrogen species is what causes the classic signs and symptoms of inflammation. In addition, harmful germs are capable of infecting wounds. Therefore, herbal medicines' anti-inflammatory, antioxidant, and antibacterial properties can aid in the entire healing process [27]. The scientists concluded that the KP extract's beneficial immunomodulating effect on the granulation and epithelialization of the lesion appeared to be more significant than its microbicide effect. At the daily dose of 1mL, KP extract did not exhibit any toxicity.

CONCLUSION

We presented the first demonstration of the topical use of the MEKP leaf and root-based cream in an in vivo excisional wound healing animal model. Given that both formulations produced identical outcomes, the cream with MEKP leaf might be more beneficial and less expensive because to the tedious, pricey, and time-consuming methods. Our findings support the possibility of using this plant therapeutically in routine medical care.
By following these recommendations, we can drive the development of safe, effective, and accessible phytoconstituent-based wound healing agents. Such advancements have the potential to revolutionize wound care and improve the quality of life for countless individuals worldwide.

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CONFLICT OF INTEREST

Authors declared for none conflict of interest.

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