

## EVALUATION OF THE WOUND HEALING PROPERTIES OF METHANOLIC BARK EXTRACT OF *SAMADERA INDICA* GAERTNER *IN VIVO*

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Received: 05 March 2019, Revised and Accepted: 11 April 2019

### ABSTRACT

**Objective:** The present study was conducted to evaluate the effect of *Samadera indica* bark extracts on experimentally induced excision wounds in Wistar rats.

**Methods:** The bark samples were collected, allowed to shade dry for a week, then coarsely powdered and were extracted with methanol by the soxhlation process. The extract was used for wound healing experiment using the excision wound model. 18 healthy Wistar albino rats divided into three groups of six rats each with an average body weight of 150–200 g were selected randomly for the study. ANOVA was used to compare the variation in the treatments in the result. The 1<sup>st</sup> group of animals is left as such for the natural healing process as control, 2<sup>nd</sup> group of animals was treated with standard ointment betadine, and 3<sup>rd</sup> group with the test drug methanolic bark ointment formulation. The methanolic extract in simple ointment base as mentioned above and betadine ointment were applied on the wound once a day for 16 days starting from the day of wounding. The percentage of wound closure was observed on 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup>, and 16<sup>th</sup> post-wounding day.

**Results:** As for the group received methanolic bark extract for wound healing, 4<sup>th</sup>-day wound mean diameter was 208.0±3.521, 8<sup>th</sup>-day the wound mean diameter was 160.66±7.89, 12<sup>th</sup>-day wound mean diameter was 87.5±5.8, and 16<sup>th</sup>-day wound mean diameter was 22.5±5.12. Thus, there was a marked decrease in wound diameter with every point of time the observation made indicating the effect of the extract on progressive healing of wound. Comparing to standard drug betadine the bark extract showed faster healing in terms of diameter of wound which was statistically signified.

**Conclusion:** From the study, *S. indica* proved its wound healing potential of the plant extract which is due to the bioactive compounds, and thus the study supports local folklore practitioners and tribal people for the use of the plant in different ailments. The extracts of *S. indica* are used in traditional medicines for the treatment of skin diseases, rheumatism, cough, and to kill head lice. The present investigation adds to the existing knowledge in the field of therapeutic medicine and may even become the base for the development of herbal based gel formulations or ointments for treating wounds and thereby continuous usage of synthetic drugs; its associated side effects could be avoided.

**Keywords:** *Samadera indica*, Wound healing, Albino rats, Methanol extract.

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### INTRODUCTION

A medicinal plant is any plant, which in one or more of its organs contains active ingredients which can be used for therapeutic purposes or contain foundational compounds that can be used for the synthesis of useful drugs. Medicinal plants have invariably been a rich source of new drugs and many drugs in use today were either obtained from plants or developed using their chemical structure as templates. Various herbal products have been used in the management and treatment of wounds over the past years [1-5].

*Samadera indica* Gaertner belongs to the family Simaroubaceae with vernacular names Niepa bark tree (English), Lokhandi (Hindi), Kaduhonge (Kannada), Karinjotta, karigotta (Malayalam), and Guchchakaranjah (Sanskrit). It is available in the coastal belt of Dakshina Kannada and Kasaragod districts mainly used by local folklore practitioners and tribal people for various disease treatments. The leaves and bark of the plant are bitter in taste due to glucoside samaderin, yield terpenoids, and flavonoids used against various disorders and skin diseases. The extracts have got antioxidant and antimicrobial activity [6-8]. *S. indica* is an evergreen tree or shrubs up to 10 m high with stout branches and pale yellow bark. Its propagation is by seeds and flowering and fruiting during April–July. *S. indica* is commonly found in backwaters and moist deciduous forests and coastal belts of Maharashtra, Kerala, and Karnataka. It is also

distributed in the forests of Africa-Madagascar, Myanmar, Papua New Guinea, and Salmon Islands.

Like, the bark is effectively used for the treatment of fever; skin diseases and management of emmenagogue. A decoction of the leaves relieves cough and extract controls head lice. The seeds extract commonly applied as an emetic and purgative; seed oil is applied externally on rheumatic joints and used as a liniment on bruises. A decoction of the leaves is used to kill termites and an infusion of leaves is used as an insecticide.

The main objective of the present study is to evaluate the wound healing properties of methanolic bark extract of *S. indica*.

### MATERIALS AND METHODS

#### Collection of sample

*S. indica* plant was identified by Dr. Rama Bhat P, specimens samples were collected, and herbarium was prepared and deposited with No. ALCMB 05/2015. The bark samples were collected from the forests of Kasaragod district, Kerala state, India, during the month of November 2015, allowed to shade dry for a week. It was then kept in a hot air oven at 60°C for 24–48 h until it was dried completely, make it coarsely powdered and stored in a closed container for further use.

### Preparation of methanolic extract

The coarse powder of the bark (25 g) was extracted by soxhlation process using 150 ml methanol. This was followed by distillation and the extract obtained was used for wound healing activity.

### Experimental animals

A total of 18 healthy albino rats of four each with an average weight of 150–200 gm were selected randomly for the study which was divided into three groups.

### Ointment preparation

Emulsifying wax and liquid paraffin were heated up to 70–75°C and stirred until a uniform mass was obtained. The extracts were incorporated into the ointment base and the composition of the ointment base and the ointment formulations are given in Table 1.

### Grouping

The grouping of the animals for this study will be control, standard, and trial which contain a minimum of six rats in each. Animals in each group were kept in separate cages after weighing and labeling with picric acid stain for their individual identity. The animals were fed with standard laboratory diet and drinking water. The grouping of the animals for the present experiment is shown in Table 2.

### Excision wound healing method

The wound model chosen for the present study was excision wound [9]. Selected animals were starved 12 h before wounding, anesthetized using diethyl ether in aseptic conditions. A circular patch of full-thickness skin (500 mm<sup>2</sup>) was cut away from a pre-determined area on the dorsal thoracic region. The animals were housed separately as grouped. The methanolic extract in simple ointment base as mentioned above and betadine ointment were applied on the wound once a day for 20 days starting from the day of wounding. The percentage of wound closure was observed on 1<sup>st</sup>, 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup>, and 16<sup>th</sup> post-wounding day by modifying the method of Madhavan *et al.* [10].

To monitor the changes in the wound and wound shape, the wound margins were traced on thin, transparent polythene sheet which was again retraced on millimeters scale graph paper on the day of wounding (0 day) and were followed until complete wound healing.

The experiment was started after obtaining the Institutional Ethical Committee consent.

### Statistical analysis

The results of the present experiment were subjected to statistical analysis such as mean, standard deviation, and ANOVA. The data were

statistically analyzed with repeated measures of ANOVA test and multiple comparison procedures test by InStat software. The result was assessed statistically in different aspects for the better understanding such as comparison within group and between groups. Each group wound closure diameter is assessed at 4<sup>th</sup> day, 8<sup>th</sup> day, 12<sup>th</sup> day, and 16<sup>th</sup> day. The results of wound healing experiment are expressed as a mean ± standard deviation.

## RESULTS

The progress of wound healing was noted from the 4<sup>th</sup> day of the experiment until the completion, where on a 16<sup>th</sup> day full healing was observed (Plate 1).

### Mean wound closure observed at different intervals of day in three groups

#### Control

As it does not receive any drug, the 4<sup>th</sup>-day wound mean diameter was 236.5±6.28, the 8<sup>th</sup> day the wound mean diameter was 205.0±2.51, 12<sup>th</sup>-day wound mean diameter was 168.83±3.71, and 16<sup>th</sup>-day wound mean diameter was 60.5±10.36.

#### Standard drug (betadine ointment)

As for the group received the standard drug (betadine ointment) for wound healing, 4<sup>th</sup>-day wound mean diameter was 228.0±8.9, the 8<sup>th</sup> day the wound mean diameter was 181.83±6.654, the 12<sup>th</sup> day the wound mean diameter was 130.33±10.33, and 16<sup>th</sup> day the wound mean diameter was 44.83±7.08. Hence, there was a marked decrease in wound diameter with every point of time the observation made.

#### Test drug (methanolic bark extract)

As for the group received test drug (methanolic bark extract) for wound healing, 4<sup>th</sup>-day wound mean diameter was 208.0±3.521, the 8<sup>th</sup> day the wound mean diameter was 160.66±7.89, 12<sup>th</sup>-day wound mean diameter was 87.5±5.8, and 16<sup>th</sup>-day wound mean diameter was 22.5±5.12. Thus, there was a marked decrease in wound diameter with every point of time the observation made indicating the effect of the extract on progressive healing of the wound (Table 3).

## DISCUSSION

Medicinal plants have invariably been a rich source of new drugs and many drugs in use today were either obtained from plants or developed using their chemical structure as templates [1]. Pharmacology is the branch of medicine and biology concerned with the study of drug action, where a drug can be broadly defined as any manmade, natural, or endogenous (from within body) molecule which exerts a biochemical

Table 1: Composition of the ointment base

Ingredients	Quantity (%)
Emulsifying wax	60
Liquid paraffin	45
Extract	5

Table 2: The groupings of the animals for the study

Groups	Drug used	Method	Number of rats
G-1 (control-1)	Natural recovery	Excision wound technique by Morton and Malone.	6
G-2 (Standard)	Betadine ointment	" "	6
G-3 (Trial drug)	Methanolic bark extract (ointment formulation)	" "	6

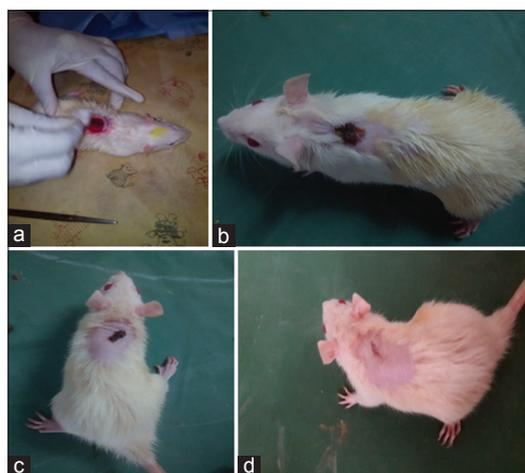


Plate 1: Different stages of wound healing – (a) 1<sup>st</sup> day, (b) 8<sup>th</sup> day, (c) 12<sup>th</sup> day, and (d) 16<sup>th</sup> day

Table 3: Mean wound closure and SD measured at different intervals of days in three groups

Groups day	Mean	Standard deviation	Standard error	95% confidence interval for mean		Minimum	Maximum
				Lower bound	Upper bound		
4 <sup>th</sup> day	236.5000	6.28490	2.56580	229.9044	243.0956	228.00	242.00
8 <sup>th</sup> day	205.0000	5.29150	2.16025	199.4469	210.5531	198.00	211.00
Normal control 12 <sup>th</sup> day	168.8333	3.71035	1.51474	164.9396	172.7271	164.00	174.00
16 <sup>th</sup> day	60.5000	10.36822	4.23281	49.6192	71.3808	41.00	71.00
Total	167.7083	68.09551	13.89994	138.9541	196.4625	41.00	242.00
4 <sup>th</sup> day	228.0000	8.96660	3.66060	218.5901	237.4099	219.00	240.00
8 <sup>th</sup> day	181.8333	6.64580	2.71314	174.8590	188.8077	174.00	189.00
Standard drug 12 <sup>th</sup> day	130.3333	10.36661	4.23215	119.4542	141.2124	116.00	142.00
16 <sup>th</sup> day	44.8333	7.08284	2.89156	37.4003	52.2663	38.00	55.00
Total	146.2500	69.88764	14.26576	116.7390	175.7610	38.00	240.00
4 <sup>th</sup> day	208.0000	3.52136	1.43759	204.3046	211.6954	202.00	212.00
8 <sup>th</sup> day	160.6667	7.89092	3.22146	152.3857	168.9477	151.00	172.00
Test drug 12 <sup>th</sup> day	87.5000	5.82237	2.37697	81.3898	93.6102	81.00	95.00
16 <sup>th</sup> day	22.5000	5.12835	2.09364	17.1181	27.8819	16.00	30.00
Total	119.6667	72.36181	14.77079	89.1110	150.2224	16.00	212.00

SD: Standard deviation

and/or physiological effect on the cell, tissue, organ, or organism; especially medicinal substances obtained from plants used as drugs. The medicinal values of the plants lie in bioactive phytochemical constituents that produce definite physiological action on the human body.

The present study is to understand the pharmacological aspects of *S. indica*, belongs to the family Simaroubaceae which is a locally available medicinal plant mainly used by local folklore practitioners and tribal peoples for various disease treatments. Viswanad *et al.* [7] reported the preliminary phytochemical analysis of the methanolic leaf extract of *S. indica* revealed the presence of alkaloids, tannins, phenolic compounds, triterpenes, carbohydrates, steroids, proteins, and flavonoids and suggested that the *S. indica* is a bitter plant and the bitterness is due to the presence of quassinoids. Quassinoids is a triterpenoid responsible for a wide variety of biological activities. Deepa *et al.* [8] undertaken qualitative and quantitative phytoconstituents of methanolic and aqueous extracts from bark, fruit, and leaf of *S. indica* exhibited the presence of major biomolecules. Raja and Ravindranath [11] reported the chemical and pharmaceutical constituents of *S. indica*.

The study design has numerical data of wound closure diameter of 18 albino rats which are equally divided into three groups, taken from normally distributed population. Hence, the size of the individual group was similar and groups were independent. Hence, ANOVA test was conducted to compare the effect of *S. indica* plant in excision wounds made using scalpel and depilator.

The acute toxicity studies of the methanolic leaf extract of *S. indica* were reported by Viswanad *et al.* [7] and all the animals tolerated the maximum test doses of the extract, as there were no clinical signs of toxicity or mortality of the animal's extract offers no potential ill effects to animals. Wound healing process consists of different phases such as granulation, collagenation, collagen maturation, and scar maturation which are concurrent but independent to each other. Biological activities in the skin are due to its interaction with various binding proteins. In the tissue repairing, inflammatory cells promote the migration and proliferation of endothelial cells leading to neovascularization of connective tissue cells which synthesize extracellular matrices including collagen resulting reepithelialization of wounded tissue [12]. The present study on the wound healing activity of methanolic bark extract excision wound animal model on the albino rats revealed that the drug extract affects the rate of wound contraction significantly as compared to the standard drug betadine. The above finding provides an insight into the usage of *S. indica* bark in traditional treatment of wounds, skin diseases. On the other hand, Singh *et al.* [13] revealed that

ethanolic leaf extract of *Elephantopus scaber* treated animals, the rate of wound contraction on day 16, skin breaking strength and weight of the granulation tissue on that day 10 was 92.4% 380 g and 67.67 mg/100 g, respectively. There are few earlier reports which support the present result [14-17]. Kodati *et al.* [18] experimented with methanolic root extracts of *Plumbago zeylanica* on rats, 10% (w/w) extract ointment treated groups showed significant wound healing from day six onward. Complete healing was noted on 16±2 days, whereas control group animals took more than 20±2 days. Babu *et al.* [19] reported wound contraction of 97.6, 95.2, and 96.2%, respectively for std, aqueous, and alcohol extract treated animals in an experiment using leaf extracts of *Wattakaka volubilis* on albino rats with framycetin sulfate cream as standard. Similarly, Garg and Paliwal [20] used aqueous and ethanolic leaf extracts of *Ficus benghalensis* in wound healing experiments using 10% ointment base IP placebo as standard. Ethanolic extract showed significant ( $p < 0.001$ ). The ethanolic extract showed wound healing in 17.16 days whereas the aqueous extract was in 18.33 days as compared with 21.50 days of control. Similar results even reported using extracts of *Ficus* spp. later by Chowdhary *et al.* [21].

Matadeen *et al.* [16] administered a single dose of alcoholic extract of *Nyctanthes arbor-tristis* orally to Wistar rats to study the wound healing effect and its effect in dexamethasone suppressed wound healing. The epithelization and wound contraction in case of excision wound showed significant ( $p < 0.001$ ) with dexamethasone-treated group. Chandra *et al.* [17] studied that the wound healing activity of methanolic and ethyl acetate extract of *Caesalpinia bonducella* and *Cyclea peltata* in PEG base was applied to wounds in diabetically induced rats. They found moderate granulation with marked epithelial enclose and moderate hyperplasia in groups treated with high dose (100 mg/kg BW) of the methanolic extract of aerial part of *C. peltata* and root of *C. bonducella*. The wound healing was found to be statistically significant. They concluded that therapeutic medicine may even become the base for the development of herbal based gel formulations or ointments for treating wounds in diabetic patients and thereby continuous usage of synthetic drugs, its associated side effects could be avoided. Similar reports were made in some earlier studies too [4,22,23].

On comparing the treatment effects as per the ANOVA, F value obtained for normal control was 746.651, for standard control 524.108, and for test control F value found to be 1183.974. Hence, there is a statistically significant difference in the efficacy of drugs in the closure of wound among the various groups at the interval of 4<sup>th</sup>-16<sup>th</sup> day, where  $p < 0.0001$ .

The group which received the test drug for wound healing the on the 4<sup>th</sup>-8<sup>th</sup> day, 12<sup>th</sup> day, and 16<sup>th</sup> day shows that there is a high significant decrease in mean diameter with every point of observation made.

The standard drug on the 4<sup>th</sup>-8<sup>th</sup> day, 12<sup>th</sup> day, and 16<sup>th</sup> day showed a decrease in wound diameter. The statistical data proves that the test drug is more effective on the 4<sup>th</sup>-8<sup>th</sup> day, 12<sup>th</sup> day, and 16<sup>th</sup> day than the standard drug used and there was a significant difference ( $p > 0.001$ ) in wound diameter which was observed.

The wound healing properties of *S. indica* may be attributed to the phytoconstituents present in the plant, and quick process of wound healing could be a function of either the individual or the additive effects of the phytoconstituents.

#### ACKNOWLEDGMENT

The authors are grateful to the Management of Alva's Education Foundation, Moodbidri, Karnataka, for providing financial support to the senior author in the form of a minor research grant.

#### AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author RBP designed the study, supervised the work, managed the analyses of the study, wrote the first draft of the manuscript, and edited the manuscript. Authors DPR carried out all laboratory work, practical work, wrote the protocol, and literature search. Author RRS designed the animal experiment and observed the study. All authors read and approved the final manuscript.

#### CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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