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Effect of *schleichera oleosa* bark extract in wound healing activity

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ABSTRACT

This study evaluates the wound healing potential of *Schleichera oleosa* bark extract using an excision wound model in albino rats. The plant material was collected, authenticated, and extracted using methanol via Soxhlet apparatus. Pharmacognostic, physicochemical, and phytochemical analyses revealed the presence of key bioactive constituents such as flavonoids, tannins, terpenoids, and saponins. Two concentrations (5% and 10%) of the extract were formulated into ointments using standard British Pharmacopoeia guidelines. These ointments were applied to wounds in rats and compared with a control group and a standard povidone-iodine treated group. The results showed a significant, dose-dependent increase in wound contraction, particularly in the 10% extract group, which exhibited nearly complete healing by day 22. Statistical analysis confirmed the extract's efficacy with p-values <0.05 and <0.0001 on the 7th and 14th days, respectively. The enhanced wound healing is attributed to the extract's antioxidant, anti-inflammatory, and antimicrobial properties. The study supports the traditional use of *S. oleosa* in wound management and underscores its therapeutic potential as a natural alternative for wound care.

Keywords:

Schleichera oleosa, wound healing, phytochemical analysis, excision model, herbal ointment.

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1. INTRODUCTION

Wound healing is a complex and dynamic biological process that involves a series of overlapping phases, including hemostasis, inflammation, proliferation, and remodeling. These processes are influenced by a variety of factors, such as the extent of injury, patient health, microbial presence, and the use of therapeutic agents. In recent years, increasing attention has been given to the role of medicinal plants in promoting and accelerating wound healing due to their accessibility, cost-effectiveness, and minimal side effects. Among such plants, *Schleichera oleosa*, commonly known as Kusum, has emerged as a promising candidate for natural wound healing therapies due to its diverse pharmacological properties. *Schleichera oleosa* is a medium to large deciduous tree native to the Indian subcontinent and Southeast Asia. Traditionally, various parts of this plant have been utilized in folk medicine for the treatment of skin disorders, inflammation, and infections. Its bark, in particular, has been reported to contain a variety of bioactive constituents such as flavonoids, tannins, saponins, glycosides, and triterpenoids, many of which are known for their antimicrobial, anti-inflammatory, and antioxidant activities **(Kirtikar & Basu, 1996).** These phytochemicals contribute significantly to wound repair by promoting fibroblast proliferation, collagen synthesis, and angiogenesis, while also preventing oxidative damage and microbial infections **(Mukherjee et al., 2000).** The present study investigates the wound healing potential of methanolic bark extract of *Schleichera oleosa* using an excision wound model in albino rats. The extract was incorporated into ointment formulations at 5% and 10% concentrations, and their efficacy was evaluated in terms of wound contraction, healing duration, and histopathological changes. The pharmacognostical and physicochemical parameters of the plant material were also assessed to ensure quality and consistency, in accordance with WHO guidelines on herbal drug standardization **(WHO, 2002).** Preliminary phytochemical screening confirmed the presence of key constituents like flavonoids and tannins, which are well-documented for their role in enhancing tissue regeneration and controlling infection **(Tiwari et al., 2011)**. Furthermore, statistical evaluation of the wound healing data demonstrated that the groups treated with *Schleichera oleosa* extract ointments showed significantly higher wound contraction rates and faster healing compared to the control. The 10% w/w extract ointment, in particular, exhibited notable efficacy, nearly matching the performance of the standard povidone-iodine treatment. This effect may be attributed to the synergistic action of the plant's phytoconstituents that promote cell migration

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and proliferation while also mitigating inflammatory responses. In light of growing antibiotic resistance and the demand for safer therapeutic options, plant-based interventions such as *Schleichera oleosa* present a valuable alternative. This research not only reinforces the ethnomedical use of *S. oleosa* bark in wound care but also paves the way for its integration into modern pharmaceutical formulations for dermal applications.

2. MATERIAL AND METHOD

2.1 Collection and Authentication of plant –

Fresh outer bark and leaves of *Schleichera oleosa* were collected from Kharsia, Raigarh district, Chhattisgarh, India, during May–June 2022. The plant material was washed, air-dried for two weeks, and then ground using a mortar and pestle. The identity of the plant was authenticated by Dr. Ranjana Shrivastava, Department of Botany, Govt. V.Y.T. PG Autonomous College, Durg, Chhattisgarh. The dried, powdered plant material was extracted using methanol as a solvent in a Soxhlet apparatus. Various chemicals, including mercuric chloride, potassium iodide, concentrated hydrochloric acid, lead acetate, ferric chloride, chloroform, acetic anhydride, concentrated sulfuric acid, and trichloroacetic acid, were used for phytochemical screening, all provided by the institute.

2.2 Extraction using soxhlet

The Soxhlet system was used to extract ten grams of powdered Schleichera oleosa plant matter using methanol. The solvent was collected for chemical analysis. Six extraction rounds were completed in three hours using both pure and blended solvents. The resulting crude extract solutions were dried in an atmosphere oven at 60°C or below in a vacuum rotary evaporator. High temperature treatment was avoided to reduce component degradation. The extract was then used for a primary phytochemical analysis to identify plant components. The extracted extract was then used for extractive value tests, powder microscopy, and phyto-chemical analysis **(Azwanida, 2015)**.

2.3 Pharmacognostical evaluation

Macroscopic and microscopic studies were conducted on freshly collected leaves of *Schleichera oleosa*. Macroscopically, the plant exhibited greenish-yellow flowers, red to green pinnate leaves with coriaceous texture, ovoid indehiscent fruits, and black seeds with a thick brown coat. The tree grows wild, reaching 10–15 m in height, with bark thickness of 10–12 mm. Microscopically, transverse sections of the leaf showed a single-layered epidermis, polygonal hypodermal cells, two layers of palisade parenchyma, spongy mesophyll, collenchymatous tissue, phloem, xylem, and a central pith. Powder microscopy

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was carried out on shade-dried and coarsely ground material, treated overnight with 25% nitric acid, then stained with safranin. The sample revealed calcium oxalate crystals, parenchyma, trichomes, sclereids, epicarp, endocarp, endosperm, perisperm, and various vessel elements, indicating the presence of key anatomical features for plant identification and quality assessment **(Narayanan et al., 2025)**.

2.4 Phytochemical investigation

By performing a thorough qualitative phytochemical analysis, the experiment was designed to determine whether or not various phytoconstituents were present. The precipitate formation or color intensity was utilized to gauge how the body would react to various tests. Standard operating procedures were employed **(Tiwari and Pandey, 2017).**

2.5 Physicochemical analysis

2.5.1 Total Ash Value

To determine the total ash value of a crude drug, approximately 10 grams of the powdered sample is accurately weighed and placed in a silica crucible. The sample is gradually incinerated by increasing the temperature to 500–600°C until it becomes carbon-free, indicating complete combustion of organic matter. The crucible is then allowed to cool in a desiccator to avoid moisture absorption. The residue, which primarily consists of inorganic salts like carbonates, phosphates, and silicates of sodium, potassium, calcium, and magnesium, is weighed. The total ash content is calculated as a percentage of the air-dried sample using the formula:

Total ash (%) = (Weight of ash / Weight of sample taken) × 100.

This method is used to assess the purity and quality of crude drugs by determining the presence of inorganic matter.

2.5.2 Ash insoluble in acid:

25ml of dil. hydrochloric acid was used to boil the solution of acid for five minutes containing ash in crucible. Unsolvable material gathered either on paper or in a melting pot that had fewer ashes, and then this Paper filters without ash was rinsed in steaming water. The unsolvable matter dried and weigh after it was cooled for minutes in the desiccator. The medicine that had been air-dried was used as a reference to calculate the proportion of acid-insoluble ash.

2.5.3 Assessment of ash's water solubility:

20ml distilled water has been used to bring the residue ash to a 5-minute boil. Insoluble

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material was gathered, rinsed with hot water, burned, and weighed either on ash-free paper filters or in melting pot. The weight from the residue, ash was deducted based on the unsolvable weight material. The amount of weight differential represents the water-soluble ash. In order to calculate the air-dried medication's proportion of water-soluble ash was utilized as a standard.

2.6 Extractive value accessible in water

Five grams of coarsely powdered, air-dried drug was macerated with 100 mL of filtered water in a closed flask for 24 hours, with mechanical shaking for the first 6 hours followed by 18 hours of standing. The mixture was then filtered using Whatman No. 41 filter paper. From the filtrate, 25 mL was evaporated to dryness in a pre-weighed flat-bottomed petri dish, dried at 105°C, and weighed. The water-soluble extractive value (% w/w) was calculated using the formula:

Extractive value = (Weight of residue / Volume of extract evaporated × Weight of sample) × 100.

2.6.1 Extractive value accessible in alcohol

Five grams of coarsely ground, air-dried medicine was soaked in 100 ml of 100% or 70% alcohol for a full day in a closed flask, shaken for a period of six hours, and then allowed to stand for 18 hours. The liquid was then rapidly filtered via Whatman filter paper No. 41. A pre-weighed, flat-bottomed petridish containing 25 milliliters of the filtrate was dried at 105°C until it was completely dry, and then it was weighed. Using the dried using air drug and the same procedure as the water soluble extractive value above, the % w/w alcoholic soluble extraction value was determined.

2.6.2 Extractive value accessible in ether

Five grams of air-dried, coarsely powdered drug was extracted using a Soxhlet apparatus with ethyl ether for 20 hours. The ether extract was collected in a petri dish and dried to a constant weight at 105°C. The percentage of ether-soluble extractive was then calculated based on the weight of the air-dried sample used **(Chandel et al., 2011)**.

2.7 Experimental animals

Either sex albino rats (100-150 g) employed in the experiment. They were given a pellet diet and access to water while being confined to cages in accordance with circumstances (22-25 degrees Celsius, 35-55 % humidity levels, and twelve-hour cycles of light and darkness). They were used to the lab environment for a week prior to the trial. Prior to the test, the animals were fasted for the whole night and all the procedures used in this The Institutional

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Animal Ethics Committee authorized the experiments.

2.8 Formulation for ointment

Trituration method is used for that mortar and pestle was used to create simple ointments containing plant extract in concentrations about 5% and 10% (w/w). The bases such as white soft paraffin, wool fat and other ingredients were combined slowly, heated slightly in hot water bath using china dish, while being stirred with glass rod to establish homogeneity, and then stirred until it cooled. After cooling of simple ointment powdered extract is added. Simple ointments made from the experimental plant materials' extracts were created using British Pharmacopoeia's recipe **(Mulisa et al., 2015).**

Table 1. Ointment formula

Ingredients	Master formula (1000g)	Reduced formula (200g)
wool fat	50g	10g
White soft paraffin	850g	170g
Hard paraffin	50g	10g
Cetostearyl alcohol	50g	10g

2.9 Grouping of animals

Excision wound models were performed on four groups of six animals each. The reference standard, control, and extract ointment (5%) and (10%), respectively, had been administered to experimental subjects within groups I, II, III, and IV. The Group 1 rats' wounds were treated topically with a vehicle containing no plant extract twice daily, whereas the Group 2 and Group 3 animals' wounds were treated topically with simple ointment containing 5% and 10% plant extract, respectively. As a reference, Group 4 rats' wounds received twice-daily topical application of commercial ointment.

Group 1 consist of control group

Group 2 served as standard drug consisting povidone iodine

Group 3 - extract *s.o.* 10% of crude drug

Group 4 - extract *s.o.* 5% of crude drug

2.10 Test for acute toxicity

The animals were split up into four groups, each with six animals. Extract applied on to the rat to determine the dermal toxicity as a single dose according to its body weight. These rats were observed at regular intervals for development of any toxicity. As such no toxicity was developed in the rat all along the monitoring period **(Pokhrel et al., 2015)**.

2.11 Excision wound model

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Six animals each made up each of the four groupings. One day before to the test, the particular area of skin was taken off. With the help of a round seal, a consistent wound area of 250 mm ^{2} in diameter was aseptically excised from the dorsal neck of all rats. The wounds were bare and open to the elements. The ointment base, standard medication ointment, and extract of plant ointment (5% w/w & 10% w/w) were topically administered to the control group, standard group, and treatment group, respectively, until the wound healed completely. The wound contraction log was kept up to date. During the first two days after wound development, the percentage of wound contraction was determined **(Meshram et al., 2015).**

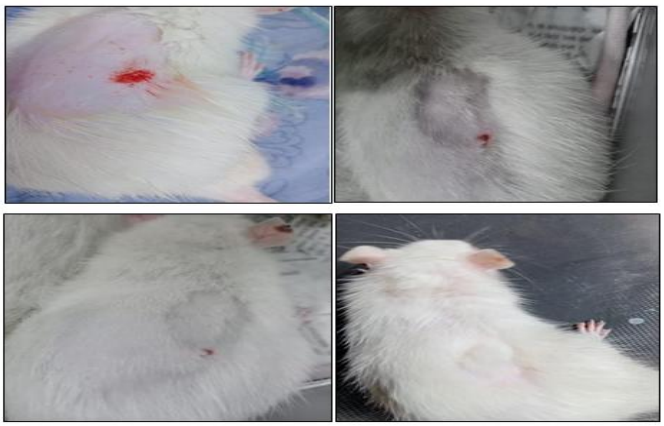

Figure 1. Excision wound in rats

2.12 Method for Evaluation of Wound Healing Activity

An excision wound model was used to evaluate the wound healing activity in rats. Twenty-four healthy rats were divided into four groups (n=6). Group I served as the control and received only the vehicle. Group II received standard treatment with povidone-iodine ointment. Groups III and IV were treated with *Schleichera oleosa* extract ointment at concentrations of 5% and 10%, respectively. A uniform excision wound (250 mm²) was created on the dorsal neck region of each rat. The wound area was traced onto transparent sheets every two days and then transferred to graph paper for surface area calculation. Wound contraction was assessed by comparing the wound size on different days with the initial wound size using the formula:

% Contraction = [(Initial wound size – wound size on specific day) / Initial wound size] × 100.

Wound healing progress was monitored from day 0 until complete recovery.

2.13 Statistical analysis

Outcomes from the two injury model were compared between the treated and control groups,

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and results were stated as mean ± SEM in a one-way ANOVA was used to statistically evaluate the data and compare the treated and control groups. At P <0.05 the data were deemed significant.

3. RESULTS

3.1 Physico Chemical Analyses

The plant extracts *Schleichera oleosa* was analysed through many different physicochemical parameters and the results obtained are mentioned below in table no.2

Table 2 – Analysis of *Schleichera oleosa* extract physicochemical parameter

S. No.	Parameter	Percentage (w/w)
1.	Total ash drying	5.90
2.	Acid-insoluble ash value	4.51
3.	Water-soluble ash value	2.97
4.	Foreign matters	2.56
4.	Moisture content	8.36

3.2 Extractive value test

The extract of *schleichera oleosa* was undergone for determination of extractive value test parameter by the use of aqueous, methanol and petroleum ether. The extractive values are determined using this calculation:

Extractive value in % = weight of dry extract obtained/weight of drug ×100

Table 3 – Analysis of extractive value obtained from extract

S.no	Chemical	Value (w/w)
1.	Petroleum ether	3.23%
2.	Methanol	8.34%
3.	Water	4.32%

3.3 Preliminary phytochemical test – Quantitative and Qualitative analysis of *Schleichera oleosa* extract

There are numerous secondary metabolites observed in the plant extract that were identified using their specific test mentioned before in the method and material of chapter no 4.

3.4 Phytochemical parameter testing

The plant, *schleichera oleosa* was extracted through using methanolic solvent for the phyto-chemical test conducted for the qualitative profiling of the plant extract which was done using the specified standard methods. The results obtained from the test after reagent addition concluded based on the colour changing properties, precipitate formation or ring formation as shown on the table below-

Table 4. Phytochemical test result of plant extract

S. no	Compounds in plant	Reagent or test	Result
1.	Alkaloids	Mayer's reagent	-
		Wagner's reagent	
		Dragendorff's test	

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2.	Flavonoids	Shinoda's test	+
		Alkaline reagent test	
3.	Glycosides	Keller killiani test	+
		Legals test	
4.	Steroids	Libermann	+
		Burchard test	
5.	Triterpenoids	Libermann Burchard test	+
6.	Saponin	Foam test	+
7.	Phenolic compound and tannins	5% FeCl3 solution	+

3.5 Observation of Pharmacological activities of wound healing

Table 5 Effect of methanolic extract of *schleichera oleosa* wound contraction on excision wound model in rats

Recovery in days	**Percentage of wound contraction**			
	Control	**Standard**	**Extract 10%**	**Extract 5%**
2	6.06±1.732%	13.10±1.202%	11.96±0.87%	8.06±0.924%
4	17.28±1.972%	35.55±3.772%	29.65±3.819%	26.25 ±3.095%
6	35.93 ±2.595%	56.28 ±1.888%	42.10 ±3.523%	40.83± 2.054%
8	45.02± 1.065%	79.00 ±1.251%	61.80 ±1.005%	59.88 ±1.158%
10	56.38± 1.935%	87.98 ±1.137%	80.66 ±2.270%	74.27 ±0.728%
12	73.91 ±1.176%	94.18 ±1.197%	88.75 ±1.172%	82.84 ±1.419%
14	81.44 ±0.704%	97.03 ±0.462%	94.78± 0.616%	85.83 ±1.048%
16	86.76± 0.754%	99.63 ±0.228%	96.58 ±0.608%	91.69 ±1.609%
18	90.31 ±0.959%	-	99.52 ±0.204%	94.63 ±0.728%
20	95.24± 0.582%	-	-	96.67 ±1.352%
22	97.14± 0.400%	-	-	99.47 ±0.211%
24	99.25±0.087%	-	-	-

Mean standard deviation (SEM; n = 6). After doing a one-way ANOVA of the outcomes, Dunnett's post hoc test was performed.

Table 6. ANOVA test result on decreased wound length

Treatment day	**Decrease of wound length**	**SS**	**DF**	**MS**	**F**	**P value**
7th day	Treatment (between columns)	2697	3	899	9.184	0.0020
	Residual (within columns)	1175	12	97		
	Total	3872	15			
14th day	Treatment (between columns)	502.5	3	167.5	170.8	<0.0001
	Residual (within columns)	11.77	12	0.9805		
	Total	514.3	15			

One way ANOVA (n=6) was used followed by dunnett's post hoc test. SS = sum of square, DF = degree of freedom, MS = mean square.

4. DISCUSSION

In this study of wound healing activity, the healing process consist of the following phases such as hemostasis, inflammatory phase, proliferative and followed by remodelling phase

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after which it is stated that the wound is completely healed. These events entail a variety of cellular processes such as migration, proliferation, adhesion, phenotypic differentiation, and so on. Clot development occurs immediately after damage, and the early stages of wound healing entail inflammation and ground material creation. The ground material is mostly composed of proteoglycans, which are heterogeneous, non-fibrillar extracellular matrix components. The glycosaminoglycans (GAGS) are intricate macromolecules composed within a tightly bound covalently peptide core bonded along straight hetero-polysaccharides. Skin provides barrier cells associated with inflammation propel endothelium cells' motility and multiplication during tissue healing, which leads to the formation of vascular of connective tissue cells—which produce extracellular matrices like collagen—and the re-epithelialization of damaged tissue. The recuperation of wounds characteristic *s.o.* might have been linked about the phytoconstituents contained within the plant's body, as faster wound healing process could involve product in two ways the addition or the person themselves actions among the active components present in plant. Faster healing of excision wound has been contribution of various phytochemical found in the plant extract.

Using the extract directly to the afflicted wound does not provide the intended effect since It leaves the laboratory animals' injured skin without staying there for an extended period of time. To induce continuous medication release at the application locations, ointment is required. As a result, hydrophobic substance basis chosen according to standard claims, considering the productive molecules in *schleichera oleosa* predominant polarized elements that could be made accessible easier starting with the nonpolar core and the other way around. The hard and white soft paraffin in the ointment base acts as an occlusive membrane for humidity as well. Ointment stability is maintained with the use of thickeners such as ceto-stearyl alcohol and wool fat.

In excision wound model, the findings of this research on the recovery of wounds activities demonstrated that the unrefined extract considerably boosts effects on wound healing in groups treated with 10% (w/w) and 5% (w/w) extract ointment. This is further supported by the fact that the more the drug's effectiveness and the faster the wound heals with more effective therapy, the greater the reduction in the rate of wound contraction.

The crude extract consisting of 80% methanol of *s.o.* bark demonstrated decrease in wound area that is sufficiently significant when compared to the negative control group in an excision wound healing model. The 10% (w/w) extract ointment treatment group had

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faster wound area shrinkage through day 6 to day 14. In contrast, the prepared 5% (w/w) extract ointment treated group began to exhibit statistically significant wound area contraction on day 8. The extract ointment's enhanced pace of wound contraction may be ascribed to its capacity to inhibit bacteria in a dose-dependent manner or to stimulate the growth of macrophage cells.

Another factor responsible for the improved impact of wound recovery might being the raw extracts of *schleichera oleosa*, have anti-oxidant, free radical scavenging, and cell proliferating characteristics. Other investigations on the *schleichera* plant have indicated plant exhibits anti-inflammatory, antipyretic, and antioxidant capabilities, which support the significance having antioxidant and free radical scavenging characteristics in the process of recovering from injuries.Several researches demonstrate the importance of phytochemicals in wound healing. Such as, tannins are strong detoxifying agents that prevent bacterial development; terpenoids enhance wound healing due to their astringent and antibacterial properties; and Flavonoids contain powerful anti-oxidants and free radical scavengers. Flavonoids and Polyphenol, which inhibit prostaglandin production, have conformed to have anti-inflammatory and anti-bacterial activity effects.

5. CONCLUSION

The study concludes that the 80% methanolic extract ointment of *Schleichera oleosa* bark is effective and safe for wound healing, likely due to its rich content of flavonoids, terpenoids, and tannins. These phytochemicals exhibit antimicrobial, anti-inflammatory, and antioxidant properties, which enhance wound recovery by reducing oxidative stress, promoting collagen formation, and accelerating tissue regeneration.

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