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Exploring *Senna Alata* Bark as a Potential Therapeutic agent for Wound Healing: A Comprehensive Pharmacognostic, Physicochemical, Phytochemical, & Pharmacological Study

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KEYWORDS

Wound healing, Senna alata, Phytochemical, Pharmacological, Gel formulation

ABSTRACT:

Wound healing is a complex biological process crucial for tissue repair following injury, involving sequential phases of hemostasis, inflammation, proliferation, and maturation. Senna alata (L.) Roxb., commonly known as candle bush, is a flowering shrub with recognized medicinal properties. This study explored the Pharmacognostic, physicochemical, phytochemical, and pharmacological characteristics of Senna alata bark, aiming to assess its potential as a therapeutic agent in wound care. Macroscopic and microscopic analyses confirmed the identity of Senna alata, revealing characteristic features such as light green color and distinct odors. Physicochemical evaluations demonstrated favorable parameters including low moisture content and significant extractive values, supporting its suitability for medicinal formulations. Hydroalcoholic extracts of Senna alata were enriched with bioactive compounds including steroids, flavonoids, and alkaloids, identified through phytochemical screening. The extracted components were utilized to formulate a gel preparation, demonstrating good consistency and stability suitable for topical application. Pharmacological investigations using an excision wound model in rats showed that Senna alata formulations facilitated accelerated wound healing compared to control groups, as evidenced by enhanced wound contraction rates over a 15-day period. These findings underscore the potential of Senna alata as a promising herbal remedy for promoting wound healing. To fully use its therapeutic potential, more investigation into its mechanisms of action and wider clinical applications is necessary. benefits.

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1. Introduction

The definition of A wound is a cut in the outer layer continuity. Moreover, muscular or thermal trauma can cause an injury to the epidermis layer of the skin or mucosa, defining a wound. In general terms, there are four distinct phases of wound healing: hemostasis, inflammation, proliferation, and maturation [1]. One of the main causes of physical impairments is injury. A wound is a disrupted condition of tissue that is often linked to loss of function and can be brought on by physical, chemical, microbiological, or immunological assaults. The Wound Healing Society defines a wound as a physical injury that results in a break or opening in the skin that disrupts the normal structure and function of the skin. The intricate interplay of cellular and biochemical processes leading to the recovery of wounded tissues' strength and structural and functional integrity is known as wound healing [2].The skin, the body's biggest organ, serves as a barrier against outside forces. Skin lesions or diseases can result from the breakdown of skin tissue integrity, such as when a wound develops [3]. Wounds are a serious public health problem in industrialized nations, causing millions of dollars in annual costs because to the microbiological complications they generate. These consequences include poor healing, the growth of multi-resistant bacteria, and local or apparent infection [4]. An individual may experience financial and social hardship as a result of wounds or skin conditions. Among the top 5 skin illnesses that cause the most financial burden are wounds and ulcers on the skin. Products and treatments for wound care can speed up the healing process, prevent new wounds from developing, and improve the characteristics of newly healed skin [5]. Many cells and a large amount of connective tissue accumulate during the proliferative period that follows. Fibroblasts, keratinocytes, and endothelial cells are all present in the wound [6]. Wound healing is the term used to describe the process of tissue repair, which consists of an ongoing series of inflammation and healing within which platelets, fibroblasts, endothelial cells, inflammatory cells, and epithelial cells momentarily assemble outside of their usual domains and interact to restore characteristics of their previously used discipline and subsequently resume their normal function [7]. One of the main causes of weakness is injury. A wound that is an altered condition of tissue brought on by chemical, physical, microbiological, or immunological assaults, or that is generally linked to function loss The Wound Healing Society defines wounds as physical injuries that result in a break or opening in the skin that disturbs the normal anatomy and function of the skin.(Deep, Bansi, and Garg, n.)

1.2 Plant profile

S. alata (L.) Roxb. is a Fabaceae family blooming shrub. It gets its name from the structure of its inflorescences, which resembles a candle. With an average height of 1 to 4 meters, this annual or occasionally biennial plant grows fastest in areas that receive lots of sunlight and humidity. The leaves are oblong in shape, with 5 to 14 leaflet sets, dense blooms (20 × 50 by 3 × 4 cm), strong petioles (2 to 3 mm), and caduceus bracts (2 × 3 by 1 × 2 cm). Bright yellow in color, zygomorphic blooms with seven stamens and a pubertal ovary. Fruit is a 10 to 16 ×1.5 cm tetragonal pod with thick, flattened wings that is brown when ripe and contains numerous brown seeds in the shape of diamonds.(Oladeji et al. 2020)

Fig 1. Plant of *Senna alata*

2 Material and Method

2.1 Collection of Plant Material

Senna alata was verified from the botanical survey of India (BSI) in the Prayagraj government in India. The dried part of the plant was collected, and the specimen was certified by Dr. Vinay Ranjan, Scientist, Botanical Survey of India Central Regional Centre, 10 Chatham Lines, Prayagraj, 212002. A specimen with voucher number SIP/2024/087 has been deposited at the Botanical Survey of India.

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2.2 Pharmacognostic study

The macroscopic, microscopic, and physiochemical parameters of *Senna alata* bark were examined in the Pharmacognostic investigation.

2.2.1 Powder microscopic feature

Microscopic powder analysis revealed the presence of various cell types, including sclerenchyme fragments, diacytic stomata, spiral vessel fragments, palm parenchyme fragments with elongated cells, hairbripers, unicellular structures with punctuated surfaces, fiber fragments, skin fragments with rounded cells, and elements to be characterized in Senna alata sheets. The same plant has powdered diacytic stomata cells in its leaves. (Kasiama et al. 2022)

2.3 Extraction

2.3.1 Preparation of Extract

The plant components were cleaned twice: once with sterilized water and once with running tap water. In a dimly lit room, the leaves were shade dried. After being dried, the leaves were ground into a mortar and pestle to a fine powder and stored in an airtight glass container at 4ºC until needed. A Soxhlet apparatus was used to extract 20 grams of dried powdered S. alata bark into a cotton thimble, which was then filled with 100 ml of ethanol solvent and left for 6–8 hours. After that, the substance that emulsified was completely with the help of a rotating vacuum evaporator, evaporated to leave just the dried-out extract behind. The final the quantity of extracts was counted., and extract concentrations were prepared using the twofold dilution procedure.(Pachorkar and Patil 2021)

2.4 Determination of Physicochemical property

2.4.1 Loss on drying

About 1.5 grams of powder were placed into a thin, flat porcelain dish that was weighted. Dry in the oven at 105 ºC until the scale stays steady. Once it cools in the desiccator, weigh it in the end. Weigh loss while drying is referred to as moisture. (Shaikh 2022)

$$\textbf{Loss on drying } \% = \frac{\textbf{Initial weight of sample} - \textbf{Weight of sample}}{\textbf{Initial weight of sample}}$$

2.4.2 Swelling index

A number of phytopharmaceuticals including prctine, cellulose, and gum mucilage. They create two to four times their initial weight or volume when they come into touch with water and absorb it. The volume measured in milliliters (ml) that results from treating a particular quantity of air-dried plant material (1 gram) with an appropriate solvent (25 ml) is known as the swelling index. 1 gram of air-dried plant material is taken and transferred into a 25 ml granulated stoperd measuring cylinder, with each division holding 02 ml. The length of the 1 gram of air-dried material in the measuring cylinder, which has a length of 125 mm and an intern diameter of 60 mm, is then measured. 25 ml of water is added, and it is shaken occasionally for at least one hour before being left to stand for the following three hours. carefully examined the plant material's length that appears in the solvent's top and lower middile portions to identify the original value is subtracted from the final reading to get the swelling index.

$$\textbf{Swelling Index } \% = \frac{\textbf{Volume of swollen material (ml)}}{\textbf{weight of dr}}$$

2.4.3 Ash value

2.4.3.1 Total ash

Ash value determination is used to identify a crude medication and assess its quality and purity.

Method- Put around 2 grams of precisely weighed ground powdered air-dried medication in a crucible and burn it on a flame 2 cm high until the fumes nearly stop. After the ash has cooled in the appropriate desiccators for thirty minutes, weigh it right away. Determine the dry material's % total ash concentration in milligrams per gram. A 2g sample was weighed and allowed to air dry in a silicon dish that had been heavily tarred. It was completely carbon-free when burned at a temperature of no more than 450 ºC. After cooling, the ash was weighed and its percentage was computed.(Shaikh 2022)

$$\% \textbf{ Total Ash Value} = \frac{\textbf{Weight of Ash}}{\textbf{Weight of Drug}} \textbf{ x 100}$$

2.4.3.2 Acid insoluble ash

The ash was collected using the procedure for total ash mentioned above. After obtaining the ash, 25 ml of 2Ml hydrochloric acid were heated for five minutes. After being filtered, the insoluble material was also collected and cleaned in a Gooch crucible using hot water, lit, let to cool in a desiccator, and then weighed. The amount of

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acid-insoluble ash was computed using the medication that had been dried by air.(Shaikh 2022)

$$\textbf{Acid Inoluble Ash}\ (\%) = \frac{\textbf{Weight of crucible + Ash − Weight of Crucibl}}{\textbf{Sample dry Weight}}$$

2.4.3.3 Water soluble ash

The ash was collected using the procedure for total ash mentioned above. After obtaining the ash, 25 ml of water were heated for five minutes. After being filtered and the insoluble material gathered in a Grouch crucible, it was heated to a temperature of no more than 450 ◦C for 15 minutes and then ignited. The weight of the ash was deducted from the weight of the obtained insoluble material. The water-soluble ash was indicated by the difference in that weight. Using the dry medication, the proportion of water-soluble ash was computed.(Shaikh 2022)

$$\textbf{Water Soluble Ash}\ (\%) = \frac{\textbf{Dish Residue Weight}}{\textbf{Weight of Sample}}\ \textbf{x 100}$$

2.4.4 Extractive value determination

This technique, which extracts the medicinal plant material with solvents, counts the number of active components in a particular amount. Any crude medication can be extracted using a particular solvent to produce a solution that has various phytoconstituents. A solution containing several phytoconstituents is produced by the combination of these Phyto-constituents in that specific solvent. The type of the medication and solvent utilized determines the makeup of these Phyto-constituents in that specific solvent.(Shaikh 2022)

2.4.4.1 Alcohol soluble extractive value

Precisely weighed 5 grams of air-dried crude medication were added to a closed flask and macerated for 24 hours with 100 ml of 95 percent ethanol. During the first six hours, shake often and filter quickly to prevent alcohol loss. A 25-milliliter sample of the filtrate was obtained, dried on a water bath, and then dried completely at 105 degrees Celsius. Then weigh after cooling in a desiccator. Using the medicine air dried as a reference, the percentage of alcohol soluble extractive value was determined.(Shaikh 2022)

$$\textbf{Alcohol − Soluble Extractive}\ (\%) = \frac{\textbf{Weight of Residue}}{\textbf{Weight of Drug}}\ \textbf{x 100}$$

2.4.4.2 Water soluble extractive value

For 24 hours, 5 grams of the coarsely ground, air-dried medication must be macerated in a closed flask with 100 millilitres of water, stirring regularly for the first 6 hours and letting stand for the remaining 18 hours. After that, filter quickly while being cautious not to lose any water, evaporate 25 millilitres of the filtrate in a shallow dish with a tarred bottom, dry at 105 ^{o}C, and weigh. Calculations are made to determine the percentage of water-soluble extractive value in relation to the air-dried drug stop.(Shaikh 2022)

2.4.5 Chromatographic Study

2.4.5.1 TLC

A technique for solid-liquid adsorption, in which a liquid mobile phase interacts with a solid stationary phase, is thin-layer chromatography. Under the influence of the solvent, solid phase, and polarity of the substance, the mobile phase passes through the stationary phase and rises. The most prevalent substance is ninhydrin, which is distinguishable with blacklight viewing. Preservatives, active components, macromolecules, active substances, and synthetic production methods are examples of biological sources that may be purified using thin-layer chromatography. Additionally, it employs mobile phases mediated by cationic and non-ionic surfactants to distinguish pesticides from complex pharmaceutical components.(Sayed 2021)

2.4.6 Phytochemical Study

1. **Test for Alkaloids**:
   To 3 ml of the extract, 1 ml of 1% HCL was added. I next added a few drops of Meyer's reagent to this combination. The presence of alkaloids was established by the formation of a creamy white precipitate. (Ogunkwe et al., 2004).
2. **Identification of anthraquinones test**
   To find the anthraquinone aglycones in the extract, Borntrager's reaction was employed. After adding 2M hydrochloric acid to the sample, the mixture was boiled for 15 minutes in a hot water bath before being cooled and

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filtered. Chromatoform was used to extract the filtrate. After being separated, the chloroform layer was shaken with a 10% potassium hydroxide solution. The uppermost layer of water turns pink red.(Kamble, Shinge, and Shinde, n.d.)

3. **Test for Saponins**: 2 ml of the plant extract were combined with 5 drops of olive oil, and the mixture was given a good shake. The development of a stable emulsion suggested saponins were present. (Trease and Evans, 1996)
4. **Test for Tannins**: 1 ml of the plant extract was mixed with two drops of 5% FeCl3. Tannins were present because a dirty-green precipitate appeared. (Trease and Evans, 1996)
5. **Test for Flavonoids**: 3 drops of ammonia solution (NH_3+) and half a ml of strong HCl were put to one milliliter of the extract. The combination as a whole turned a pale brown colour, which suggested the presence of flavonoids. (Odebiyi and Sofowora, 1978).
6. **Test for Steroids**: 1 ml of pure tetra-oxo-sulphate (vi) acid (H2SO4) was added to one ml of the plant extract. The presence of steroids was established by a red coloring. (G., J.E, and J. M 2014)
7. **Test for Protein:** To 2 ml of extract, add 2 ml of the Biuret reagent. Give it a good shake and reheat in a bath of water. The appearance of red or violet indicates the presence of proteins. colour.
8. **Test for Resins**: 5 ml solution of copper acetate was added to 5 ml of the extract. After giving the mixture a good shake, it was let to separate. There were resins present because a reddish-brown precipitate appeared. (Elmahmood and Doughari, 2008).
9. **Carbohydrate test:** Add 1 ml Fehling's A with 1 ml. The Fehling's B solutions require a minute to boil. Pour in the same amount of test extract solution. Take a 5-to 10-minute bath in hot water to heat. Carbohydrates are present when an orange-red precipitate appears.

2.5 Pharmacological activity

2.5.1 Experimental animals

The protocol of the experiment SIP/IAEC/028/3/24 was approved by Institutional Animal Ethics Committee (IAEC) of Pravara Rural College of Pharmacy, Loni and was conducted in accordance with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.4.7 Preparation of gel

Carefully weighted in a beaker, carbopol 934 was added and mixed with 50 milliliters of pure water. After setting the beaker aside to allow the carbopol to expand for 30 minutes, stir the mixture with a mechanical or lab stirrer set at 1200 rpm for 30 minutes. Take the necessary amount of Extract and 5 milliliters of propylene glycol. Transfer 5 milliliters of propylene glycol to a separate beaker, then accurately weigh out and mix in methyl and propyl paraben. Ultimately, 1 gram of extract, Carbopol was disseminated, and solutions of preservatives were added while continuously stirring. Lastly, the remaining distilled water was added to bring the amount up to 100 ml, then triethanolamine was added drop by drop.(Jamadar and Husen Shaikh 2017)

Table: -1 Formulation of Gel

Sr.no.	Ingredients	Quantity
1	Carbopol-934	2gm
2	Propyl paraben	0.1ml
3	triethanolamine	Required quantity
4	Distilled water	q.s to 100ml
5	Extract of *senna alata*	1gm

2.5.2 Excision wound model

Rats in the excision wound model were depilated prior to wounding by shaving off hairs in the dorsal thoracic region. Diethyl ether was used to numb the rats' bodies before they were removed. During this

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phase of the investigation, rats with depilated dorsal thoracic regions had an aseptic circular wound of 2.5 cm in diameter created on them. The wounds were promptly measured (in millimeters squared) by covering the wound with a transparent polythene graph paper and drawing the approximate 500 mm2 area of the wound on it. This was the first measurement of the wound area. There are four groups (n=6) created from the rats. I used the animal in group I as a control, applying topically just ointment base.(Dubey et al. 2022)

2.5.3 Group Divided of animals

The following organized medication was given to the rats, who were split into three groups (n = 6).

Table:2 Group of Animals

Group-I:	Control (diseased).
Group-II:	Standard (5% w/w povidone iodine ointment USP applied topically)
Group-III:	*Senna alata* Bark (5%) applied topically

photographed throughout the measuring process using an appropriate scale and image.(Dadgar, Noori-Zadeh, and Pakzad 2014)

2.5.5 Treatment protocol

Rats in the models of excision wounds were separated into three different groups at random (n = 6/groups). (18) rats in all received the following treatment. (Shaikh 2022)

2.5.4 Wound healing evaluation

During surgery, the medication was administered, and the margins of the excised incision were tracked every two days. For 21 days, measurements of the wounds were made utilizing digital image analysis. Up to 21 days were allowed for between measurements. The proportion of the wound area that has healed was used to indicate the wound contraction. Using a template and calliper, the wound surface was measured every several days until full healing. In order to ensure the validity of manual measurement, each rat was

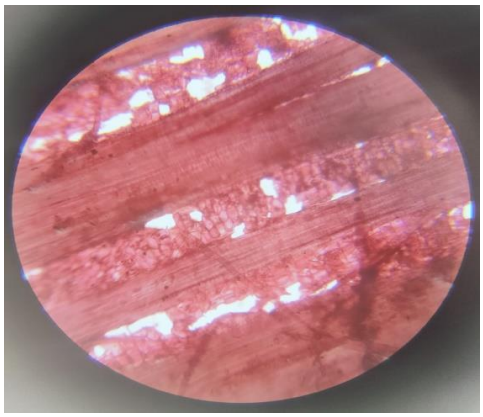
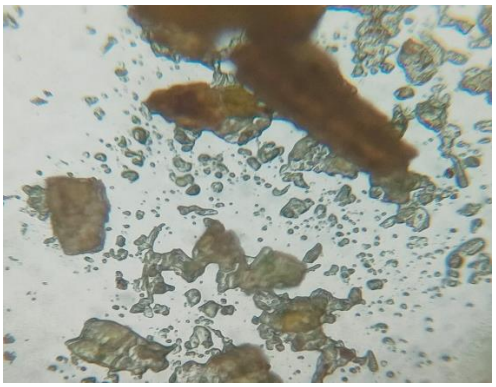


Fig 2. Compound Microscope of *Senna alata* bark



3. Result

3.1 Plant authentication

Plant materials were authenticated at Botanical Survey of India Central Regional Centre, 10 Chatham Lines, Prayagraj, 212002. A specimen with voucher number SIP/2024/087 has been deposited at the Botanical Survey of India.

3.2 Experimental animals' approval

The experimental procedure was approved by the Institutional Animal Ethics Committee of Shambhunath Institute of Pharmacy provide approved number SIP/IAEC/028/03

3.3 Pharmacognostic study

3.4 Microscopic examination of Bark

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Fig 3. Powder Microscopic of *senna alata* bark

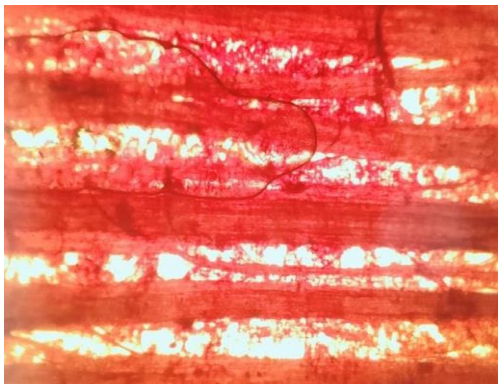

Fig 4. Projection Microscopic of *Senna alata* bark

Table 3-: Characterization for bark of *senna alata*

Sr. no.	Bark powder	Characteristics
1	Colour	Light green
2	Odour	Faint
3	Test	Pungent

3.5 Preparation of Extract

3.5.1 Find of percentage yield

A higher percentage yield indicates that a researcher obtained a greater amount of product after extraction. The formula below is used to calculate percentage yield, which is a measure of the effectiveness of the entire extraction process. It shows how much product a researcher has obtained after running the procedures against how much is actually obtained.(May and Bandiola, n.d.)

% **yield** = Weight of actual dried extract x 100 % of weight

Table: 4 Extractive values of bark of *senna alata*

Sample	Extraction method	Solvent	w.t of sample	Yield (%w/w)
Senna alata bark powder	Soxhlet extraction	Ethanol	20gm	22.8

3.5.2 Determination of Physicochemical property

Table-: 5 Physio-chemical properties

Sr. no.	Test	Observed value in %
1	Total ash	11
2	Acid in soluble ash	0.75
3	Alcohol soluble extractive value	22.8
4	Water soluble extractive	14
5	Water soluble ash	0.5
6	Loss on Drying	6.52
7	Swelling index	0.7

3.5.2 Determination of solubility for Bark of *senna alata*

Table: - 7 Solubility of extract

Sr. no.	Solvent	Extract
1	Ethanol	Soluble
2	Distilled water	Soluble
3	Chloroform	Soluble
4	Propylene glycol	Soluble

3.5.3 Chromatographic studies

Table 8 TLC with solvent system I Ethyl acetate: n-hexane (5:10)

Sr. no.	Plant species	Extract	Distance travelled by solute (cm)	Distance travelled by solvent (cm)	R_f value
1	*Senna alata*	Ethanol	11.2	18	0.62
			16.2	18	0.9

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2	***Senna alata***	**Aqueous**	**11.4**	**18**	**0.63**
3	***Senna alata***	**Hydroalcoholic**	**15**	**18**	**0.83**
			12.4	**18**	**0.68**
4	***Senna alata***	**Ethyl acetate**	**17**	**18**	**0.94**
			12.6	**18**	**0.7**

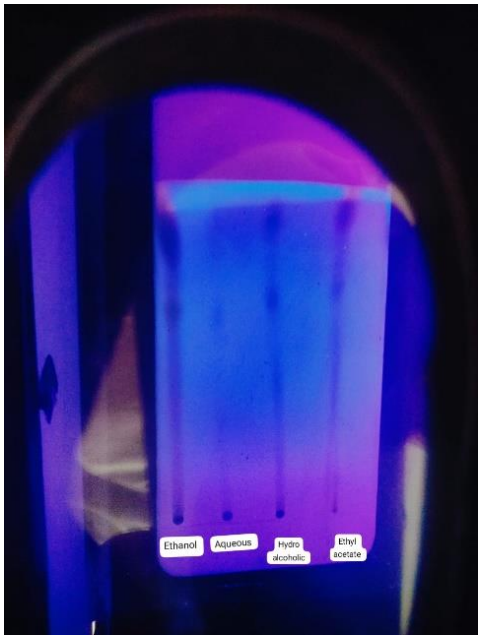


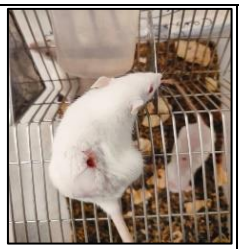
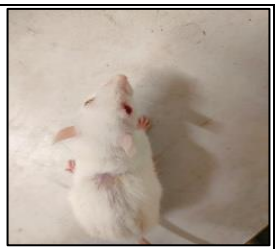
Fig 3. Thin layer Chromatography

Table: - 8 Phytochemical screening of *senna alata*

S.no.	Phytochemical Chemical Constituents	Observation	Ethanol extract
1	Alkaloids	The test solution gives cream colour ppt	+
2	Saponins	dirty-green precipitate appeared	+
3	Flavonoids	pale brown colour	+
4	Steroids	red colour	+
5	Resins	reddish-brown precipitate appeared	+
6	Tannins	dirty-green precipitate appeared	+
7	Protein	red or violet colour	–
8	Carbohydrate	orange-red precipitate	–

Phytochemical Study

3.6 Pharmacological activity

Control group	 **Day 3**	 **Day 6**	 **Day 9**	 **Day 12**	 **Day 15**

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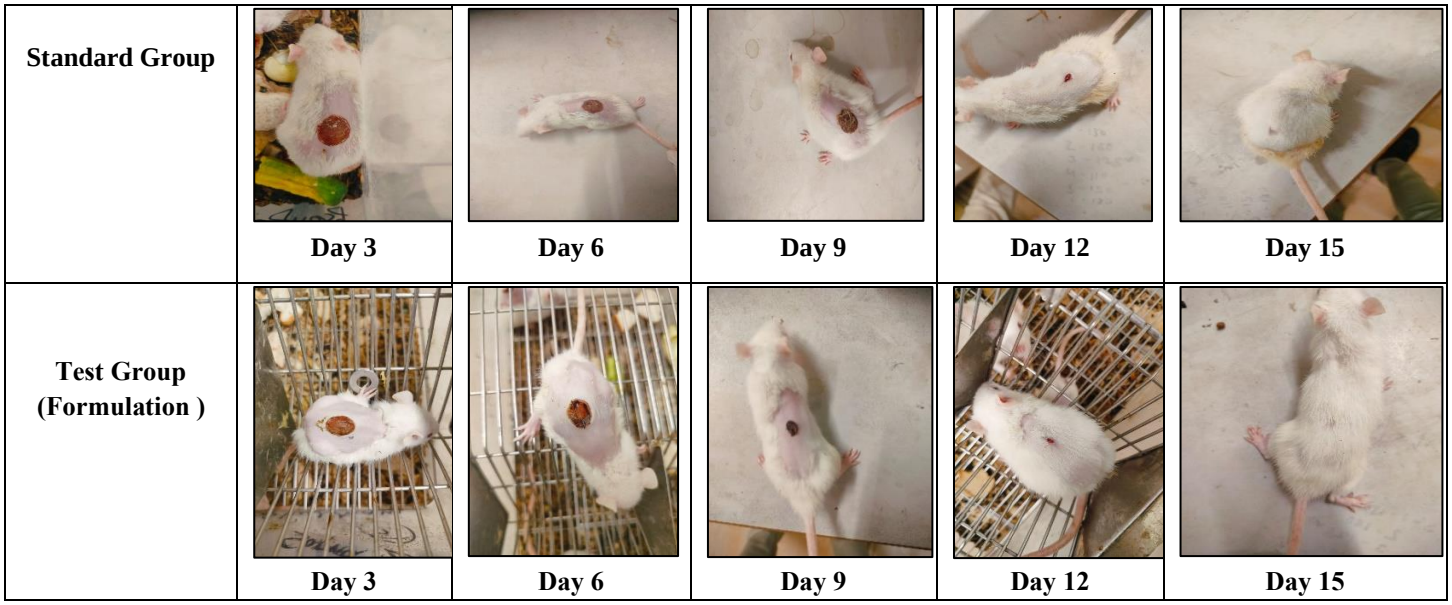

Fig 4. Impact of herbal formulation on excision wound model healing

3.6.1 Wound Healing Activity

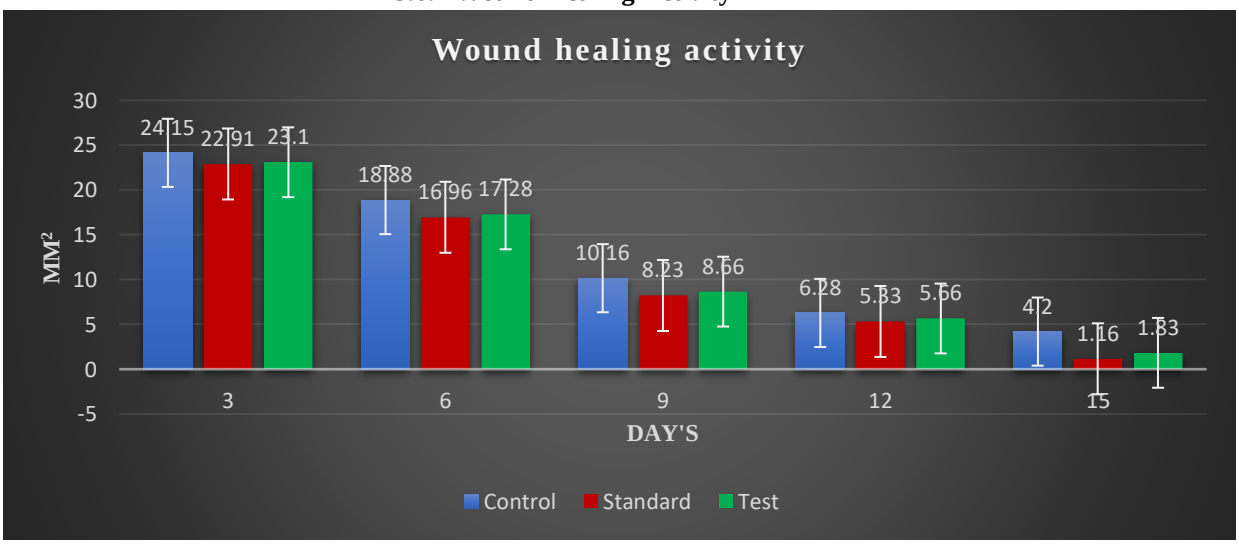

Fig 5. Impact of standard, Test 1, Test 2 on the size of the wound

3.6.2 Wound Contraction Studies

Photographs were used to display the findings of investigations on wound contraction. Based on data on constriction of the wound area, it was determined that formulation group 3 caused a larger amount of wound contraction than the other tested formulations, and that the amount of wound contraction increased throughout the course of the day. Based on the data shown in the table, it was also determined that the ointment formulation significantly ($p<0.08$) accelerated the healing of wounds in comparison to the control group.(Kolhe 2018)

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Table 9 Impact of Herbal Formulations Applied on Rat Excision Wound Models

Group	Treatment	Wound Contraction(mm^2) on a day				
		3 days	6 days	9 days	12 days	15 days
Control	-	24.15 ± 0.35	18.88 ± 0.44	10.16 ± 0.57	6.28 ± 0.39	4.2 ± 0.51
Standard	5 % w/w Povidone iodine Ointments USP	22.91 ± 0.48	16.96 ± 0.51	8.23 ± 0.47	5.33 ± 0.82	1.18 ± 0.44
Test	*Senna alata* bark	23.1 ± 0.56	17.28 ± 0.61	8.6 ± 0.46	5.6 ± 0.45	1.83 ± 0.43

(Mean ± SD, n = 6) expressed as period of epithelization in each group on the post wounding day * P

3

.6.3 FTIR Analysis of S. alata Extracts:

An infrared absorption spectrum is produced by the Fourier Transform Infrared Spectroscopy (FTIR), which is used to determine the chemical bonds of a molecule. FTIR spectrophotometer was used to identify the bioactive components of S. alata in ethanolic plant extracts.

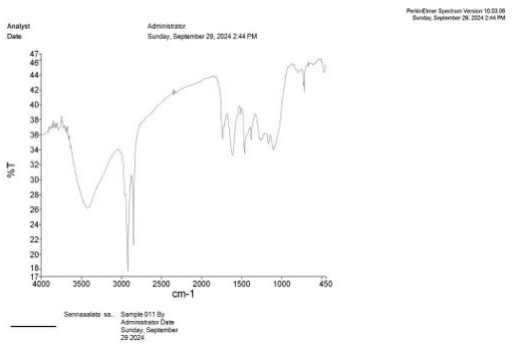

Fig. 5 FTIR ANALYSIS OF ETHANOLIC EXTRACT OF S. ALATA (Bark)

4. Discussion

The Pharmacognostic study included both macroscopic and microscopic analyses, confirming the identity of Senna alata through distinctive odors and light green color. Physicochemical evaluations showed low moisture content and significant extractive values, indicating favorable parameters for medicinal formulations. Phytochemical screening identified bioactive compounds in hydroalcoholic extracts of Senna alata, including steroids, flavonoids, and alkaloids. Formulation studies showed that Senna alata extracts could be included into gel preparations with good consistency and stability suitable for topical applications. Pharmacological investigations using an excision wound model in rats revealed that Senna alata formulations promoted accelerated wound healing. In summary, the study highlights the potential of Senna alata as a viable herbal remedy for wound healing, bolstered by its identified bioactive compounds and favorable formulation characteristics. To fully realize its therapeutic potential, more research into its mechanisms of action and wider clinical applications is advised.

5. Conclusion

In this study examined *Senna alata* capacity for wound healing using a thorough Pharmacognostic, physicochemical, phytochemical, and pharmacological assessment. The plant, which has long been used medicinally, was examined in terms of both its macroscopic and microscopic features. This analysis

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provided important information on the identity and composition of the plant. Hydroalcoholic extracts rich in bioactive substances with therapeutic potential, including steroids, flavonoids, and alkaloids, were produced during the extraction process. The plant's favorable qualities, such as its low moisture content and significant extractive values, were shown by physicochemical tests, demonstrating its appropriateness for use in medicinal formulations. *Senna alata* extract was used to create the gel formulation, which showed encouraging consistency and stability and suggested prospective uses in topical applications. *Senna alata* extract-containing formulations facilitated quicker wound healing than control groups, according to pharmacological experiments conducted on an excision wound model in rats. This was demonstrated by considerable wound contraction rates over a 15-day period. These results imply that *Senna alata* has potential as a homeopathic treatment for hastening the healing of wounds. Overall, the potential of *Senna alata* as a useful therapeutic agent in wound care is highlighted by the fusion of traditional herbal therapy with contemporary scientific approaches. To further understand its methods of action and investigate its wider therapeutic potential in clinical settings, more study is necessary.

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