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Original Article

In vitro and *in vivo* Wound Healing Potential of Plant *Commiphora caudata*

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ABSTRACT

Background: *Commiphora caudata* is commonly called hill mango, an incredibly available species of *Commiphora*, and belongs to Burseraceae. *Commiphora caudata* is commonly used in Ayurveda and Siddha medicines. Different parts of the plant have many pharmacological activities like antiviral, antispasmodic, cytotoxic, hypothermic activity, antiacne, hepatoprotective, febrifuge, antibacterial, antioxidant, and anti-inflammatory activities. **Materials and Methods:** Extracts were prepared by successive solvent extraction, *In vitro* studies like, MTT assay, scratch assay, Estimation of Inflammatory Marker IL-6 by Enzyme-Linked Immuno sorbent Assay (ELISA test), determination of hydroxyproline, estimation of total protein, *in vivo* excision wound model and diabetic model were done. **Results:** In all the chloroform extract of *Commiphora caudata* has shown a good wound-healing property. **Conclusion:** Based on the ethnic medical uses and proven biological activities, the gum blended in with water is utilized as a mouthwash to cure mouth ulcers and is utilized for wound healing and rheumatoid arthritis., current work focused on proving wound healing potential of plant *Commiphora caudata.*

Keywords: *Commiphora caudata*, MTT, Scratch assay, IL-6, Excision, Diabetic model.

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INTRODUCTION

Wound healing refers to a living system's replacement of destroyed or damaged tissue by newly produced tissue.[1] Wound healing is a complex biological process that consists of hemostasis, inflammation, proliferation, and remodeling Many types of cells include neutrophils, macrophages, lymphocytes, keratinocytes, fibroblasts, and endothelial cells-these all are involved in the wound healing process. Several elements can cause reduced wound healing by affecting single or multiple phases of the process and are divided into local and systemic factors. One or more factors may play a role in any single or multiple individual phases, leading to the overall healing process.[2,3] Medicinal plants with wound healing activity were shown in Table 1.[4]

The *Commiphora caudata* plant contains various beneficial components, including alkaloids, amino acids, flavonoids, glycosides, proteins, reducing sugars, starch, steroids, tannins, and terpenoids.[5] This plant is commonly used in Ayurveda and Siddha medicine and is known for its liver-protecting, antibacterial, antipyretic, and antioxidant properties. Additionally, a gum and water mixture made from the plant is used as a mouthwash to treat mouth ulcers and promote wound healing and relief from rheumatoid arthritis. When combined with goat milk, the leaves can act as a stimulant for sexual activity. The roots of the plant have astringent, sweet, cooling, aphrodisiac, and diuretic properties and are used in treating diabetes.[6]

Commiphora caudata is a deciduous tree with a short height and papery bark. Its compound leaves have 3-5 leaflets that are ovate or elliptic, glabrous, acute apex, or acuminate. The leaflets have a long petiole and are terminal. The small flowers are held up in a long-peduncle paniculate dichasium with a length of 10 cm. The fruit is a subglobose drupe with a diameter of 1.2 cm.[7]

The ethanolic extract of *Commiphora caudata* leaves has antiarthritic activity, which has been tested in rats induced by Freund's adjuvant arthritis. The *Commiphora caudata* leaves extract of 200 and 400mg/kg were tested for their anti-arthritic activity and proved to have good anti-arthritic activity.[8] Studies show that *Commiphora caudata* has antiarthritic, antispasmodic, cytotoxic, hypothermic, anti-inflammatory, anti-ulcer, and memory-enhancing properties.[9] The plant's oils have antioxidant activity and can be used to store and prepare food products. Fruit oil has more antimicrobial and antioxidant properties, and further research is needed to prove this.[10]

Commiphora caudata methanol extract also shows significant anti-inflammatory activities when compared to the control, with Indomethacin used as an anti-inflammatory drug.[11] *Commiphora*

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caudata at 200 and 400 mg/kg body weight, administered orally, improved learning and memory in rats with scopolamine-induced amnesia, possibly due to its antioxidant properties containing flavonoids, tannins, and polyphenols.[12] The plant's roots have been shown to have antidiabetic activity in a high-fat diet and Streptozotocin-induced model.[13] *Commiphora caudata* has significant anti-inflammatory effects when administered orally, outperforming external application in pharmacological tests.[14] *Commiphora caudata* bark ethanol extracts have also been shown to reduce the formation of peptic ulcers induced by absolute ethanol, demonstrating its anti-ulcer activity.[15] However, despite the traditional and biological evidence supporting the various claims and benefits of this plant, proper evaluation and exploitation are necessary.[16]

Current estimates indicate that approximately 6 million people suffer from chronic wounds Worldwide.[17] Today, the principles of topical wound therapy involve the elimination of necrotic tissue, control of bacterial loads, management of wound exudates, maintenance of open proliferative wound edges, and provision of a moist and protected wound surface.[18] It is estimated that 95% of the medicinal plants used in the Indian herbal industry today are collected from the wild. Although there are around 8,000 medicinal plant species used by different communities in India across different ecosystems, only around 10% of them are in active trade. In the current study, the *Commiphora caudata* plant is chosen based on the ethnomedicinal uses shared by the kuruma tribes of the Wayanadu districts of Kerala, India. They were using leaf juice for treating wounds.[19]

MATERIALS AND METHODS

Collection and Identification of plant material

The leaves of *Commiphora caudata* were collected from Sanjeevaiah Park Necklace Road, Hussain Sagar, Khairatabad, Hyderabad, Telangana, 500003, India in December 2021 and authenticated by Mr. L. Rasingam, Scientist, Botanical Survey of India, Deccan Regional Centre, Hyderabad, Telangana (No. BSI/DRC/2021-22/Tech/Identification/452). The collected leaves were made free from extraneous matter, washed, and air-dried. The dried leaves were powdered and stored in a well-closed container.

Preparation of Plant Extracts

Successive solvent extraction

The method is based on the extraction of active constituents present in the drug using various solvents ranging from non-polar to polar.[20] A 100 gm of dried powder was extracted successively by a Soxhlet apparatus using dissimilar organic solvents like chloroform, ethyl acetate, alcohol, and distilled water. For effective extraction, in-depth extraction was done for about 6-8 cycles for 3-4 hr with each solvent. The solvents used were chloroform, ethyl acetate, alcohol, and finally with distilled water. Extracts of dissimilar organic solvents were received separately into clean and dry beakers. A rotary evaporator was used to recover solvents at 60°C, then dried them in desiccators for 1 hr ultimately the extracts were weighed and the percentage yield was calculated.[21]

Cell viability by MTT Assay

The cell growth rate is measured using a 3-(4,5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) proliferation assay kit according to the manufacturer's instructions (Sigma Aldrich). Keratinocytes were isolated from wister rats' skin tissue (Centre for Cellular and Molecular Biology, Habsiguda, Uppal Road, Hyderabad, Telangana, India,) and cultured in a keratinocyte serum-free medium (KSFM life tech). Briefly, 3000 cells/well keratinocytes are cultured in 96-well plates and incubated at 37°C in 5% CO_2 for 24 hr. The keratinocyte cells were suspended in media prepared with control, hordenine, and rotenone. The liquid content of the wells was aspirated off and 100 µL DMSO was added to dissolve the formazan crystals (attached to the wells) produced by viable cells. The absorbance was read using an ELISA plate reader at 540 nm with a reference wavelength of 720 nm. The cell viability of different extracts of *Commiphora caudata*, mega heal as a positive control, and hordenine on keratinocytes was determined.[22,23] The percentage of cell viability at different extract concentrations was obtained using this formula:

$$\% \text{ of Wound Closure: } \frac{\text{Wound area on day 0} - \text{Wound area on a specific day}}{\text{Wound area on day 0}} \text{ X } 100$$

In vitro Wound Healing Scratch Assay

Cultured and Isolated primary cells from Umbilical cord tissue mesenchymal stem cells from a 60mm tissue culture dish. Subcultured in T25 flasks at Passage 1 to maximize the proliferation of cells. Culture passage 2 Mesenchymal Stem Cells (MSC) in a 12-well culture plate and 2 mL of 20% culture media containing antibiotics (120 ug of Gentamycin sulfate and 2.5 ug of Amphotericin B) added to each well. For MSC cells, recommend plating 100k to 200 k cells in each well of a 12-well plate to get confluence by the next day. Each well of a 12-well culture plate has about 4 cm^2 of the growth area. Once confluence is at 70 to 80%, scrape the cell layer in a straight line using a 1 mL pipette tip.[24,25] Keep the tip perpendicular to the bottom of the well. Scratch another line perpendicular to the first line to create a cross in each well. When making a scratch, the tip needs to maintain contact with the bottom of the well to remove the cell layer, but pressure should not be excessive. After a scratch, gently wash the cell monolayer to remove detached cells using DPBS, then replenish with fresh culture medium. Added hordenine and all the extracts to test wells.

12 Well Culture plate

Culture cells: Cord tissue human mesenchymal stem cells.

Negative Control: α-MEM+MSC Cells.

Positive Control: α-MEM+FBS+MSC Cells.

Test well 1: α-MEM+FBS+MSC Cells+Hordenine 10 µg, 30 µg, 50µg and 100µg.

Test well 2: α-MEM+FBS+MSC Cells+Chloroform extract 10 µg, 30 µg, 50µg and 100µg.

Test well 3: α-MEM+FBS+MSC Cells+Ethyl acetate extract 10 µg, 30 µg.

Test well 4: α-MEM+FBS+MSC Cells+Alcoholic extract 10 µg, 30 µg.

Test well 5: α-MEM+FBS+MSC Cells+Aqueous extract 10 µg, 30 µg.

Image using phase contrast inverted microscope on 4x or 10x magnification at 0 hr. Make a note of where images are taken and take an image at the same spot each time. Place in a CO_2 incubator and image on phase contrast Inverted microscope at 36 hr until cells migrate to meet in the middle. Measure the scratch distance of 0hr and 36hr by using Image J software and calculate the wound closure percentage.[26]

$$\% \text{ of Wound Closure: } \frac{\text{Wound area on day 0 - Wound area on a specific day}}{\text{Wound area on day 0}} \text{ X } 100$$

Estimation of Inflammatory Marker Interleukin 6

The keratinocytes were seeded in 12 well plates, and one well containing 1X104 cells was incubated at 37°C, after 80% confluency 1µg/mL of lipopolysaccharide in each well was added to create inflammation. After 24 hr removed media, washed with Phosphate-buffered saline, and added fresh media, different concentrations of drugs were added and incubated for 24 hr. After incubation, the ELISA procedure was followed.[27,28]

Gene expression studies MMP1 and MMP9

Quantitative RT-PCR

The measurement of mRNA levels for matrix metalloproteinases (MMPs) MMP-1 and MMP-9 is done through real-time polymerase chain reaction (RT-PCR). MMP-1, also known as fibroblast collagenase, is a zinc-dependent enzyme that causes collagen breakdown. MMP-9, on the other hand, is a 92 kDa protein with protease activity that mainly targets extracellular matrix and basement membrane attachment.[29] keratinocyte cell lines were treated with DMSO or the optimized concentration of and its analogs for 24-36 hr. Cell line or tissue total RNA were extracted with RNA Sure mini isolation kit Nucleo-pore, Genetix, cDNA solution with Emerald Amp® GT PCR Master Mix (Takara) instructions and cDNA were prepared using Thermo fisher cDNA synthesis kit with primers. The analysis of gene expression was performed using gene-specific primers (Table 1). The qRT-PCR steps were: 1) Denaturation at 95°C for 3 min, 2) 30 cycles at 95°C for 1 min, 3) 57°C for 30 sec (depending on primer sets), 4) 72°C for 1 min, and 5) Extension at 72°C for 7 min. Melting curve examination verified a single product. Relative expression quantities were evaluated and normalized by comparing them to action.[30,31]

Ointment formulation

A simple ointment of the 95% extracts was prepared following the formula described in the British Pharmacopoeia.[32] Ointment preparations with (5% w/w) and without (simple ointment only and served as a control) the extract were formulated using the reduced formula from the master formula. All ingredients of the ointment base were weighed, mixed, and heated gently with continuous stirring until a homogenous base formed and then cooled. For preparing medicated ointment, 5 g, 10g, and 15 g of chloroform extract was mixed with 95 g of the ointment base by levigation on the surface of the ointment lab to make an ointment of 5%, 10%, and 15% of uniform consistency and smooth texture.[33] In preparing the control ointment, 100 g of the base was taken and treated in the same manner to formulate an ointment without an active ingredient.[34] The formula is expressed in Table 2.

Experimental animals

Wister rats weighing between 150-200 g, and 6 animals in each group were used. Experimentation was done at Jeeva Life Sciences, Shanthi Nagar, Uppal, Medchal-Malkajgiri District, Telangana. They were maintained at standard environmental conditions of temperature, and dark/light cycles and had free access to feed (nutrient animal feed) and water *ad libitum* during the quarantine period. The animal was fasted 12 hr before experimentation but had been allowed free access to water. The study protocol was approved by the Ethics Committee IAEC approval no. CPCSEA/IAEC/JLS/17/03/22/013. Animal handling and care were carried out throughout the experiment following international laboratory animal use and care guidelines.[35]

Acute toxicity studies

An acute oral toxicity study was performed as per Organization for Economic Cooperation and Development [OECD] guidelines 423, ointment of *Commiphora caudata* extract ointments applied to Wistar rats. Animals were observed individually during the first 30 min and periodically during the first 24 hr, with special attention given during the first 4 hr and daily thereafter, for a total of 14 days. The rats were found to be safe and no toxicity was observed. There were no toxic effects of mortality observed up to 12 days.[36]

Experiment Models

Wound healing activity was evaluated by:

1. Excision wound model

Table 1: Medicinal Plants having wound healing activity.[4]

Sl. No.	Plant name	Family	Useful part
	Adhatoda vasica Linn.	Acanthaceae	Leaves, Stem
	Hippophaerhamnoides L	Elaeagnaceae	Leaves, Fruit
	Aloe Vera	Liliaceae	Leaves
	Hibiscus Rosa sinensis	Malvaceae	Leaves, Root
	Tribulus terrestris linn.	Zygophyllaceae	Leaves
	Gymnema sylvestre	Asclepiadaceae	Leaves
	Lawsonia inermis Linn	Lythraceae	Leaves
	Euphorbia Hirtal	Euphorbiaceae	Leaves
	Adhatoda zeylanica M.	Acanthaceae	Leaves
	Agrimonia Pilosa ledeb	Rosaceae	Whole Plant
	Alstonia scholaris R. Br	Apocynaceae	Latex
	Anacardium occidentale L	Anacardiaceae	Fruit
	Areca Catechu L.	Arecaceae	Fruit
	Argemone mexicana L	Papaveraceae	Latex
	Aristida setacea Retz.	Poaceae	Leaves
	Barleria prionitis L.	Acanthaceae	Leaves
	Begonia Fallox DC	Begoniaceae	Stem
	Betula alnoides B.H.	Betulaceae	Bark
	Brassica Juncea L.	Brassicaceae	Fruit
	Buxus wallichiana	Buxaceae	Bark
	Calendula Officinalis L.	Asteraceae	Flower
	Callicarpa arborea roxb.	Verbenaceae	Bark
	Calotropis gigantea L.	Asclepiadaceae	Stem
	Calotropis procera Br	Asclepidaceae	Leaves
	Cassia Alata L.	Caesalpinae	Leaves
	Cassia Auriculata L.	Caesalpinae	Leaves, Bark
	Chasalia curviflora Wall.	Rubiaceae	Root
	Chenopodium Album Linn.	Chenopodiaceae	Leaves
	Combretum flagrocarpum	Combretaceae	Leaves
	Commelina benghalensis	Commelinaceae	Stem
	Eupatorium Odoratum L.	Asteraceae	Leaves
	Euphorbia Antiquorum L	Euphorbiaceae	Stem
	Ficus bengalensis L.,	Moraceae	Leaves
	Solanum xanthocarpum linn.	Solanaceae	Fruit
	Murraya paniculate linn.	Rutaceae	Leaves

2. Diabetic wound model

Excision wound model

Wistar rats weighing between 150-200g were selected. They were divided into groups. Each group contains 6 animals.

Group-I-Control.

Group II-Ointment base.

Group-III-Standard (Mega heal ointment).

Group-IV-5%w/w chloroform extract ointment.

Group-V-5% w/w ethyl acetate extract ointment.

Group-VI-5%w/w alcoholic extract ointment.

Group-VII- Enriched diet.

Table 2: Ointment Formula.[33]

Ingredients	Master Formula	Reduced formula
Wool fat	50 g	10 g
Hard paraffin	50 g	10 g
White soft paraffin	850 g	170 g
Cetostearyl alcohol	50 g	10 g
	1000 g	200 g

Ointment preparations with (5% w/w) and without (simple ointment only and served as a control) the extract were formulated using the reduced formula from the master formula.

The method of Mortan and Malone was adopted. The hair on the skin of the back surface of animals was removed by using a depilatory agent (veet hair remover). Circular wounds of 10mm diameter were inflicted on the cleared skin by cutting under mild ether anesthesia. The areas of the wounds were measured (sq mm) immediately by placing a transparent polythene graph paper over the wound and then tracing the area of the wound on it. This was taken as the initial wound area reading. The wound was left undressed in an open environment. The ointments were applied topically with a fine brush twice a day, starting from the day of operation till the complete epithelisation occurs. The parameters studied were the percentage closure of the excision wound and the time of epithelisation. The wounds were traced on sq mm graph paper on 0, 2nd, 4th, 6th, 8th, 10th, and 12th days until healing was completed. The period of epithelisation was calculated as the number of days required for falling of scar without any residual raw wound. The degree of wound healing was calculated as percentage closure in the wound area from the original wound area using the formula:[37]

$$\% \text{ of Wound Closure: } \frac{\text{Wound area on day 0} - \text{Wound area on a specific day}}{\text{Wound area on day 0}} \times 100$$

The means, S.E.M values were calculated.

Diabetic wound model

Streptozotocin (STZ) was used to induce diabetes in rats. The Type 1 diabetic model was induced by a single, relatively high dose (60 mg/kg) of STZ (dissolved in 0.1 M citrate buffer, pH 4) intraperitoneal injection with normal chow (12% calories as fat). Before STZ injection, the rats were fasted overnight but were given access to water to prevent dehydration. STZ was given the following morning with a recovery diet. For the Type 2 diabetic model, male SD rats were fed a high-fat diet (60% calories as fat, 58Y1, Test Diet) for 2 weeks. A single intraperitoneal injection of 30 mg/kg STZ was given with a recovery high-fat diet during the experiment. Blood glucose and insulin were measured to confirm whether the Type 1 and Type 2 diabetic models were successfully established. A week after the STZ injection, the blood glucose levels of Type 1 and Type 2 diabetic rats were measured using a glucometer, we made a small spaceman on the rat tail using a surgical blade and absorbed the blood with blood glucose test strips to monitor glucose levels. When the glucose level was higher than 300 mg/dL, the diabetic model was confirmed to be established. Type 1 and Type 2 diabetes can be easily distinguished by blood glucose levels. During the experiments, the wounds were traced on sq mm graph paper on 0, 2nd, 4th, 6th, 8th, 10th, and 12th days until healing was completed.[38]

Biochemical estimation

The granulation tissue formed on day 8 of wound creation was used for further biochemical studies. About 50 mg of tissue was washed in physiological saline and cut into pieces, defatted with chloroform: methanol (2:1), and lyophilized with 5 mL of 6N Hydrochloric acid for 20 hr in sealed tubes. After hydrolysis, the sample was evaporated to dryness to get the residue. The residue obtained was dissolved in water and made up to 3 mL which was used for the estimation of hydroxyproline and total protein.

Determination of hydroxyproline

Reagents

0.01 M copper sulfate solution, 2.5 N sodium hydroxide, 6% hydrogen peroxide, 3 N sulphuric acid, 5% p-dimethylamino benzaldehyde in n-propanol. Procedure: For the assay of the unknown solution, nine dry test tubes were used. Standard hydroxyproline 5,10,15 and 20 µg/mL and distilled water(blank) and unknown solution are taken in different test tubes (in duplicate) and added 1 mL of 0.001 M copper sulfate solution, 1 mL of 2.5 N sodium hydroxide and 1 mL of 6% hydrogen peroxide were added successively. The solutions were mixed and shaken occasionally for 5 min and then placed in a water bath at 80°C for 5 min with frequent vigorous shaking. The heating and shaking destroy the excess peroxide. Traces of peroxide that remain will decrease the color formation and produce an Orange-red blue. The tubes were chilled in an ice and water bath and 4 mL of 3 N sulphuric acid was added with agitation, then 2 mL of 5% p-dimethylamino benzaldehyde solution was added with thorough mixing. The tubes were placed in a water bath at 70°C for 16 min and then cooled in tap water. The contents were transferred to selected absorption tubes and light transmission was read at 540 nm with an Elico double-beam spectrophotometer. The amount of hydroxyproline (measured in micrograms) in 1 mL of the unknown solution was established

by finding the point corresponding to its optical density on the standard curve prepared at the same site.

Estimation of Total protein

Godkar *et al.*, 1994 modified biuret endpoint assay method was used for the estimation of total proteins and analyzed in an auto-analyzer (STAR 21 PLUS, manufactured by Aspen Diagnostics Pvt. Ltd.,). The peptide bonds of proteins react with cupric ions in an alkaline solution to form a colored chelates, the absorbance of which was measured at 578nm. The biuret reagent contains sodium potassium tartrate, which helps in maintaining the solubility of this complex at alkaline pH. The absorbance of the final color is proportional to the concentration of total protein in the sample. All the above solutions were mixed well and incubated at 37°C for 5 min and analyzed in the auto analyzer.[39,40]

$$\text{Total protein concentration (g/dl)} = \text{Absorbance of test} \times 6.5$$

$$\text{The standard concentration of protein} = 6.5$$

Histopathological studies

The wound tissue collected was subjected to detailed histopathological processing. Tissue was fixed in formalin, trimmed, and processed, i.e., dehydrated in graded alcohol, cleared in xylene, and embedded at 58°C-60°C in paraffin. These tissue blocks were cut at 4-5 µ in thickness stained with hematoxylin and eosin and finally mounted with digital picture exchange. Slides were then studied under a light microscope and photographs were taken.[41]

RESULTS

Evaluation of cell viability by MTT Assay

We have carried out *in vitro* studies for the assessment of viable cells, on keratinocytes (5, 10, 20, 30 µg/mL) after 24 hr of treatment with control, mega heal, rotenone, different extracts of *Commiphora caudata*, and hordenine, cell viability is examined at the 24 hr post-exposure time point. Each time point should be taken in the triplicate wells and each experiment is repeated twice. The percentage of cell viability is high about 80.42% with the chloroform extract when compared to others shown in Table 3 and Figure 1.

In vitro Wound Healing Scratch Assay

The percentage wound closure results for *Commiphora caudata* extracts and hordenine at 10 µg/mL, 30 µg/mL, 50 µg/mL, and 100 µg/mL, compared to untreated control, at 0 hr, 26 and 36 hr and microscopic images depicting the wound gap closure results after 0, 26 and 36 hr of treatment with *Commiphora caudata* extracts and hordenine are depicted. It is evident that the application of *Commiphora caudata* extracts ointment on human mesenchymal stem cells with scratched wound gaps resulted in notable improvement in wound gap closure in comparison to the untreated control. The wound closure results indicated the effectiveness of *Commiphora caudata* extracts ointment concerning wound healing, exhibiting 2-fold to 2.5-fold higher wound closure in comparison to the untreated control shown in Tables 4-6.

Estimation of Inflammatory Markers IL-6(Interleukin 6) of Hordenine and Chloroform extract

The cells were induced with lipopolysaccharide incubated for 24 hr and treated with chloroform extract, hordenine, and rotenone. The chloroform extract of *Commiphora caudata* on inflammation was significantly reduced when compared to the compound hordenine and rotenone. Cells treated with chloroform extract showed decreased IL-6 secretion expressed in Table 7, Figure 2.

Gene expression studies MMP1 and MMP9

Quantitative RT-PCR

Real-Time Polymerase Chain Reaction (RT-PCR) was used to measure mRNA amounts of Matrix Metalloproteinases (MMPs): MMP-1, and MMP-9, genes in keratinocytes. When studying gene expression using real-time Polymerase Chain Reaction (PCR), researchers typically examine changes in the expression of specific genes by measuring their transcript abundance. It is recommended to design amplicons that are within the range of 200 to 1000 base pairs. We observed a remarkable (about 15-fold) increase in the expression level of matrix metalloproteinase MMP-1 and MMP-9 genes. The results of our studies of MMP gene expression in cultured primary human keratinocytes treated with chloroform extract of *Commiphora caudata* have shown upregulation of MMP gene expression in cultured keratinocytes. Therefore, upregulation of MMP genes in the skin could result from chloroform extract of *Commiphora caudata* on skin cells shown in Table 8.

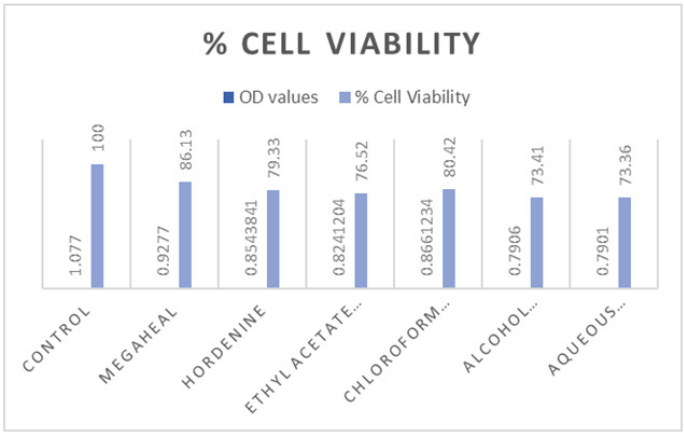

Figure 1: Percentage viability of keratinocytes after 24 hr incubation supplemented with mega heal, rotenone, hordenine, ethyl acetate extract, chloroform extract, aqueous extract, and alcoholic extract using the MTT assay.

Wwound healing activity

Wound healing activity was done by excision and diabetic model with *Commiphora caudata* extract.

Excision and diabetic wound models

The 5%, 10%, and 15% *Commiphora caudata* extract ointment were tested for wound healing activity. Quantitative measurements of wound size are routinely used to assess initial wound size before and after debridement, as well as progress toward wound closure. The wound contraction rate was measured as the percentage reduction in wound size on days 2, 4, 6, 8, and 12. Significant progress in the percentage of wound contraction was observed in the treated excision wounds compared with the untreated control. On days 6, 8, 10, and 12, the 15% chloroform extract treated significantly increased the percentage of wound contraction compared to 5 and 10%. Results were comparable to that of the standard. The percentage of wound contraction for chloroform extract, hordenine, and the standard is 95.1%, 93%, and 95% respectively. Results are shown in Table 9.

Biochemical estimation

Estimation of hydroxyproline content in granulation tissue

Hydroxyproline is one of the biochemical parameters implicated in the wound-healing process. Hydroxyproline stimulates collagen formation. Therefore, the estimation of hydroxyproline is yet another evidence for the wound-healing effect of the

Table 3: Percentage of cell viability.[22]

Compound	OD values	% Cell Viability
Control	1.077	100
Megaheal	0.9277	86.13
Hordenine	0.8543841	79.33
Ethyl acetate Extract	0.8241204	76.52
Chloroform Extract	0.8661234	80.42
Alcohol Extract	0.7906	73.41
Aqueous Extract	0.7901	73.36

Table 4: Percentage wound closure results after exposure to *Commiphora caudata* extracts and hordenine ointment at 10 µg/mL at 0 hr and 26 hr treatment periods.[26]

Scratch assay-Length (mm)			
Sample	**0 hr**	**26 hr**	**% Wound Closure**
NC	0.903	0.593	34.35
Megaheal	1.06	0.33	68.87
Aqueous extract (10 µg)	1.088	0.933	14.25
Chloroform extract (10 µg)	1.048	0.875	16.47
Ethanolic extract (10 µg)	0.87	0.853	2.01
Ethyl acetate extract (10 µg)	0.9	0.755	16.11
Hordenine extract (10 µg)	0.933	0.903	3.22

Table 5: Percentage wound closure results after exposure to *Commiphora caudata* extracts and hordenine ointment at 30 µg/mL at 0 hr and 26 hr treatment periods.[26]

Scratch assay-Length (mm)			
Sample	**0 hr**	**26 hr**	**% Wound Closure**
NC	0.903	0.593	34.35
Megaheal	1.06	0.33	68.87
Aqueous extract (30 µg)	0.985	0.978	0.76
Chloroform extract (30 µg)	1.033	0.795	23.00
Ethanolic extract (30 µg)	0.983	0.945	3.82
Ethyl acetate extract (30 µg)	0.83	0.73	12.05
Hordenine extract (30 µg)	0.94	0.828	11.97

samples. Hydroxyproline is estimated in the granulation tissue of the wound as per the literature-reported procedure.

Hydroxyproline was higher in animals treated with chloroform extract, hordenine, and mega heal to alcohol, aqueous, and ethyl acetate extracts of *Commiphora caudata*. Results are shown in Table 10, Figure 3.

A representative calibration graph of absorbance versus concentration resulted in the regression equation. (Y=mx).

$$Y=0.006x, R^2=0.998$$

Where Y=Absorbance, m=slope=0.006, x=Concentration.

The calibration curve was found to be linear from 5-15 µg/mL with a coefficient of 0.998.

Estimation of Total protein

The total protein is another biochemical parameter implicated in the wound-healing process. There is an increase in protein content, which was predominantly due to enhanced collagen synthesis. Total protein content was higher in animals treated with chloroform, and hordenine compared to ethyl acetate, alcohol, and aqueous extracts of *Commiphora caudata* shown in Table 11.

Histopathological Studies of *Commiphora caudata* extracts and Hordenine

Control: Day 7: Normal control: Severe abscesses were observed epidermal layer of skin along with infiltration of inflammatory cells [Neutrophils and lymphocytes]-red arrow. Day 14: Severe dermal inflammation with infiltration of inflammatory cells [lymphocytes and neutrophils] was observed-yellow arrow.

Table 6: Percentage wound closure results after exposure to *Commiphora caudata* extracts and hordenine ointment at 50 µg/mL and 100 µg/mL at 0 hr and 36 hr treatment periods.[26]

Scratch assay-Length (mm)			
Sample	**0 hr**	**36 hr**	**% Wound Closure**
NC	1.095	1.003	8.45%
Mega heal	1.025	0.563	45.12%
Chloroform extract (50 µg)	1.003	0.573	42.89%
Chloroform extract (100 µg)	0.908	0.475	47.66%
Hordenine extract (50 µg)	1.013	0.713	29.63%
Hordenine extract (100 µg)	0.830	0.595	28.31%

Table 7: Inflammatory response Chloroform extract of *Commiphora caudata* and hordenine.[27]

	STD	LPS	Hordenine	Mega heal	Chloroform extract
2000 (pg/mL)	1.9551	1.3	0.3024	0.7654	0.1689
1000 (pg/mL)	1.904	1.5083	0.3975	0.8956	0.1943
500 (pg/mL)	1.4	1.3447	0.3497	0.7656	0.3023
250 (pg/mL)	1.2	1.0562	0.2241	0.5577	0.1399
125 (pg/mL)	1	1	0.3	0.7	0.1

Table 8: List of primers employed for assessing the gene expression of the proposed genes matrix metalloproteinases, MMP1, MMP9, IL 6, and GAPDH) using real-time PCR.[30]

Gene	Sequence direction 5′-3′	Product size
MMP-2 sense Antisense	GTGCTGAAGGACACACTAAAGA CCTACAACTTTGAGAAGGATGGCAA	201 bp
IL-6 sense Antisense	GAACTCCTTCTCCACAAG CTGAAGAGGTGAGTGGCTG	178 bp
GAPDH sense Antisense	GGCTCTCCAGAACATCATCCCTGC GGGTGTCGCTGTTGAAGTCAGAGG	215 bp
MMP-9 sense Antisense	GTCTTCCAGTACCGAGAGAAAG GTTTGTATCCGGCAAACTGG	191 bp

Standard: Day 7: Mild dermal inflammation. Day 14: Complete re-epithelization of the epidermal layer [Red arrow] of skin, but the lack of hair follicles and sebaceous glands with dermal fibrosis was observed in the dermal layer [green arrow].

Chloroform extract: Day 7: Mild dermal inflammation [red arrow] and [Acanthosis-green arrow] along with vacuolization of the basal layer of epithelial cells was observed in the epidermal layer of skin. Day 14: Normal morphology of epidermal layer of skin-red arrow Normal morphology of dermal region with hair follicles and sebaceous glands-green arrow

Hordenine: Day 7: mild abscesses were observed epidermal layer of skin. Day 14: Complete re-epithelization of the epidermal layer with vacuolization of epithelial cells [Red arrow] of skin, but the lack of hair follicles and sebaceous glands was observed in the dermal layer [green arrow]

DISCUSSION

Wound healing is a step-by-step process, which constitutes phases like hemostasis, inflammation, proliferative, and remodeling or maturation. The genetic response regulating the body's cellular resistance mechanisms contributes to the wound and its repair. Skin wounds and imperiled wound healing were the majority concerns for the public health sector. Complicated and long treatments cause increased healthcare expenses. Even in uncomplicated cases, burns, chronic and other difficult-to-treat wounds require surgery and extended hospitalization periods.[42] Therefore, this study worked on two different models i.e excision wound model and the diabetic model to establish the wound healing potential of chloroform, extract of *Commiphora caudata*, and hordenine.[43]

Cell viability is examined at the 24 hr post-exposure time point. Each time point should be taken in the triplicate wells and each experiment is repeated twice. The percentage of cell viability is

Table 9: Effect of *Commiphora caudata* extracts and hordenine on the closure of wound area on post-wounding days.[37,38]

Post Wounding days	Wound area -mm²±S.E.M (% closure of wound area)						
	Group-1	Group-2	Group-3	Group-4	Group-5	Group-6	Group-7
	Control	Ointment base	Standard mega heal	Chloroform extract 15%w/w ointment	Chloroform extract 5%w/w ointment	Diabetic	Chloroform extract 10%w/w ointment
Day 0	78.50±2.6 (0%)	79.65±3.2 (0%)	78.95±2.4 (0%)	78.35±2.9 (0%)	79.85±3.1 (0%)	80.05±3.4 (0%)	80.35±2.7 (0%)
Day 2	74.25±2.9 (5.4%)	71.66±3.6 (10%)	69.37±2.8 (12.1%)	67.90±3.4 (13.3%)	72.36±3.1 (9.38%)	73.87±3.4 (7.72%)	68.39±2.9 (14.8%)
Day 4	68.32±3.2 (12.9%)	65.25±2.7 (18%)	52.39±3.5 (33.6%)	49.15±3.6 (37.2%)	61.27±2.8 (23.2%)	63.86±2.5 (19.82%)	51.12±2.4 (36.37%)
Day 6	65.56±2.7 (16.48%)	63.86±2.5 (19.82%)	35.79±3.1 (54.6%)	34.54±2.2 (55.9%)	48.19±2.8 (39.6%)	55.40±3.4 (30.7%)	34.90±3.5 (56.5%)
Day 8	62.47±3.1 (20.42%)	59.37±2.2 (25.4%)	24.53±2.98 (68.9%)	23.90±2.4 (69.4%)	36.19±3.4 (54.6%)	45.20±3.2 (43.1%)	24.51±2.2 (69.4%)
Day 10	59.54±2.3 (24.15%)	56.49±3.1 (29%)	15.13±2.4 (80.8%)	15.07±2.8 (80.7%)	26.73±3.2 (66.5%)	24.41±2.2 (69.3%)	16.25±2.4 (79.7%)
Day 12	56.84±2.56 (27.5%)	54.37±2.9 (31.7%)	3.31±2.6 (95%)	3.78±3.4 (95.1%)	18.35±2.8 (77%)	16.20±2.3 (79.3%)	5.03±2.2 (93%)

Table 10: Hydroxyproline content in the granulation tissue.[39]

Group	Hydroxyproline in µg/50mg tissue
Control	±3.38 µg/mL
Ointment base	±3.70 µg/mL
Standard (Mega heal)	±13.38 µg/mL
Chloroform extract (5%)	±12.74 µg/mL
Diabetic	±9.64 µg/mL
Hordenine	±8.38 µg/mL
Chloroform extract (10%)	±13.06 µg/mL

Table 11: Total protein content in granulation tissue.[40]

Group	Total protein concentration (g/L)
Control	±16.50
Ointment base	±17.20
Standard (Mega heal)	±48.18
Chloroform extract (5%)	±47.20
Diabetic	±36.38
Hordenine	±30.48
Chloroform extract (10%)	±47.69

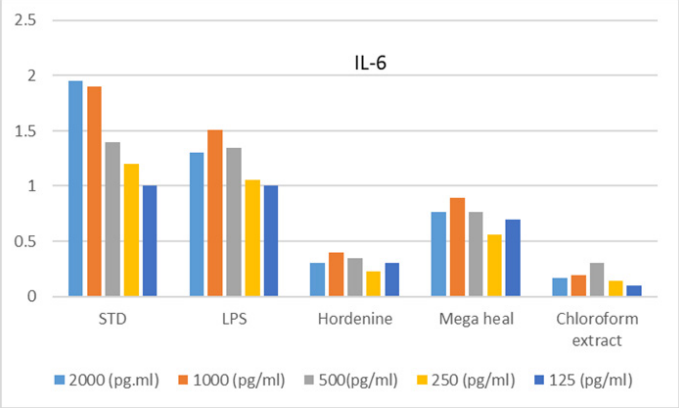

Figure 2: Inflammatory response Chloroform extract of *Commiphora caudata* and hordenine.

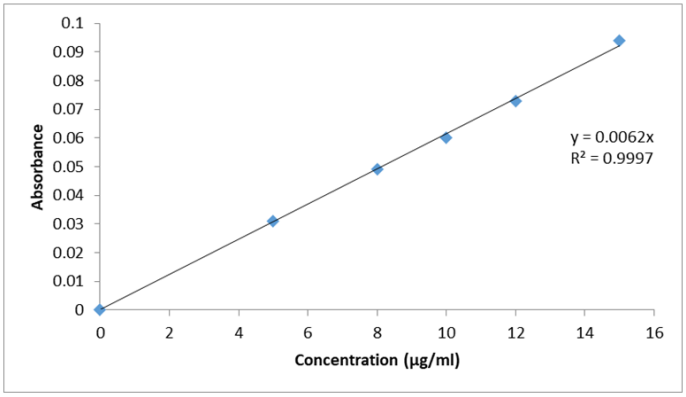

Figure 3: Standard calibration curve of Hydroxyproline content.

high about 80.42% with the chloroform extract when compared to others. Cell viability is crucial for successful wound healing, as immune cells, fibroblasts, endothelial cells, and keratinocytes must function properly and survive.

It is evident that the application of *Commiphora caudata* extracts ointment on human mesenchymal stem cells with scratched wound gaps resulted in notable improvement in wound gap closure in comparison to the untreated control. Hence this study helps to do further research on animals to prove that it has wound healing properties.

In the excision wound healing model, the chloroform extract of *Commiphora caudata* and hordenine showed a significant increase in percentage wound closure by enhanced epithelialization. This enhanced epithelialization may be due to the effect of *Commiphora caudata* extracts on enhanced collagen synthesis.[44]

In the diabetic wound model, the wounds were treated perhaps due to the increase in collagen concentration and fibres stabilization. A healing tissue collagen synthesizes, it is a main constituent of the growing cell. The increase of blood vessels and antioxidants role were proved experimentally. Thus, it proves the wound-healing activity of *Commiphora caudata*.

From the observations, it was evident that *Commiphora caudata* possesses a definite potential healing action. The excision wound model and diabetic wound model showed increased wound closure in chloroform extract and hordenine groups. The wound-healing activity of extracts is further evidenced by the estimation of hydroxyproline and total protein content which were found to be at higher levels in chloroform extract and hordenine-treated animals compared to control.[45]

From histopathological observations, with chloroform extract, it was observed that normal morphology of the epidermal layer of skin, normal morphology of dermal region with hair follicles and sebaceous glands, with hordenine complete re-epithelization of the epidermal layer with vacuolization of epithelial cells of the skin, but the lack of hair follicles and sebaceous glands were observed in the dermal layer.

CONCLUSION

Extracts from *Commiphora caudata* have been found to contain phytochemicals like flavonoids, alkaloids, saponins, steroids, carbohydrates, proteins, and amino acids that aid in the process of wound healing. Cell viability is a crucial factor in promoting wound healing, and *Commiphora caudata* has been shown to maintain it efficiently. Through *in vitro* studies using scratch assays on various extracts, we found that chloroform extract was the most efficient in promoting wound healing, leading us to exclude all other extracts when working on animals. *In vivo* investigations on different concentrations of chloroform extracts using an excision wound healing model and a diabetic wound model revealed that

the extract possesses good wound healing properties, likely due to the presence of phytoconstituents like flavonoids, alkaloids, saponins, steroids, carbohydrates, proteins, and amino acids. Our work was guided by the traditional use of *Commiphora caudata* for treating mouth ulcers and promoting wound healing. Based on our findings, we believe that *Commiphora caudata* has good wound-healing properties and has the potential for developing new products like antiseptic solutions, silver-based products, hydrocolloid dressings, foam dressings, gauze or non-adherent dressings, biological dressings. Further research is necessary to formulate these products for the market.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

IL-6: Interleukin 6; **ELISA test:** Enzyme-linked immunosorbent assay; **MTT assay:** (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay; **MEM:** Minimum Essential Medium; **FBS:** Foetal bovine serum; **MSC:** Mesenchymal stem cells; **MMP1**: Matrix metallopeptidase 1; **MMP9:** Matrix metallopeptidase 9; **RT-PCR:** Reverse transcription-polymerase chain reaction; **DMSO:** Dimethyl sulfoxide; **cDNA:** Complementary DNA; **OD values:** Optical density Values; **LPS:** Lipopolysaccharide; **HCL:** Hydrochloric acid.

SUMMARY

The process of skin wound healing has been studied for many years, but the molecular mechanisms involved are not yet fully understood. This is a growing concern, especially due to the increasing prevalence of obesity, and diabetes, and individuals working in coal mining, fire services, and those with skin allergies. However, our *in vitro* and *in vivo* study on the wound-healing potential of the plant *Commiphora caudata*, which contains multiple phytoconstituents, has shown promising results. Our experiments using the scratch assay, excision wound model, and diabetic wound model all demonstrated the plant's ability to effectively heal wounds.

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