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Research Article

Formulation and of *Commiphora caudata* (Wight and Arn.) Engl.:- Herbal Wound Healing Ointment

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ABSTRACT

Aim: The present study explores compatibility studies, analysis, physiochemical parameters of formulated ointment with herbal wound healing ointment activity. **Materials and Methods:** *Commiphora caudata* bark ethanolic extract used to prepare ointment after studies different solvents phytoconstituents potential. Ethanolic extract of the bark of the *Commiphora caudata* was studies for its *in vitro* wound healing activity through scratch wound assay performed on 3T3-CCL92 cells. The rate of healing was examined at regular intervals and determined using ImageJ software. **Results:** The concentrations (50-1000 µg/mL) with the fibroblast cells (3T3-CCL92) caused a large spike in the proportion of wound healing activity levels ranging from 30.75%-59.15%. The percentage inhibitory concentrations, CTC_{50} values, for the wound healing concentration in fibroblast cells (3T3-CCL92) were calculated from the dose-response curve produced linear. The microscopic image represents increase the concentration of the plant extract result increase the wound healing activity linearly and also number of viable cells also increases. **Conclusion:** *Commiphora caudata* ethanolic extract shows the presence of majority of phytoconstituents as well the microscopic image represents increase the concentration of the plant extract result increase the wound healing activity linearly and also number of viable cells also increase.

Keywords: *Commiphora caudata* (Wight and Arn.) Engl., Herbal Wound healing ointment, Cell Line - 3T3 - CCL92, *In vitro* Wound healing activity.

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INTRODUCTION

Nature is always a golden sign to show the prominent phenomena of coexistence. Natural products from plants, animals and minerals are the basis for treating human diseases.[1] In fact, the use of medicinal plants for the treatment of diseases dates back to the history of human life, that is, since human beings have sought a tool in their environment to recover from a disease, the use of plants was their only choice of treatment.[2] The term medicinal plant refers to a variety of plants that have medicinal properties. These plants are a rich source of compounds that can be used to develop drug synthesis.[3] Medicinal herbs are mainly collected from the wildlife population. Indeed, the demand for wildlife sources has increased by 8%-15% per year in Europe, North America and Asia in recent decades.[4] *Commiphora caudate* belongs to the family Burseraceae is a deciduous tree growing from 12 to 20 m tall. The bole can be 15-25 cm in diameter. The tree is sometimes harvested from the wild for local medicinal use. It is occasionally used as an avenue tree and is often planted as an ornamental. The endosperm obtained from four or five fresh or dried seeds is taken two times a day for 2-3 days to relieve stomachache.[5] The heartwood is gray with darker streaks; the sapwood is white. In addition, these major compounds extracted from seeds of *Commiphora caudata* have important pharmacological effects, thereby aiding in the understanding of the physiology of organisms and in the treatment of various pathologies.[6] *Commiphora caudata* is has hepatoprotective, febrifuge, antibacterial and antioxidantanti inflammatory activity.[7] Therapeutic potential and medicinal use of *Commiphora caudate* in traditional system of medicine dates back to 3000 years ago.

MATERIALS AND METHODS

Chemical and reagents

Petroleum Ether, Chloroform, Acetone, Ethanol and Distilled water.

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Collection and authentication of plant material

For the present study, clean and healthy bark part of *Commiphora caudata* collected from Naigao, Taluka-patoda, District-beed, Maharashtra, India. The identification and authentication of specimen were done by Dilip Shirodkar, Botanist, Botanical Survey of India, Western Regional Centre, 7, Koregaon Park, Pune-411001.

Cell lines

The cell lines were purchased from MTCC (Microbial type culture collection) Cell lines.

Methods

Identification of Phytochemical Active Constituents

The following preliminary phytochemical analyses were performed on the obtained extracts (Petroleum ether, Chloroform, Acetone, Ethanol, and Aqueous).[8-10] The extracts therapeutic potential and pharmacological impact are due to the presence of certain phytoconstituents. To this end, it is crucial to conduct a chemical analysis of the extracts upon which the pharmacological activity tests were conducted. Each extract was then put through a battery of tests designed to help identify any potential phytochemicals it contained. The findings from both the ethanol and aqueous extracts confirmed the existence of the same types of components in the aforementioned samples. Therefore, the polarity-neutral ethanolic extract was chosen for additional pharmacological testing.

Fibroblast Cell Line for Assessing Healing Time

The capacity of murine embryonic fibroblast cells (3T3-CCL92) to heal wounds and proliferate was investigated by selecting medicinal plant extracts with viability results at or above the control.[11] To learn more about the limiting phase of an injury, a scratch wound closure measure was carried out. Using Image J, we determined the proportion of the wound that had healed and the rate of healing acceleration in comparison to the control group at various doses and times. After harvesting the cells with 0.25% trypsin-EDTA and centrifuging them at 1,500 revolutions per minute for 5 min, the supernatant was discarded, and new media was added to make a suspension containing 2 x 10^5 cells per milliliter. The 24-well plates were then seeded with 500 µL of cell suspension and incubated for 24 hr. In order to facilitate the cells ability to form a monolayer. Using a sterile 200 µL plastic pipette tip, a linear wound was made on the monolayer with a regulated width of 550±50 µM. Phosphate-Buffered Saline (PBS) was used to remove the debris from the cells. Photographs of wound regions were obtained at 0 and 24 hr after adding the medicinal plant extract to basic medium (DMEM with 0.5% fetal bovine serum and 1% penicillin-streptomycin). 0.2% DMEM medium. The positive control, asiaticoside, was compared to the DMSO group. To determine the rate of acceleration, photographs were taken every 3 hr for up to 24 hr using an extract concentration shown to promote the most rapid wound healing. To determine if the plant extracts had the potential to promote wound closure, we determined the percentage of recovery area in each image and compared it to the control group.

Compatibility Studies

Physical compatibility studies

Petri dishes with the plant extract and excipient inside of them were placed in a stability chamber set to 45°C and 75% relative humidity for a short period of time.[12] After a period of storage, the samples are analyzed for any outward signs of change, such as color shifts, odor development, and so on.

Chemical compatibility studies

These tests of compatibility were captured in the wave range of 4000 to 400 cm^{-1} using an ATR-FTIR spectrophotometer. The mortar was used to thoroughly combine the natural oils and excipients. The spectrum was captured when the sample was taken from the mortar and put in the sample container.

Procedure for preparation of herbal ointment

Before the ointment base could be made, grated hard paraffin had to be precisely measured and then evaporated in a dish set over a water bath. And then melt it in a water bath at 70°C. The dish that was evaporating was taken out of the water bath. The ointment base was melted, then cetosteryl alcohol, wool fat, and white soft paraffin were added in decreasing order, and the mixture was agitated gently to provide an even melting and mixing. Herbal ointment was made by adding *Commiphora caudata* extract, which was accurately measured, to the ointment base using the levigation method to create a smooth paste with 2 or 3 times its weight of the base, then adding more base until a homogenous ointment was formed and finally transferring it to a suitable container. The formulation table was mentioned in Table 1.

The analysis of the Formulated Ointment

Appearance

Visual inspection was used to examine actual limits, such as volume and odor.[13] The color, clarity, and consistency of the manufactured herbal ointment were all assessed visually. By rubbing the formulation between the fingertips, we were able to assess its smoothness, clumpiness, roughness, and homogeneity.

Consistency

We rubbed the cream between our fingers and evaluated its smoothness, clumpiness, roughness, and homogeneity to determine its consistency.

pH

A digital pH meter was used to calculate the approximate pH of the curated natural therapy.[14] The salve was made using 100 cc of distilled water and stored for 2 hr. We measured the solution's pH three times to get an accurate average.

Viscosity

Ointment viscosity was measured using a Brookfield viscometer (s-62, model LVDV-E) at 25°C and 100 rpm for the spindle no.1 speed.[15]

The herbal ointment's viscosity was measured using a Brookfield viscometer. The first spindle on the Brookfield viscometer was set to 100 revolutions per minute. After 2 min, the sample was in a stable state, and impressions were obtained. The experiment was performed three times, and the average was recorded.

Spreadability

The glass slides were measured twice to ensure consistency.[16] One of the slides had the herbal ointment composition applied to it. When the second slide was placed on top of the gel, there was enough space between them to measure 7.5 cm along each side. 100 g of gel were spread on the top slides, and the gel in the space between the slides was pushed down evenly to produce a thin layer. The gel that had adhered to the slides was scraped off, and the weight was taken away. The two slides in place were secured to a platform such that only the top slides could fall off easily due to the weight tied on them, and this was done without causing the least bit of disruption. Carefully attached to the top was a 20 g weight. Weight was applied to a slider system, and the time it took for the top slide to travel the full 7.5 cm and disengage from the bottom slider was recorded. The study was gone through many times, and in the meanwhile, an estimate was made.

Spreadability was calculated by using the following formula:

$$S=M\times 1/T$$

Where,

S- Spreadability,

M- Weight tied to upper slides,

L- Length of the glass slide,

T- Time was taken in sec.

Extrudability

A container made of collapsible tube was used to hold the formulation.[17] The extrudability was measured by the amount of ointment needed to produce a ribbon measuring 0.5 cm in length during 10 sec. The crimped end of a collapsible tube carrying 30 g of ointment was pushed forcefully, and the tube was clamped shut to prevent it from unrolling. The gel was released once the cap was taken off. The quantity of ointment that was squeezed out was determined. All above Results are mentioned in Table 5.

Washability

Ointment was applied to the skin, and the formulation's washability was evaluated based on how well and how much the ointment washed off with distilled water.[18]

Stability Study

The purpose of stability testing is to determine how the quality of a drug substance or drug product will change over time in response to storage circumstances,[13] re-test intervals, and shelf-lives. Determining how fast a product deteriorates while stored at room temperature is a time-consuming process. To prevent this unnecessary lag, we use the concepts of rapid stability studies.

Guidelines published by the International Conference on Harmonization (ICH) under the heading "stability testing of New Drug substance and products" (QIA) outline what must be tested for in a stable drug product.

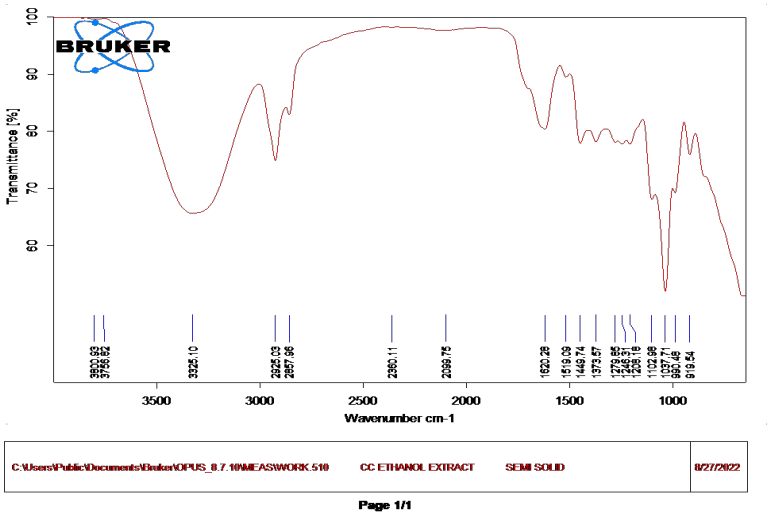

Figure 1: ATR-FTIR of Ethanolic bark extract of *Commiphora caudata*.

Table 1: Formulation Development of Herbal Ointment.

Ingredients	F1	F2	F3
Commiphora caudata	2 g	2 g	2 g
Hard Paraffin	2 g	3 g	4 g
Cetostearyl Alcohol	2 g	3 g	4 g
Wool Fat	2 g	3 g	4 g
White Soft Paraffin	21 g	18 g	15 g
Vitamin E	1 g	1 g	1 g
Span 80	q.s	q.s	q.s
Lavender Oil	q.s	q.s	q.s

Table 2: Colour, consistency and percentage yield of *Commiphora caudata* (Wight and Arn.) Eng.

Plant name	Part used	Solvent	Colour of solvent	Nature of extract	% yield of extract
Commiphora caudata	Bark	Petroleum ether	Dark green	Stiff paste	4.5%
		Chloroform	Dark Green	Stiff paste	7.5%
		Acetone	Green	Sticky	5.7%
		Ethanol	Dark Green	Stiff paste	13.5%
		Aqueous	ReddishBrown	Sticky semiSolid	10.8%

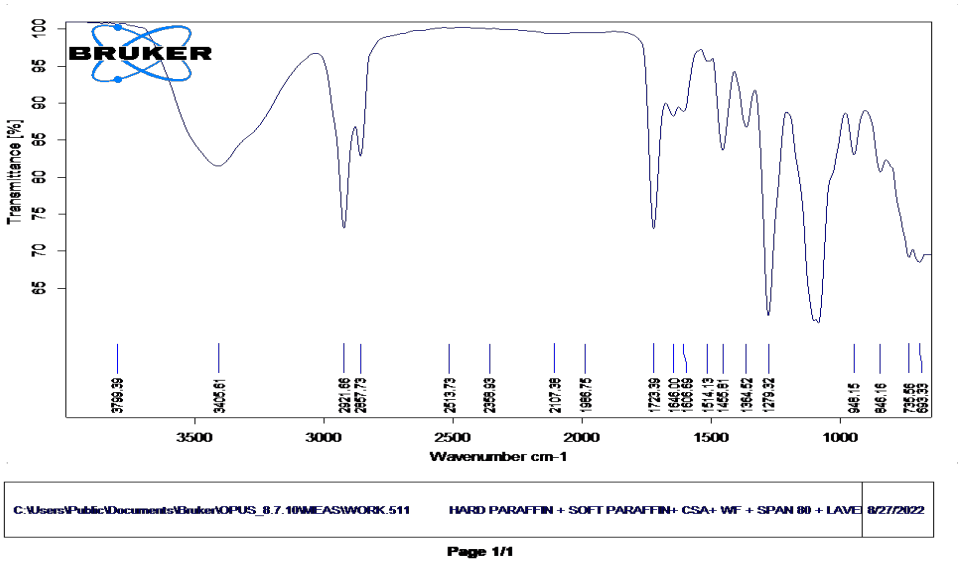

Figure 2: ATR-FTIR of white soft paraffin + hard paraffin + cetostearyl alcohol + wool fat + white paraffin + vitamin E.

The improved formulation underwent a one-month stability investigation in the current work, with testing conducted at 40°C±2°C/75%RH±5%RH (accelerated stability testing conditions for pharmaceutical products, specifically for products intended for Zones III and IV). The formulation table was mentioned in Tables 2-4.

ATR-FTIR of plant extract

The spectra were captured between 4000 and 400 cm^{-1}. An ATR-FTIR spectrophotometer was used to record an IR spectrum of a drug sample placed directly into the sample holder's cavity as described in Figures 1-5.

The linearity graph of wound healing analysis

The percentage inhibitory concentrations, CTC_{50} (Cytotoxic Concentration 50) values, for the wound healing concentration in fibroblast cells (3T3-CCL92) were derived from the dose-response curve by the process of interpolation using the linear regression analysis method, as described in Figures 6 and 7.

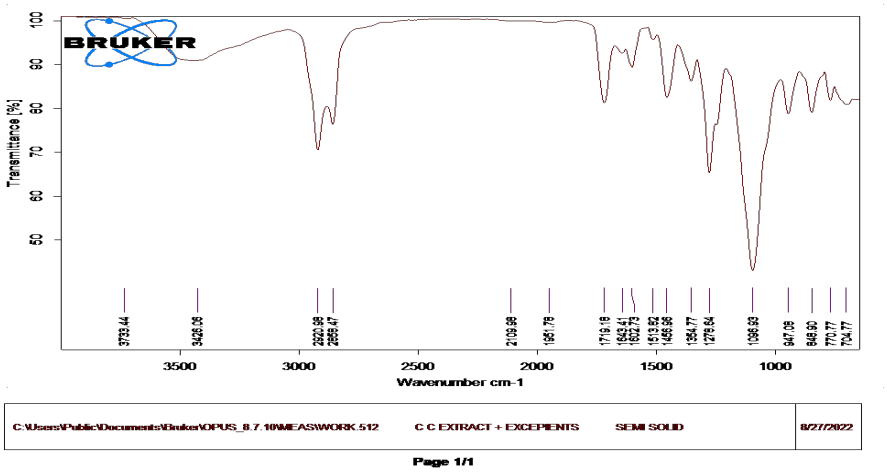

Figure 3: ATR-FTIR of white soft paraffin + hard paraffin + cetostearyl alcohol + wool fat + hard paraffin + vitamin E.

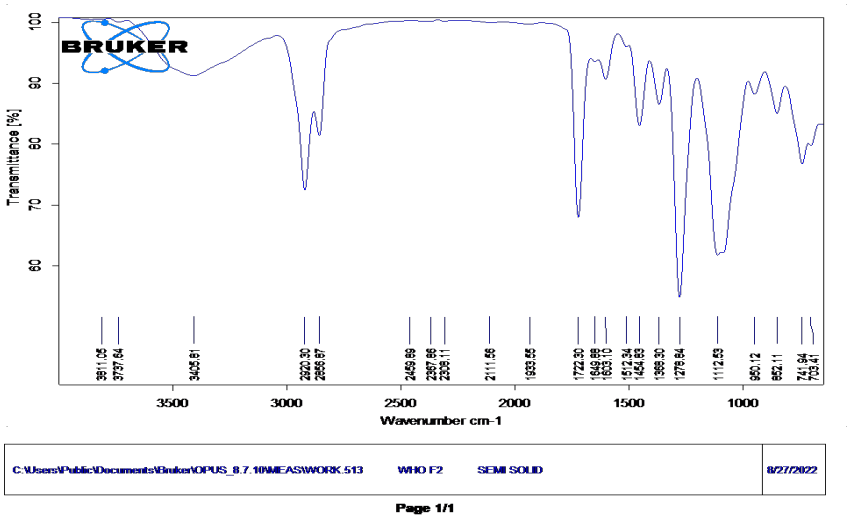

Figure 4: ATR-FTIR of *Commiphora caudata* bark extract + white soft paraffin + hard paraffin + cetostearyl alcohol + wool fat + hard paraffin + vitamin E.

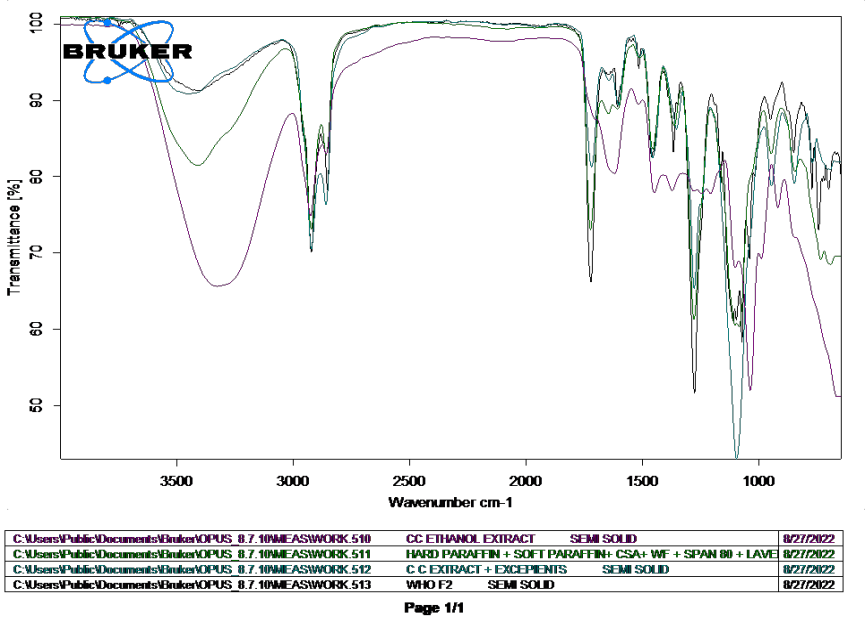

Figure 5: ATR-FTIR of extract and excipients compatibility study.

Table 3: Evaluation of active phytochemical constituents.

Solvent	Phytochemical constituents
Petroleum ether extract	Alkaloids, Steroids, Phenolic compound, Tannin, Glycosides, Terpenoids, Triterpenes.
Chloroform extract	Alkaloids, Steroids, carbohydrates, Phenolic compounds, Tannin, Glycosides, Terpenoids, saponins.
Acetone extract	Alkaloids, Steroids, carbohydrates, Phenolic compounds, Tannin, Glycosides, Terpenoids, and Triterpenes.
Ethanolic extract	Alkaloids, Steroids, carbohydrates, Tannin, Terpenoids, Triterpenes, and Proteins.
Aqueous extract	Alkaloids, Steroids, carbohydrates, Phenolic compounds, Tannin, Glycosides, Terpenoids, and Triterpenes.

Table 4: Wound Healing Analysis Cell Line - 3T3 - CCL92 OD Value, %CTC_{50} CTC_{50}.

Concentration	OD Value	%CTC_{50}
50	0.295	30.75
250	0.261	38.73
500	0.237	44.37
750	0.199	53.29
1000	0.174	59.15

Table 5: Evaluation parameters of ointments formulations.

Formulation	pH	Viscosity	Spreadability (g.cm/sec)	Extrudability (g)
F1	7.3	64.1	12 g	1.268
F2	7.4	60.1	10.05 g	0.536
F3	7.4	62.0	8.06 g	0.215

Evaluation Of Ointment Formulation With Regards To Physiochemical Parameters

Appearance

The finished ointment was examined for defects in terms of its color, consistency, and overall look. The finished product had a pleasant light green hue, a thick viscosity, and a decent consistency with no lumps.

Washability

All the formulations were easily washable with normal water.

pH Measurement

The pH of the finished ointment was measured using a digital pH meter by completely submerging a glass

Determination of Viscosity

The rotating viscometer (model LMDV-100) was set to 99.9 rpm, and the viscosities of formulations F1, F2, F3, ointment, and RV (Rotational Viscometer)-7's spindle were measured. For each formulation, 100 g of ointment was poured in a beaker, and the spindle was plunged in it, spinning for 5 min to get readings. The results of all the formulations are shown in Table 5 electrode into the ointment. The following is a summary of the study results shown in Table 5.

Determination of Spreadability

In order to calculate Spreadability, the formula was used.

$$S = ML / T$$

Where 'S' represents spreadability, 'M' is the mass applied, 'L' is the length of spread, and 'T' is the time taken for the spread (The result was mentioned in Table 5).

Extrudability

The extrudability of the ointment was determined by measuring its viscosity throughout a spectrum from 1.2680 to 0.21500 g (The result is mentioned in Table 5).

Stability study

The optimal formulation underwent a one-month stability testing at 40°C and 75% relative humidity, per ICH norms (The International Council for Harmonisation of Technical Requirements of Pharmaceuticals for Human Use).

RESULTS AND DISCUSSION

We can be certain that there have been no new peaks added or removed from the plant extract and excipient combination by comparing the ATR-FTIR spectra of the drug to those of the combination of the plant extract and the excipient. This gives us peace of mind. Because of this outcome, it is clear that the combination of the plant extract and the excipient is entirely stable. The concentrations (50-1000 µg/mL) with the fibroblast cells (3T3-CCL92) caused a large spike in the proportion of wound healing activity levels ranging from 30.75% - 59.15%. The percentage inhibitory concentrations, CTC_{50} values, for the wound healing concentration in fibroblast cells (3T3-CCL92) were calculated from the dose-response curve produced linear. The microscopic image represents increase the concentration of the plant extract result increases wound healing activity linearly and also number of viable cells also increases. pH is one of the major evaluation factors in the ointment preparation purpose of avoiding skin irritation upon application. All of the different formulations have a viscosity of between (60.1) and (64.1) mPas. Ointment has a viscosity of (60.1) mPas thanks to the agent reduction used in the (F2) formulation, which also has the best consistency. The three different formulas were tested for their potential to spread depending on their viscosity. The improved consistency of the new formulation allowed the ointment to be distributed with little shearing force. Extrudability was determined by measuring the ointment's viscosity throughout a spectrum from 1.2680 to 0.21500 g. The formulation (F2) has better consistency than the ointment. The optimal formulation

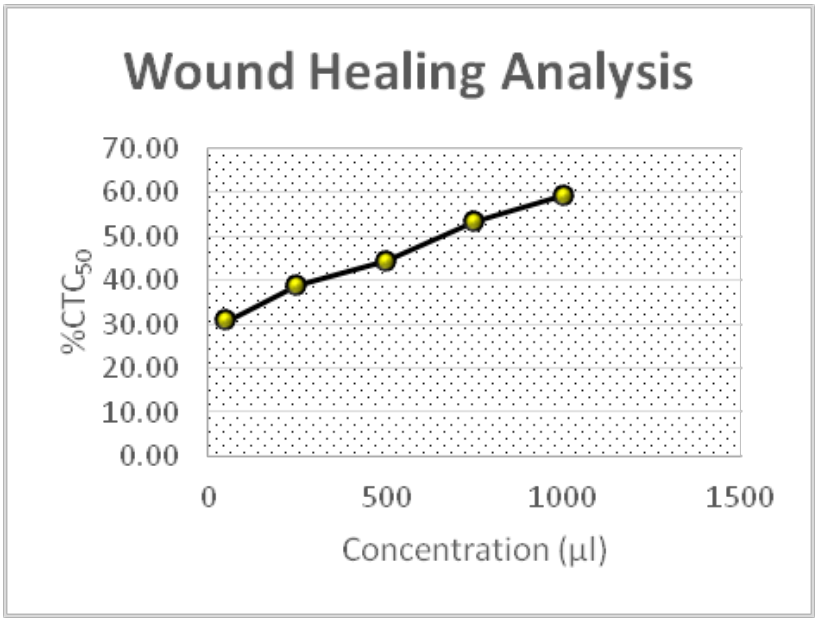

Figure 6: Linearity graph of wound healing analysis.

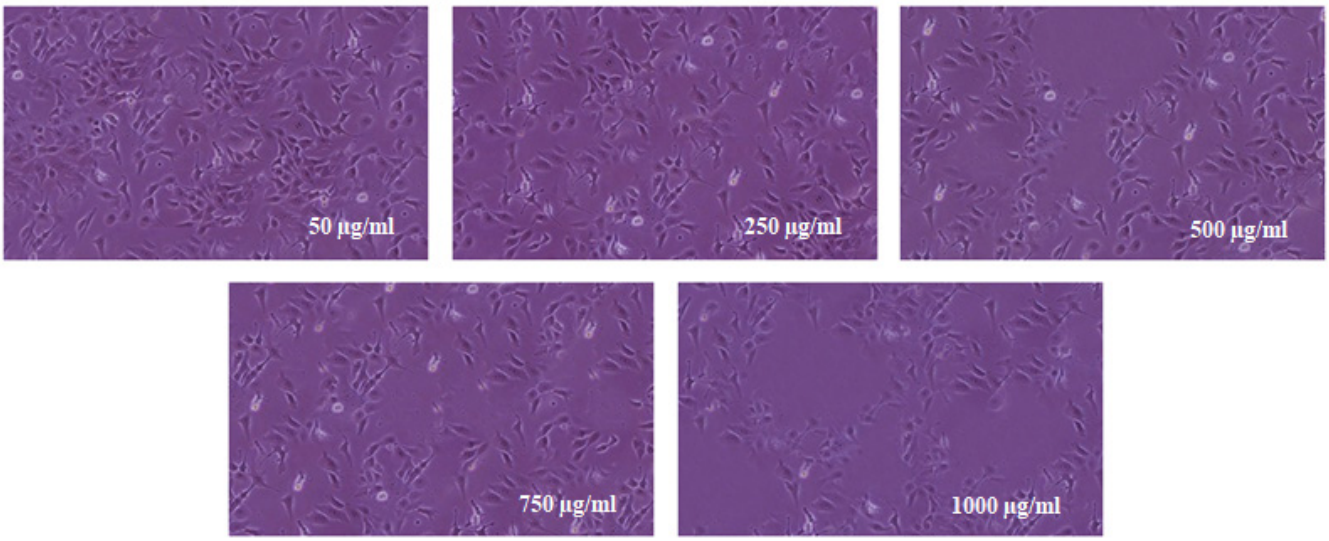

Figure 7: (A, B, C, D, E) Microscopic image of Wound Healing Analysis Cell Line - 3T3 - CCL92 microscopic image.

underwent a one-month stability testing at 40ºC and 75% relative humidity, per ICH norms. The physical and chemical characteristics of the cream were not significantly altered. Therefore, it was determined that the formulation (F2) was stable.

CONCLUSION

The plant *Commiphora caudata* was chosen for the current inquiry for its phytochemical and pharmacological assessment based on the traditional usage of the plant as well as a review of the literature about previous investigations. In future, the characterized compounds can be studied for their chemical modification which can lead to the synthesis of more active compounds. Ethanolic extract shows the presence of majority of phytoconstituents. Hence it was selected for pharmacological evaluation. Furthermore, a detailed mechanistic study of these extracts, and pharmacological activities can help in a better understanding of their exact mode of action and help in future work in this direction.

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ABBREVIATIONS

DMEM: Dulbecco's Modified Eagle Medium; **DMSO:** Dimethyl sulfoxide; **ATR-FTIR:** Attenuated Total Reflectance-Fourier Transform Infrared spectroscopy; **QIA:** Quantitative Image Analysis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The present study provides an insight into the ability of Crude extract obtained from *Commiphora caudata* as the wound healing activity. This study might help in developing drugs that can aid therapeutic needs.

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