

WOUND HEALING POTENTIAL OF *ACACIA SUMA* ROXB LEAF

Sumanta Mondal*, Padilam Suresh and G. Shiva Kumar

GITAM Institute of Pharmacy, GITAM University, Visakhapatnam, A.P., India, Email: phytochemistry@rediffmail.com

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ABSTRACT

India has rich tradition of plants based knowledge on healthcare. The aim of present study to assess the wound healing activity of various extracts of *Acacia suma* Roxb. leaf (Fabaceae). The chloroform, ethanol and aqueous extracts of *A. suma* leaves were evaluated for their wound healing activities in rats using excision and incision wound models respectively. The effects of test samples on the rate of wound healing were assessed by the rate of wound closure, period of epithelialisation and wound breaking strength. Povidone-iodine ointment (0.2% w/w) was used as reference standard for the activity comparison. The results of the study revealed that the animals treated with ethanol and aqueous extracts of *A. suma* leaf showed faster rate of wound healing compared to chloroform extract under study. The chloroform extract of the selected plant also produced promising results but the effects are seen to be of lesser extent than the corresponding ethanol and aqueous extracts. The present work justifies the use of *A. suma* leaves for wound healing activity as claimed in the folklore literature.

Keywords: *Acacia suma* Roxb, Wound healing activity, Povidone-Iodine, Excision wound, Incision wound.

INTRODUCTION

The use of herbal heritage has become a part of general health care by the tribes since time immemorial. The use of modern medicines of synthetic origin imparting dramatic results in a short span in the therapeutic field laid several side effects upon long term use. Traditional medicaments, chiefly obtained from plants have played a vital role in sustaining disease free human existence on this planet^{1,2}.

Wound healing is the process of repair that follows injury to the skin and proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin³. Several medicinal plants have been used since time immemorial for treatment of cuts, wounds and burns and showed promising effects. Some very common plants like *Aloe vera*, *Azadirachta indica*, *Carica papaya*, *Celosia argentea*, *Centella asiatica*, *Cinnamomum zeylanicum*, *Curcuma longa*, *Nelumbo nucifera*, *Ocimum sanctum*, *Phyllanthus emblica*, *Plumbago zeylanica*, *Pterocarpus santalinus*, *Terminalia arjuna* and *Terminalia chebula* have been extensively reported in Ayurveda, Siddha and Unani systems of medicines for their wound healing potential⁴.

Acacia suma (Roxb.) var. *Acacia polyacantha* (Family- Fabaceae) is a medium sized erect tree; trunk with fissured bark and knobby persistent prickles found in the greater part of India and coastal districts of Orissa^{5,6}. The dried stem bark is used as folklore medicine in the treatment of anemia, uterine complaints and reported to possess astringent, analgesic, anti-inflammatory and antiseptic properties⁷. The seeds are reported to have hypoglycaemic effect and bark is reported to be used as blood purifier, possesses anti-cancer and astringent properties⁸, similarly the various extracts of stem bark is also reported for hypoglycaemic activity in normoglycaemic and alloxan induced hyperglycaemic rats⁹, and the aqueous extract of the bark also possess diuretic and laxative effect¹⁰. *Acacia* polyphenol also inhibited the lipase and glucosidase activities¹¹. Indole alkaloid namely tryptamine, N-N- dimethyl is isolated from leaves¹². Presence of proanthocyanidin, quercetin¹³ and 5, 4'-dihydroxy-7, 3'-dimethoxyflavone-3-O-D galactopyranoside in the stem bark have been reported earlier¹⁴.

As per the folklore information the tribal people apply the juice of leaves over severs open wounds and claim for its promising effectiveness towards healing of wounds, so the present study to regenerate and reconstruct the disrupted anatomical continuity and functional status of the skin and to investigate the medicinal use of *A. suma* as wound healing promoter that had been cited in folkloric literature.

MATERIALS AND METHODS

Plant material

The plant material (leaves) were collected from the rural belt of Vishakhapatnam district of Andhra Pradesh during November 2011 and authenticated. The leaves were washed, shade dried and pulverized to coarse powder. The powdered leaves (500 g) was defatted with petroleum ether (40 - 60° C) for 72 h and then successively extracted with chloroform, ethanol and water for 48 h in a Soxhlet extractor. Following extraction, the liquid extracts were concentrated under vacuum to yield dry extracts. Standard methods¹⁵ were used for preliminary phytochemical screening of the different extracts to know the nature of phytoconstituents present within them.

Animals

Healthy Wistar albino rats (150-250 g) of either sex and of approximately the same age were used for the study. They were individually housed, maintained in clean polypropylene cages and fed with commercially pellet diet (M/s Hindustan Lever Ltd., Mumbai) and water *ad libitum*. The experimental protocols were subjected to scrutiny of Institutional Animal Ethics Committee for experimental clearance (Protocol No.: IAEC/GIP-1287/M Pharm/IP/SM-SA/2011-12). Five groups, each containing six animals were used for each of the excision and incision wound models. A 10% w/w of the test extracts in simple ointment I.P. was applied topically for each animal once daily (morning).

Excision wound model

Animals were anesthetized prior to and during creation of the wounds, with 1 ml of intravenous ketamine hydrochloride (10 mg/kg). The rats were inflicted with excision wounds^{16,17}, impression was made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear on the anaesthetized rat. The dorsal fur of the animals was shaved with an electric clipper and the anticipated area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of circular area of 500 mm² and 2 mm depth was created along the markings using toothed forceps, scalpel and pointed scissors. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. The entire wound was left open; all surgical procedures were performed under aseptic conditions¹⁸. The control group animals (Group I) were treated with the vehicle (Simple ointment I. P.), the positive control (Group II) was applied with 0.2% w/w Povidone-iodine ointment¹⁹. Other groups of animals were treated with the following: chloroform, ethanol or aqueous extracts of *A. suma* at a

concentration of 10% w/w in simple ointment I.P. in a similar manner.

The wound closure rate was assessed by tracing the wound on days 4, 7, 10, 13 and 16 post wounding days using transparent paper and a permanent marker. The wound areas recorded were measured using graph paper²⁰. The percentage of wound healing was calculated of original wound size for each animal of group on predetermined days i.e. 4, 7, 10, 13 and 16 days post-wounding for final analysis of results. Changes in wound area were calculated, giving an indication of the rate of wound contraction²¹. The results are tabulated in Table 1.

Incision wound model

The rats were anaesthetized prior to and during creation of the wounds, with 1 ml of intravenous ketamine hydrochloride (10 mg/kg). The dorsal fur of the animals was shaved with an electric clipper. A longitudinal paravertebral incision of 6 cm long was made through the skin and cutaneous tissue on the back²¹. After the incision, the parted skin was sutured 1 cm apart using a surgical thread and curved needle. The wounds were left undressed. Extracts were topically applied to the wound once a day. The sutures were removed on 8th post wound day and continued the application of the extract. The wound breaking strength was measured on the 10th day evening after the last application²². The results are tabulated in Table 2.

Statistical analysis

The data obtained in the studies were subjected to one way of analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed using Dunnet's t-test. A p-value <0.05 was considered to be significant. All the values were expressed as Mean \pm SEM.

RESULTS

The preliminary phytochemical screening of *A. suma* leaves extracts showed presence of steroids and sterols, triterpenoids, flavonoids, saponins, tannins and phenolic substances, gums and mucilages, carbohydrates and proteins respectively in different extracts.

The results of wound healing effects of *A. suma* showed significant promotion of wound healing activity with both aqueous and ethanol extracts in the excision and incision wound models. In excision wound model, the mean percentage closure of wound area was calculated on the 4, 7, 10, 13 and 16 post wounding days as shown in Table 1. The ethanol extract treated animals showed faster epithelialisation of wound than the animals treated with aqueous leaves extract. The percentage of wound closure was 100 \pm 00 in the case of standard drug povidone-iodine ointment (0.2% w/w) on 13th day of treatment, whereas the ethanol extract demonstrated similar effects on 16th day. The period of epithelialization was 16.53 \pm 1.98 days for the ethanol extract treated group of animals as against 14.28 \pm 3.47 for the standards drug treated group.

In incision wound model (Table 2), the chloroform, ethanol and aqueous extract treated animals showed significant increase in breaking strength (348.63 \pm 22.82, 408.55 \pm 10.18 and 365.5 \pm 17.04 respectively), when compared to the control (305.23 \pm 13.64). The mean breaking strength was also significant in animals treated with standard drug povidone-iodine (418.51 \pm 18.36) whereas the ethanol extract showed better activity (p<0.01) than the chloroform and aqueous extracts (p<0.05).

DISCUSSION

The results of the present study revealed that, animals treated with ethanol and aqueous extracts of *A. suma* showed faster rate of epithelialization in excision wound model compared to other extracts under study. The chloroform extract of the selected plant also produced promising results but the effects are seen to be of lesser extent than the corresponding ethanol and aqueous extracts. The wound healing effects of the chloroform, ethanol and aqueous extracts may be attributed to the presence of phytoconstituents like triterpenoids, tannins and flavonoids in the extracts which are known to promote the wound healing process mainly due to their antimicrobial property. Flavonoids and triterpenoids are also known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialisation^{3,18}.

Increase in skin breaking strength and tissue breaking strength in incision wound model respectively indicated enhanced collagen maturation. The high collagen turnover which may be due to the activity of some phytoconstituents like flavonoids which are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity²³.

Thus, wound-healing property of the ethanol and aqueous extracts may be attributed to the phytoconstituents they contain, which may be either due to their individual or additive effect that fastens the process of wound healing. The ethanol extract of *Acacia suma* leaves were found to possess better wound-healing property over other extracts. At this stage, it is difficult to say which component(s) of the extracts are responsible for the wound healing activity. However, further phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities.

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Table 1: Effect of various extracts of *A. suma* leaves on percentage (%) wound closure (Excision Wound Model)

Group	Treatment	Concentration	Percentage (%) wound closure.					Period of epithelialization (No. of days)
			4 th days	7 th days	10 th days	13 th days	16 th days	
I	Control	-	22.35 \pm 1.66	51.92 \pm 1.71	65.28 \pm 2.23	72.24 \pm 1.18	80.56 \pm 1.03	25.22 \pm 2.92
II	Povidone-iodine	0.2%	48.53 \pm 2.87*	84.8 \pm 1.26**	96.54 \pm 1.29**	100 \pm 00**	-	14.28 \pm 3.47 **
III	Chloroform extract	10% w/w	23.52 \pm 1.21	56.95 \pm 6.82	72.33 \pm 2.33*	81.35 \pm 1.51*	88.46 \pm 1.37*	20.16 \pm 2.05*
IV	Ethanol extract	10% w/w	27.69 \pm 1.57	71.9 \pm 3.3**	85.71 \pm 3.59**	94.12 \pm 1.38**	100 \pm 00**	16.53 \pm 1.98**
V	Aqueous Extract	10% w/w	24.33 \pm 1.45	63.24 \pm 1.39*	80.15 \pm 4.22**	88.15 \pm 2.4**	92.16 \pm 3.28**	18.5 \pm 0.78*

Values are expressed as mean \pm S.E. (n = 6). All columns are significant using ANOVA.

* P<0.05, ** P<0.01 when compared to control; Dunnet's t-test.

Table 2: Effect of various extracts of *A. suma* leaves on wound breaking strength (Incision Wound Model)

Group	Treatment	Breaking strength (g)
I	Control	305.23±13.64
II	Povidone-iodine (0.2% w/w)	418.51±18.36**
III	Chloroform extract (10% w/w)	348.63±22.82*
IV	Ethanol extract (10% w/w)	408.55±10.18**
V	Aqueous extract (10% w/w)	365.5±17.04*

Values are expressed as mean ± S.E. (n = 6). All columns are significant using ANOVA.

*P<0.05, ** P<0.01 when compared to control; Dunnet's t-test.

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