RESEARCH PAPER

Pharmacognostic Study, Phytochemical Screening and Formulation Development from Achras Sapota Bark Extract for Wound Healing Model.

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Abstract

This study examines the pharmacognostic, phytochemical, and pharmacological characteristics of the bark extract of Achras sapota (Sapodilla) to facilitate the development of a phytopharmaceutical formulation. Extraction utilized solvents of increasing polarity petroleum ether, chloroform, and methanol—resulting in yields of 2.5%, 1.9%, and 10.0% (w/w), respectively. Initial phytochemical analysis indicated the presence of alkaloids, steroids, and Triterpenoids in the petroleum ether extract; glycosides, alkaloids, and carbohydrates in the chloroform extract; and tannins and flavonoids in the methanol extract. The chromatographic analysis via TLC and HPTLC revealed unique phytoconstituents with RF values between 0.79 and 0.98. UV spectral analysis indicated a peak absorption at 229.8 nm, implying the existence of conjugated systems. IR spectroscopy of the isolated product identified typical functional groups, including hydroxyl, carbonyl, and aromatic moieties, signifying the presence of phenolic and carbonyl-containing compounds. ^1H NMR spectroscopy validated the identification of Gallic acid, a Polyphenolic molecule, as a principal ingredient. The antioxidant capacity was evaluated with the DPPH radical scavenging test. The bark extract demonstrated significant free radical scavenging activity, with percent inhibition between 39% and 52.89%, in comparison to normal ascorbic acid (40.5%–65.04%), suggesting considerable antioxidant capacity. In conclusion, the results indicate that Achras sapota bark is a substantial source of bioactive phytochemicals, including gallic acid, which may possess antioxidant properties. These findings warrant additional investigation and application in the manufacture of herbal remedies for therapeutic purposes.

Keywords:

Achras sapota, pharmacognosy, phytochemicals, herbal formulation, bark extract, medicinal plants, skin irritation test, anti-inflammatory activity, anti-arthritis activity.

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Introduction

Plants have been the main source of healthcare for most of the last two thousand years of human civilization. [2] Approximately 6,000 of the numerous species of plants that have been found are recognized to offer some kind of medical use. This is true even if a lot of different types of higher plants have been found. There has been a significant rise in the need for studies on these plants because of the huge potential they have to improve medical care throughout the world.[2,17] The World Health Organization (WHO) says that almost 80% of people on Earth depend on traditional medicine to take care of their fundamental health needs.[8] Traditional medicine often uses plant-based extracts or the active parts of plants.[17] In the last several decades, a lot of work has gone into finding out how these more common methods work.[7,8] The commercial value of these plants has risen significantly in recent decades as a direct result of the meteoric expansion of the acceptance of alternative medical practices. In the Himalayan region specifically, the use of herbal remedies plays a very vital role. [9, 10] These regions are home to a plethora of plant species, many of which have the ability to treat a range of diseases. [11] The increasing demand for plant-based

therapeutics has revitalized interest in the pharmacognostical and phytochemical study of traditionally used medicinal plants. [8,14] Among these, *Achras sapota* (L.), also known as *Manilkara zapota*, is a member of the Sapotaceae family and is widely grown in tropical and subtropical regions. [2,17] Though primarily cultivated for its sweet and fleshy fruit commonly referred to as sapodilla or chikoo various other parts of the plant, including the bark, leaves, and seeds, have been employed in folk medicine across Asia and Central America for their potential therapeutic benefits [12, 13].The bark of *A. sapota* has been traditionally used to treat ailments such as diarrhea, inflammation, and fever [14]. Once the pharmacognostical and chemical properties of A. sapota bark are known, extracts from it might be used in topical or oral medicines. The Achras sapota tree is an evergreen tree that grows in tropical areas. It is grown a lot in tropical areas for its edible fruit, which is also called chikoo. People eat and study the fruit a lot, but folk medicine has utilized different parts of the plant, such the bark, to cure diarrhea, fever, wounds, and inflammation for a long time [2,17].

MATERIALS

Standardization of plant material

Collection-plant material was collected from local supplier in Pune, Maharashtra, India.

Plant material was authenticated by Botanist.

METHODS

Extraction methodology [14, 15, 17]

I) Petroleum Ether (60-80°C) Extract

II) Chloroform Extract

III) Methanol Extract

Preliminary phytochemical screening for various extracts of plant Achras sapota

Test for Carbohydrates, reducing sugars, monosaccharides, Proteins, amino acids Steroids Cardiac Glycosides Anthraquinones Glycosides saponin Glycoside Tannins and Phenolic compounds and Flavonoids. [14,15]

Phytochemical investigations

a) TLC of methanolic & EAS extract of plant Achras *Sapota*

b) Development of HPTLC Technique of methanolic extract of plant of *Achras Sapota*.

c) Column Chromatography

d) Spectral analysis of isolated fraction of extract *of Achras Sapota* bark [14,15]

CHROMATOGRAPHIC SEPARATION:

A. Thin Layer Chromatography: [14,37]

Methanolic extract and Ethyl acetate soluble fraction of methanolic extract was evaluated by TLC to identify the presence of number of phytoconstituents present in an extract using specific solvent system which was found to give proper separation.

Steps involved in performing TLC of extracts:

Preparation of TLC plate: Prepared the slurry of adsorbent media (silica gel-G) in distilled water and poured the slurry on the TLC glass plates to obtain a thin layer.

Activation of TLC plate: Heating in oven for 30 min. at $105^{O}C$ activated TLC plate.

Sample application: Dipping the capillary into the solution to be examined and applied the sample by capillary touched to the thin layer plate at a point about 2 cm from the bottom. Air-dried the spot.

Chromatogram development: After the saturation of chamber and spotting of samples on plate, it was kept in chamber. The solvent level in the bottom of the chamber must not be above the spot that was applied to the plate, as the spotted material will dissolve in the pool of solvent instead of undergoing chromatography. Allowed the solvent to run around 10-15 cm on the silica plate

Chamber saturation: The glass chamber for TLC should be saturated with mobile phase. Mobile phase was poured into the chamber and capped with lid. Allowed saturating about 30 min.

Visualization: Plates were removed and were examined visually, under UV and suitable visualizing agent (Alcoholic ferric chloride solution) after that R_F was calculated by following formula.

$$R_F = \frac{\text{Distance traveled by solute from origin line}}{\text{Distance traveled by solvent from origin line}}$$

B. High performance thin layer chromatography (HPTLC):[37]

Reagents and other materials.

STD Gallic acid and, toluene, ethyl acetate, and methanol [all reagents of analytical grade, E-Merck] and silica gel 60F254 precoated TLC aluminium plates [E-Merck].

Apparatus

Spotting device: Linomat V Automatic Sample Spotter; CAMAG (Muttenz, Switzerland)

Syringe: 100µL Hamilton (Bonadzu, Switzerland)

Thin layer chromatography (TLC) Chamber: Glass with trough chamber (20×10 ×4 cm) (CAMAG)

Densitometer: TLC scanner 3 linked to WinCats Software (CAMAG)

HPTLC plates: 10×10 cm, 0.2 mm thickness precoated with silica gel 60 F254 (E. Merck, Mumbai, India)

Experimental conditions: Temperature 25 ± 2 ºC, relative humidity 40 %

Mobile phase: - Toluene: Ethyl acetate: methanol (5:4:1)

Preparation of standard and sample solutions

Standard solution of Gallic acid

A stock solution of Gallic acid was prepared by dissolving 10 mg of accurately weighed gallic acid in methanol and making up the volume to 10 ml with methanol to get the final concentration of 1mg/ml.

Preparation of sample solutions.

Sample solution was prepared by dissolving 50 mg of the ethyl acetate fraction of methanolic extract in methanol and making up the volume to 5 ml to get the concentration of 10 mg/ml.

Methodology:

Exactly 10 µL of each of the standard solution of Gallic acid, methanol sample solution were applied in four spots of each on 10×10 cm TLC plates. The plates were developed in a solvent system of toluene: ethyl acetate: methanol (5:4:1) at 25 ± 20C temperature and 40% relative humidity and allowed to travel up to a distance of 8 cm. After development the plates were dried in air and scanned densitometrically at 254 nm for Gallic acid, & sample solution. The colour of the spots, peak areas and peak heights were recorded. [22,29]

Specificity:

The specificity of the method was ascertained by analyzing the standard drug and extract. The spot for Gallic acid in the sample was confirmed by comparing the R_F values and spectra of the spot with that of the standard. The peak purity of the Gallic acid was assessed by comparing the spectra at three different levels, viz. peak start, and peak apex and peak end positions of the spot.

C. Column chromatography

Preparation of column: [14,37]

150 Gms of silica gel for column chromatography was activated in hot air oven at 110ºC for 1 hr. The adsorbent bed was developed in mobile phase which was initially packed with glass wool. The glass wool is fixed at the bottom of the column. The slurry of activated silica gel was made in Toluene : Ethyl acetate : methanol (5 : 4: 1) & charged into column in small portions by keeping knob open with gentle taping after each addition in order to ensure uniform packing. The small quantity of solvent was allowed to remain on the top of the column. The air bubbles present in the column were removed by gentle tapping to get uniform bed of adsorbent. The adsorbent bed was allowed to develop overnight taking care to prevent the drying of column by plugging open end with cotton and aluminium foil. The column was run fast for some times with mobile phase in order to remove any impurities. Prepared sample was then charged in to the column & was allowed to settle. A small cotton pad was placed above the sample to prevent the mixing of dust particles with the sample. Then it was eluted with mobile phase to collect fractions. Fractions collected were further concentrated. Each fraction was evaluated by TLC to detect the number of phytoconstituents present in it.

TLC of Isolated fraction after column chromatography:

Stationary phase : Silica gel G

Mobile phase : Toluene: Ethyl acetate: methanol

Proportion : 5: 4: 1

Visualizing agents: Alcoholic Ferric chloride reagents.

All fraction showed two spots on TLC plates.

Recolumn:

Preparation of sample for Recolumn: All fraction are collected in a beaker & concentrate on water bath to about 5ml fraction are left in a beaker. These fraction was mixed well with 2gm silica gel & dried in vacuum at 45^0C. The adsorbed material obtained was transferred to the column. Then it was eluted with mobile phase to collect fractions. Fractions collected were further concentrated. Each fraction was evaluated by TLC to detect the number of phytoconstituents present in it.

TLC of Isolated fraction after Recolumn:

Stationary phase : Silica gel G

Mobile phase : Toluene: Ethyl acetate: methanol

Proportion : 5: 4: 1

Visualizing agents: U.V 254nm

Fraction showing same number of compounds with same R_F values were combined, concentrated & evaporated to dryness.

Spectral analysis of isolated fraction-A:- [22,29]

1. U.V. Spectrum:
2. Fourier transform infrared spectroscopy (FTIR):
3. H_1 Nuclear magnetic resonance. (NMR):

FORMULATION AND EVALUATION OF GEL

Preparation of Topical Gel

Different combinations of *Achras Sapota* Bark Extract (2.5%, 5%) were tried with different types of polymers (Sodium CMC, Carbopol 934) using various formulae. The following few combination with Carbopol 934 resulted in the best gel formulation, which was smooth and stable. Control sample also was prepared for testing of animal to check the activity of control ingredients.

Method for Preparation of Gel Containing Extract

1. 1 g of Carbopol 934 was dispersed in 50 ml of distilled water with continuous stirring.
2. 5 ml of distilled water was taken and required quantity of methyl paraben and propyl paraben were dissolved by heating on water bath.
3. Cool the solution, then to that added Propylene glycol 400.
4. Further required quantity of *Achras Sapota* Bark Extract was mixed to the above mixture and volume made up to 100 ml by adding remaining distilled water.
5. Finally full mixed ingredients were mixed properly to the Carbopol 934 gel with continuous stirring and triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency.

Experimental design

The response surface methodology (RSM) was employed to perform Quality by Design approach for constructing and investigating the polynomial models, using fewer experimental runs. Central composite Design comprising of 2-factors and 3- levels was employed to examine the quadratic response surfaces by assessing the effect of pre-defined independent variables on different response dependent variables pH, Viscosity and Spreadability was coded as Y1 and Y2 and Y3. Two independent variables namely concentration of Bark Extract (%) and Concentration of carbopol (mg) were chosen. Each of the variables was varied at three different levels, known as high, medium and low levels. All the finalized independent variables and the response variables are described in Table 1.

Table No.1: dependent and independent variables

1. **Independent variables**

Coded level	-1 (low)	+1 (high)
X1- Concentration of bark extract (%)	2.5	7.5
X2- Concentration of Carbopol 934 (mg)	500	1000
Dependent variables		
pH	Optimum	
Viscosity	Optimum	
Spreadability	Optimum	

Table No.2 Formulation run of designed batches

Formulation Code	Concentration Bark extract (%)	Concentration of carbopol 934
F1	2.5	500
F2	2.5	1000
F3	8.53553	750
F4	7.5	1000
F5	1.46447	750
F6	5	1103.55
F7	7.5	500
F8	5	396.447
F9	5	750

EVALUATION OF TOPICAL GEL FORMULATION

A. Physical Evaluation

Physical parameters such as color and appearance were checked.

B. Measurement of pH

pH of the gel was measured by using pH meter.

C. Spreadability

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end5. By this method spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2g) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A. 1 kg weighted was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then

subjected to pull of 80 Gms. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better spreadability. Spreadability was calculated using the following formula:

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

Where,
S = Spreadability,
M = Weight in the pan (tied to the upper slide),
L = Length moved by the glass slide and
T = Time (in sec.) taken to separate the slide completely each other.

D. Viscosity

Viscosity of gel was measured by using Brookfield viscometer with spindle.

E. Stability Study

The stability study was performed as per ICH guidelines 6. The formulated gel was filled in the collapsible tubes and stored at different temperatures and humidity conditions, viz. 250 C ± 20C/ 60% ± 5% RH, 300 C ± 20C/ 65% ± 5% RH, 400 C ± 20C/ 75% ± 5% RH for a period of three months and studied for appearance, pH, viscosity and spreadability.

F. *In vitro* studies

Antimicrobial Assay

This experiment was performed according to the method of Andrews,(2001)[12]. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using broth micro dilution method. Briefly, a standardized inoculum (10 µl of a 1–5×10^5 CFU/ml suspension) was added to the appropriate growth medium, supplemented with two fold serially diluted fraction (F8). DMSO (0.1% v/v) was used as the negative control. After 16 hrs of incubation, the optical density was measured at 590nm by Spectramax M5. The lowest concentration of F8 showing complete inhibition of bacterial growth was considered as the MIC.[28,36]

For determination of MBC, the broth from the wells were spread on nutrient agar plates and was incubated at 37°C for 24 hrs. The minimum concentration of sample at which the visual bacterial colony was reduced to 99.9% was considered as the MBC [12,28,36].

Evaluation of angiogenesis activity by CAM (Chorioallantoic membrane) model

This experiment was performed according to the method [33]. In this method, embryonated chicken eggs (9 days old) were selected and a small window (1cm^2) was made on the outer shell. Through the window, a sterile disc of methyl cellulose treated with 200 to 400 µg of F8 was placed at the junction of two blood vessels. The opening (window) was then sealed and the eggs were incubated at 37° C for 72 hr. The window was then opened and the growth of new capillary blood vessels were observed and finally compared with the control eggs.[33]

G. *In vivo* studies

Wound healing studies

I. Dead space wound model

Dead space wounds were inflicted by implanting sterile cotton pellets (5mg each), one on either side of the groin and axial on the ventral surface of each [31]. The animals were randomly divided into two groups with five RATS each. Animals in the control group were provided with normal drinking water and the test group were fed with F8 (in 5%w/v gum acacia suspension; dose 50mg/kg) daily. After 10 days, the granulation tissue formed on the implanted cotton pellets along with the formed granulation tissue was carefully removed under ether anesthesia. The granulation tissue impregnated cotton pellet were collected and dried at 60^0 C for 12 hrs to determine the dry weight of the granulation tissue formed. Dried tissue was then mixed with 5 ml of 6N HCl and was kept at 110^0C for 24 hrs. Thereafter, the neutralized acid hydrolysate of the dry tissue was used for the determination of Hydroxyproline content. Then the tissue was preserved in 10% formalin [6,31].

II. Excision wound model

The excision wound healing activity of F8 was evaluated [26,24,29]

Grouping of animals

After creation of wound, the experimental animals were divided into three groups, each consisting of 5 RATS. Group A was treated with gel base (control), Group B was treated with Megaheal gel as (standard) and Group C was treated with 0.5% (w/w) F8 gel. [26,24,29]

Table 7.1. Grouping of animals

Group I	Group II	Group III
Topical Application	Control	Standard – Megaheal Gel

Determination of wound contraction

The wound contraction was determined according to

the method [26] as described earlier , The wound contraction percentage was determined using the following formula:

Percent wound contraction = (Healed area / Total area) × 100

III. Incision wound model [26,24,29]

The incision wound healing was evaluated according to the method

Determination of tensile strength

Tensile strength was measured according to the method

IV. Infectious wound model

This wound model was performed according to the method [28,36]. Experimental RATS were anaesthetized by open mask method using anesthetic ether, which were depilated on the dorsal back. Ethyl alcohol (70% v/v) was used to disinfect the shaved region before creation of wound. Thereafter, excision wound was created by removing a 7mm x 7mm full thickness piece of the skin from the shaved area of each animal. These wounds were then infected with *Staphylococcus aureus* (20 µl of 10^7 CFU/ml), and then left undressed. The RATS were distributed in groups and each mouse was placed in separate cages. This model was used to monitor the rate of wound contraction (produced following topical application of this test drug F8 or the the standard mega-heal gel). The experimental animals were divided into three groups, each consisting of 5 RATS. Group A was treated with gel base (control), Group B was treated with Megaheal gel as (standard) and Group C was treated with 0.5% (w/w) F8 gel. [24,26,]

H. Estimation of collagen content (hydroxyproline)

Hydroxyproline is a basic constitutent of collagen [1,34]. The collagen content of the granulation tissue was determined by estimating hydroxyproline content. Regenerated tissues (100mg) from the healed lesions of wound samples on 11^{th} day were collected and washed with physiological saline. They were cut in to small pieces, defatted with chloroform: methanol (2:1v/v) and lyophilized. 5mg of lyophilized sample was hydrolysed with 6N hydrochloric acid at 110°C for 4 hr in sealed tubes. The hydrolysate was neutralized to pH 7.0 and subjected to chloramine-T oxidation; all the test tubes were placed in a water bath at 60°C for 20min. The reaction was terminated by addition of 1ml (0.4M) of perchloric acid, after 3min 1ml of PDAB (p-dimethylaminobenzaldehyde) reagent was added in each tube. Then the colour development was read spectrometrically at 557nm. The collagen content of granulation tissue samples was calculated [1,34].

I. Histopathology study

The histopathology study was carried out according to the methods [24,26]

J. Statistical analysis

Results were expressed as mean ± S.E.M; (n=5). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by post hoc Dunnett's test. Student't' test was used for *in vitro* experiments. Unless otherwise mentioned, 'p' < 0.05 were considered to be statistically significant.

RESULT AND DISCUSSION

A. Physical Evaluation

Figure: Physical Evaluation

Batch Code	Color	Appearance / Texture
F1	Pale brown	Runny, slightly fluid gel
F2	Brown	Thick, opaque, uniform
F3	Light brown	Smooth, semi-translucent
F4	Dark brown	Thick, slightly opaque
F5	Light tan	Smooth, glossy gel
F6	Deep brown	Very thick, slightly lumpy
F7	Medium brown	Smooth, spreadable
F8	Light beige	Smooth, translucent gel
F9	Brown	Smooth, homogeneous gel

Discussion: The color of the formulations ranged from pale brown to deep brown, which is primarily attributed to the concentration of *Achras sapota* bark extract. Extracts rich in phenolic compounds, flavonoids, and tannins typically impart a brownish hue due to their inherent chemical composition. Formulations with higher extract concentrations such as F4 and F6 exhibited darker shades (dark brown and deep brown respectively), whereas lower extract concentrations (e.g., F1, F5, and F8) resulted in lighter colors like pale brown, light tan, and light beige. This progressive color deepening is an expected outcome and can serve as a preliminary indicator of phytochemical load. Formulations such as

F3, F5, F7, and F9 displayed smooth, semi-translucent to homogeneous textures, which are ideal characteristics for topical application. These batches balanced extract concentration and polymer content effectively to provide a stable and aesthetically pleasing formulation. Specifically, F5 and F9 were noted for their glossy and homogeneous appearance, respectively, which may indicate proper gelling and compatibility between components without phase separation or precipitation.

B. Measurement of pH

Table: Measurement of pH

Batch Code	pH
F1	6.9
F2	6.7
F3	6.8
F4	6.6
F5	7.1
F6	6.5
F7	6.9
F8	7.0
F9	6.8

Discussion: All batches exhibited pH values within the acceptable range for topical application (6.5–7.1).Batch F5 (7.1**)** was slightly above the upper limit of the ideal pH range, which may be attributed to lower extract concentration and possibly higher neutralization with triethanolamine. However, it is still generally safe for short-term skin application. Batch F6 (6.5) showed the lowest pH, possibly due to a higher extract concentration and lower amount of triethanolamine added for neutralization. The other formulations (F1, F2, F3, F4, F7, F8, and F9) were well-balanced, with pH values between 6.6 and 6.9, which is considered optimal for dermal compatibility. There was no significant deviation in pH values, indicating a consistent formulation process and effective buffering capacity of the gel base.

C. Spreadability

Figure: Spreadability

Batch Code	Spreadability *(g·cm/s)*
F1	13.5
F2	10.2
F3	12.4
F4	9.5
F5	14.2
F6	9.0
F7	13.0
F8	15.0
F9	12.8

Discussion: F8 (15.0 g·cm/s) showed the highest spreadability, indicating an excellent balance of gel consistency and polymer concentration, allowing for smooth and easy application. This may be due to the lower concentration of Carbopol 934 (396.447 mg) used, which resulted in a softer gel matrix.F5 (14.2), F1 (13.5), and F7 (13.0) also exhibited good spreadability, suggesting that moderate levels of Carbopol and extract resulted in gels with desirable texture and spreadability. F6 (9.0) showed the lowest spreadability, likely due to the highest concentration of Carbopol 934 (1103.55 mg), making the gel very viscous and resistant to spreading.F2 (10.2) and F4 (9.5) also had relatively lower spreadability, correlating with their higher polymer concentration (1000 mg), which increased viscosity and hindered smooth application. F3 (12.4) and F9 (12.8) presented intermediate values, indicating a satisfactory gel structure that balances consistency with ease of application.

D. Viscosity

Table: Viscosity

Batch Code	Viscosity *(cps)*
F1	3200
F2	4200
F3	3700
F4	4700
F5	3000
F6	4900
F7	3400
F8	3800
F9	3600

Discussion: Highest viscosity was observed in Batch F6 (4900 cps)**,** followed by F4 (4700 cps) and F2 (4200 cps)**.** These formulations had high concentrations of Carbopol

934 (1000 mg and above), which is directly responsible for increasing the gel matrix density and resistance to flow. Lowest viscosity was found in Batch F5 (3000 cps) and F1 (3200 cps), which corresponds with lower polymer concentrations. These lower values may lead to runny gels with reduced physical stability, although they often show better spreadability. Intermediate viscosities were recorded for Batches F3 (3700 cps), F8 (3800 cps), and F9 (3600 cps). These batches demonstrate a good balance of viscosity and spreadability, Batch F8 (3600 cps) also falls within an acceptable range, with a viscosity that allows for ease of application while maintaining adequate gel structure.

E. Stability study

Table: Stability study

Batch Code	Stability (3 Months)
F1	Stable
F2	Stable
F3	Stable
F4	Slight phase separation
F5	Stable
F6	Unstable (viscosity drop)
F7	Slight separation
F8	Stable
F9	Stable

Discussion: Formulations F1, F2, F3, F5, F8, and F9 exhibited no significant changes in their physical characteristics over the test period and were categorized as stable. This indicates that the formulation components in these batches were compatible and maintained their integrity under storage conditions. Formulation F4 showed slight phase separation, suggesting a potential incompatibility between the emulsifying agents or inadequate homogenization. This could compromise product consistency and necessitates reformulation or adjustment of stabilizers. Formulation F6 was found to be unstable due to a noticeable drop in viscosity. A decrease in viscosity may indicate polymer degradation, improper gelling agent concentration, or breakdown of emulsion structure, which may affect spreadability and application performance. Formulation F7 exhibited slight separation, possibly due to borderline emulsion instability. Although minor, this may progress over time and should be monitored further or improved via optimization of surfactant levels or processing conditions.

F. *In vivo* studies

Evaluation of wound healing activity of F8

I. Dead space wound model

The result of dead space wound model revealed significant increase in tensile strength and wet granulation tissue formation in the test group (F8) when compared to the control (Table 7.4, Fig.7.5).

Table 7.4. Wound healing activity of F8 by dead space wound model in RATS

Treatment	Tensile strength (gm)	Weight of Granulation tissue
	(mean ± SEM)	(mg) (mean ± SEM)
Control	148.75 ± 1.82	77.69±3.99
F8 (50mg/kg)	179.75±2.82**	95.84±2.24**

Values are expressed as mean ± S.E.M; (n=5). **p<0.01 (Vs. control).

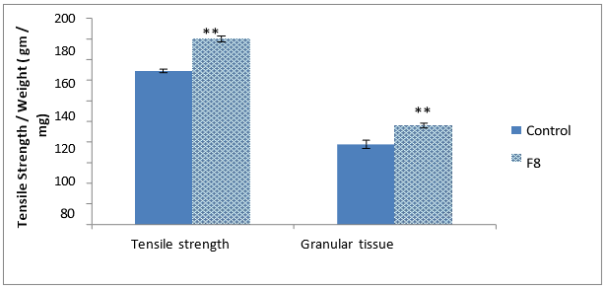


Fig.7.5. Wound healing activity of F8 studied using dead space wound model in RATS. Values are expressed as mean ± S.E.M; (n=5). **p<0.01 (Vs. control)

Estimation of hydroxyproline and collagen content

Hydroxylproline assay performed on tissue samples from the dead space wound model, exhibited significant increase in collagen content (as estimated from hydroxylproline) in F8 treated group when compared to control . (Table 7.5, Fig.7.4).

Table 7.5. Estimation of collagen content of F8 treated RATS.

Treatment	Collagen Content (mg/100mg of tissue)
Control (normal Saline)	21.2±1.2

F8 (50mg/kg/ oral)	46.2±1.6**

Values are expressed as mean ± S.E.M; (n=5). **p<0.01 (Vs. control)

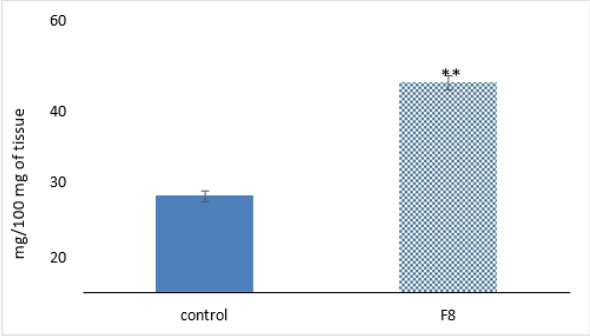


Fig.7.4. Estimation of collagen content of F8 treated RATS. Values are expressed as mean ± S.E.M; (n=5). **p<0.01 (Vs. control).

II. Effect of F8 on excision wound model

F8 treatment in the excision wound model resulted in a significant increase in the percentage of wound contraction as compared to control group. Moreover, the wound healing activity of the F8 gel (0.5 %) was found to be similar to that of the standard drug (Megaheal gel) (Table 7.6, Fig.7.5).

Table 7.6. Effect of F8 and standard drug on excision wound model

Post wounding days	Percentage wound contraction (mean±SEM)		
	Control	F8 gel	Megaheal gel
1	1.025±0.165	1.962±0.272	1.735±0.756
3	5.630±0.739	19.445±3.032	22.032±2.225
5	32.125±2.18	43.917±3.174	59.321±3.152
7	49.123±3.251	69.572±2.884	74.511±2.541
9	57.215±3.211	88.192±2.415	90.101±1.219
11	63.321±2.456	91.83±0.557	96.284±0.342
13	69.053±1.234	95.69±0.808	97.075±0.524
15	74.457±1.015	97.77±0.443	98.233±0.127

Values are expressed as mean ± S.E.M; (n=5)*P<0.05, **p<0.01 (Vs. Control).

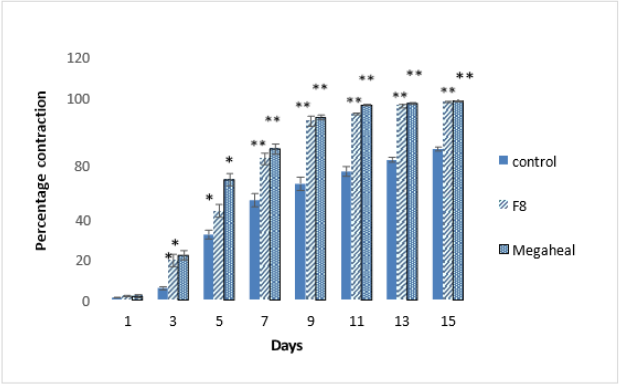


Fig 7.5. Effect of F8 gel by excision wound model. Values are expressed as mean ± S.E.M; (n=5)*p<0.05, **p<0.01 (Vs. Control).

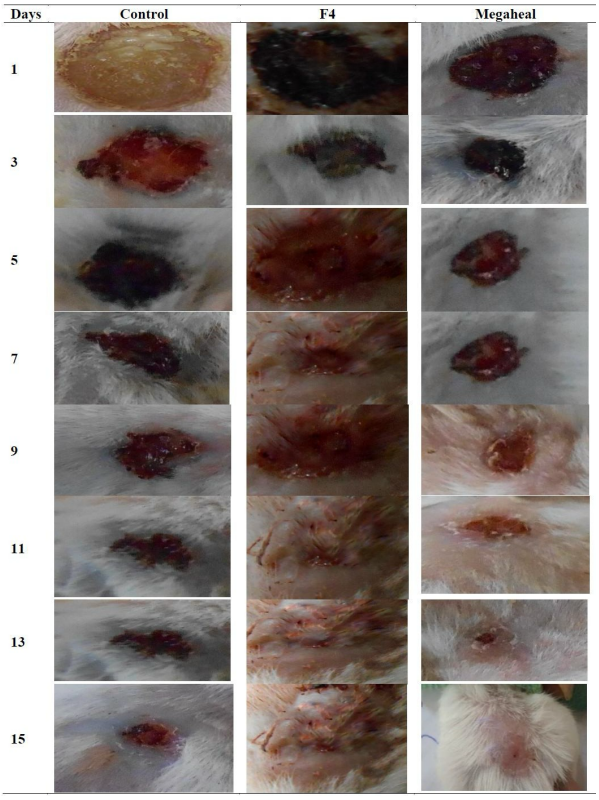


Fig 7.6. RATS dorsal wound area photographed at different days (1, 3, 5, 7, 9, 11, 13 and 15) representing the control treated group, F8 treated group and Megaheal treated group

III. Effect of F8 gel on incisional wound model

Study on the incision wound model revealed significant increase in tensile strength of test (F8; 237.75±2.82; p<0.001) and standard (mega-heal; 261.75±2.63; p<0.001) treated animals as compared to the control group measured on the 11th day (Table 7.7, Fig.7.7).

Table-7.7. Wound healing activity of F8 gel by incision wound model in RATS

Treatment	Tensile Strength (gm) (mean ± SEM)
Control	188.75±3.82
F8 gel (5%)	237.75±2.82**
Mega heal Gel	261.75±2.63**

Values are expressed as mean ± S.E.M ;(n=5), **p<0.01 (Vs. control).

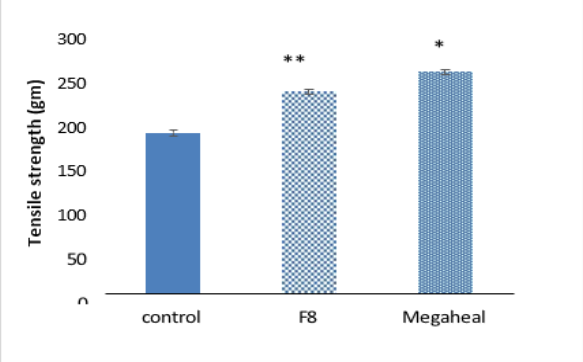


Fig.7.7. Wound healing activity of F8 gel and standard in the incision wound model in RATS. (Wound contraction was measured on the 11th day; Tensile strength expressed as gm ±SEM). Values are expressed as mean ± S.E.M ;(n=5) **p<0.01 (Vs. control).

Estimation of collagen content of F8 gel

Treatment with either F8 or the standard (Megaheal gel) produced significant increase in collagen synthesis (as evident from increased hydroxyl proline level) during the process of wound healing activity (Table 7.8, Fig.7.8).

Table-7.8. Estimation of collagen content of F8 gel

Treatment	Collagen Content (mg/100 mg of tissue)
Control	16.27±2.3
F8(5% w/w gel)	35.16±2.8**
Standard(Mega Heal)	53.22±2.5**

Values are expressed as mean ± S.E.M; (n=5),*p<0.05 and **p<0.01 (Vs. control).

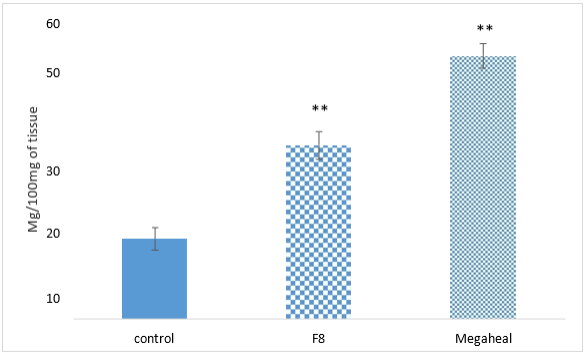


Fig.7.8. Estimation of collagen content of F8 gel. Values are expressed as mean ± S.E.M; (n=5), p<0.05 and **p<0.01 (Vs. control).

IV. Effect of F8 gel on infected wound model

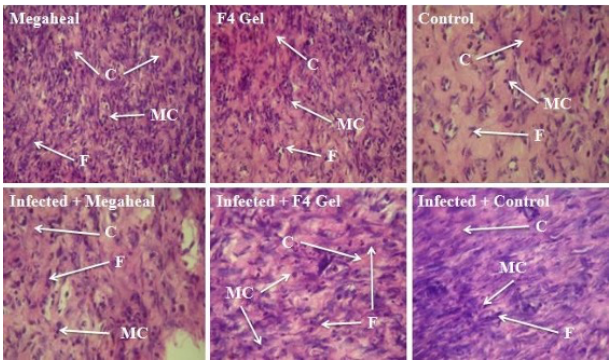
Following topical application of F8 to the infected wound, the contraction of wound (similar to excision model) was found to be significant from the 11th day onwards.

However, with standard (Mega-heal) significant wound contraction was detected from the 7th day (Table 7.9, Fig.7.9).

Table 7.9. Wound healing activity of F8 by excision wound (infected) model in RATS

Post wounding days	Percentage wound contraction (mean±SEM)		
	Control	F8 gel	Megaheal gel
1	0.98± 0.21	1.34± 0.32	1.42±0.15
3	4.32±1.25	5.20±1.81	5.41±2.74
5	6.82±0.85	6.54±0.95	7.58±0.86
7	14.71± 3.47	17.14± 3.84	22.36±2.21
9	39.58±3.68	43.32±2.38	64.99±2.14
11	52.41±2.002	64.11±2.46	89.13±3.23
13	63.05±0.89	75.77±0.45	97.25±0.51

Values are expressed as mean ± S.E.M; (n=5), *p<0.05and **p<0.01 (Vs. control).



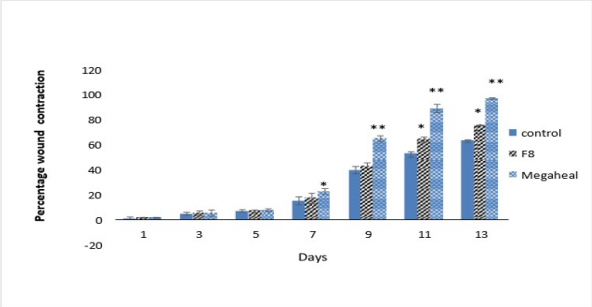


Fig.7.9. Wound healing activity of F8 by excision wound infected model in RATS. Values are expressed as mean ± S.E.M; (n=5), *p<0.05, **p<0.01 (Vs. control).

J. Histological evaluation of wound treated with F8 topical formulation

The histological evaluation of wound following 10 days topical treatment with either F8 or the Standard (Mega heal gel), were also performed and the results were discussed in the following paragraphs.

Histopathological evaluation

The normal tissue architecture of the RATS wound on the 10th post wounding day has been depictated in Fig.7.9. Histopathological changes were observed on wounds treated with F8 topical formulation and also the standard drug treated group. The wound showed effective neovascularization and significant formation of collagen after 10 days of treatment [37]. Epithelialization was observed to be faster in both F8 and the standard groups when compared to the control group. However, on comparison of the F8 treated excision wound model (non-infected), it was observed that despite F8 treatment , the healing of infected wound was found to be delayed (as evident from tissue collagen and fibroblasts) (Fig.7.10).[26]

Fig.7.10. Histopathological view of normal and infected wound treated with F8, control and standard (Megaheal) of 10th day wound of excision wound model. Skin section show hematoxylin and eosin stained epidermis and dermis, in which (C:Collagen, F: Fibroblast, MC: Mononuclear cells).[6,7]

Conclusion

This study demonstrates that *Achras sapota* bark possesses significant wound healing potential supported by pharmacognostical, phytochemical, and formulation evaluations. The methanolic extract showed a rich presence of bioactive compounds, and gallic acid was identified as a major polyphenolic constituent with strong antioxidant activity. The optimized topical gel (F8) exhibited suitable physicochemical properties and stability.

In vitro antimicrobial and angiogenic studies, along with in vivo wound models, confirmed enhanced wound contraction, tensile strength, collagen deposition, and epithelialization. Histological findings further supported improved tissue regeneration. Overall, the wound healing activity of *Achras sapota* bark appears to be mediated through antioxidant, antimicrobial, and collagen-promoting mechanisms, supporting its potential as an effective herbal wound care formulation.

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