

# Evaluation of wound-healing potency of *Vernonia arborea* Hk.

B. K. Manjunatha, S. M. Vidya, K.V. Rashmi, K.L. Mankani<sup>1</sup>, H.J. Shilpa, S.D. Jagadeesh Singh<sup>1</sup>

Department of Botany,  
S.R.N.M.N. College of  
Applied Sciences,  
Shimoga, 577 201,  
Karnataka.

<sup>1</sup>Department of  
Pharmacology, National  
College of Pharmacy,  
Shimoga, 577 201,  
Karnataka.

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Correspondence to:  
B.K. Manjunatha  
E-Mail:  
doctor\_bkm@yahoo.com

## ABSTRACT

**Objective:** To investigate the comparative wound-healing potency of aqueous and methanol leaf extracts of *Vernonia arborea* Hk.

**Materials and Methods:** Excision, incision and dead space wound models were used to evaluate the wound-healing activity of *Vernonia arborea* Hk., on Swiss Wistar strain rats of either sex. In excision wound model, treatment was continued till the complete healing of the wound, in incision and dead space wound models the treatment was continued for 10 days. For topical application, 5% w/w ointment of aqueous and methanol leaf extracts was prepared in 2% sodium alginate and for oral administration suspensions containing 30 mg/ml of each of the extracts in 1% gum tragacanth were prepared. In excision and incision wound models, the control group of animals were left untreated and in dead space wound models the animals were treated with 1 ml of 1% gum tragacanth / kg, b.w. The healing of the wound was assessed by the rate of wound contraction, period of epithelialisation, skin breaking strength, granulation strength, dry granulation tissue weight, hydroxyproline estimation and histopathology of the granulation tissue.

**Results:** Aqueous and methanol leaf extracts promoted the wound-healing activity significantly in all the wound models studied. High rate of wound contraction, decrease in the period for epithelialisation, high skin breaking strength and granulation strength, increase in dry granulation tissue weight, elevated hydroxyproline content and increased collagenation in histopathological section were observed in animals treated with methanol leaf extract and aqueous leaf extract when compared to the control group of animals.

**Conclusion:** Methanol and aqueous leaf extracts of *Vernonia arborea* Hk. promote wound-healing activity. Methanol extract possesses better wound-healing property than the aqueous extract.

**KEY WORDS:** Skin breaking strength, wound contraction, wound models.

## Introduction

The plant *Vernonia arborea* Hk. is a moderate sized tree belonging to the family Asteraceae. It is distributed in the coastal districts of Karnataka State, especially in the South Canara district.<sup>[1]</sup> The plant has many medicinal properties viz., leaf juice is used to treat worms, infusion of roots or decoction of bark in fever. In Southern Sumatra, the bark is chewed at the first sign of sprue. It is chewed as a substitute for pan by Nagas.<sup>[2]</sup> This plant contains sesquiterpene 'zaluzanin D', which is a potent antifungal agent.<sup>[3]</sup>

Tribal groups residing in the Western Ghat region of Chikmagalur district, Karnataka State, use the leaf extract for septic wounds, in treating jaundice fever and rheumatic pains (Personal communication).

A review of the literature revealed that the wound-healing property of this plant has not been subjected to scientific evaluation. This paper reports the wound-healing property of this rare plant.

## Materials and methods

### Plant material

Leaves of *Vernonia arborea* were collected from the Western Ghat range of Chikmagalur district of Karnataka State, during December 2003 and identified by the first author. The voucher specimens (BKM-533, BKM-554) were deposited in the Kuvempu University herbaria.

### Extraction

Leaves were shade dried and powdered mechanically. About 250 g of powder was subjected to soxhlet extraction with 70% methanol for about 48 h. The extract was filtered and concentrated in vacuum under reduced pressure using a rotary flash evaporator (Buchi, Flawil, Switzerland) and dried in a desiccator (yield 22.6 % w/w).

For aqueous extract, 250g of powdered leaves was macerated with 1000 ml of distilled water for three days with intermittent stirring, filtered and concentration (yield 18% w/w). Both the extracts were subjected to preliminary phytochemical tests.<sup>[4]</sup>

### Drug formulations

Two types of drug formulations were prepared from each of the extracts. For topical administration, 5% w/w ointment was prepared in 2% sodium alginate. For oral administration, 30 mg/ml of aqueous and methanol suspensions of leaf extracts were prepared in 1% gum tragacanth.

### Animals

Swiss Wistar strain rats of either sex weighing 150-200 g were procured from the National College of Pharmacy, Shimoga, Karnataka and were maintained at standard housing conditions. The animals were fed with a commercial diet (Hindustan Lever Ltd., Bangalore, India) and water *ad libitum* during the experiment. The study was permitted by the Institutional Animal Ethics Committee, National College of Pharmacy, Shimoga. Acute toxicity study was conducted for both the extracts by the stair-case method.<sup>[5]</sup>

### Wound-healing activity

Excision, incision and dead space wound models were used to evaluate the wound-healing activity.

#### Excision wound

The rats were inflicted with excision wounds as described by Morton and Malone (1972)<sup>[6]</sup> under light ether anaesthesia. A circular wound of about 500 sq. mm was made on depilated ethanol sterilized dorsal thoracic region of the rats. The animals were divided into four groups of six each. The animals of group I were left untreated and considered as the control, group II served as reference standard and treated with 1% w/w framycetin sulphate cream (FSC), animals of group III and IV were treated with 50 mg of ointment prepared from aqueous and methanol leaf extract of *Vernonia arborea*. The ointment was topically applied once a day, starting from the day of the operation, till complete epithelialisation. The parameters studied were wound closure and epithelialisation time. The wounds were traced on mm<sup>2</sup> graph paper on days 3, 6, 9, 12, 15 and 18 and thereafter on alternate days until healing was complete. The percentage of wound closure was calculated. The period of epithelialisation was calculated as the number of days required for falling of the dead tissue remnants of the wound without any residual raw wound.

#### Incision wound

In incision wound model, 6 cm long paravertebral incisions were made through the full thickness of the skin on either side of the vertebral column of the rat as described by Ehrlich and Hunt *et al.*<sup>[7]</sup> The wounds were closed with interrupted sutures of 1 cm apart. The animals were divided into four groups of six animals each. The animals of group I were left untreated and considered as the control, the group II served as reference standard and received 1% w/w framycetin sulphate cream (FSC) ointment, animals in groups III and IV were treated with 50 mg of ointment prepared from aqueous and methanol leaf extract. The ointment was topically applied once in a day. The sutures were removed on the 8<sup>th</sup> post wound day. The skin breaking strength of the wounds was measured on the 10th day as described in the method of Lee *et al.*<sup>[8]</sup>

#### Dead space wound

The animals were divided into three groups of 6 rats in each group. Group I served as the control, which received 1ml of 1% gum tragacanth/kg, b.w., p.o. The animals of group II and III received oral suspensions of aqueous and methanol

leaf extracts respectively (30 mg/kg, b.w., p.o.). Under light ether anaesthesia, dead space wounds were created by subcutaneous implantation of sterilized cylindrical grass piths (2.5 cm X 0.3 cm), one on either side of the dorsal paravertebral surface of the rat.<sup>[9]</sup> The granulation tissues formed on the grass piths were excised on the 10<sup>th</sup> post wounding day and the breaking strength was measured. Simultaneously, granulation tissue so harvested was subjected to hydroxyproline estimation following the method of Woessner *et al.*,<sup>[10]</sup> and histopathological study to evaluate the effect of the extracts on collagen formation.

### Statistical analysis

The data were subjected to ANOVA followed by Dunnett's test and the values of  $P \leq 0.001$  were considered statistically significant.

## Results

The LD<sub>50</sub> of aqueous and methanol leaf extracts were found to be 300 mg/kg, b.w. One tenth of the dose was selected<sup>[5]</sup> for the evaluation of wound-healing activity i.e., 30 mg/kg, b.w.

Significant promotion of wound-healing activity was observed in both aqueous and methanol leaf extracts in all the three wound models such as excision, incision and dead space wound. In excision wound model, the mean percentage closure of wound area was calculated on the 3, 6, 9, 12, 15 and 18 post wounding days as shown in Table 1. The methanol leaf extract treated animals showed faster epithelialisation of wound ( $17.86 \pm 0.19$ ) than the animals treated with aqueous leaf extract ( $19.03 \pm 0.59$ ). The period of epithelialisation was  $16.15 \pm 0.21$  in the case of standard drug 1% w/w framycetin sulphate cream (FSC) ointment.

In incision wound model, methanol and aqueous leaf extract treated animals showed increase in breaking strength ( $494.41 \pm 4.30$ ), ( $463.74 \pm 3.63$ ), respectively when compared to the control ( $240.46 \pm 3.28$ ). The mean breaking strength was also significant in animals treated with standard drug FSC ( $567.02 \pm 2.12$ ).

In dead space wound model, histological studies of the granulation tissue of the control group of animals showed more aggregation of macrophages with few collagen fibres. [Figure 1] In the case of aqueous leaf extract treated animal groups, moderate collagen deposition, macrophages and fibroblasts were noticed Figure 2 whereas the methanol leaf extract treated animal group evidenced significant increase in collagen deposition showing lesser macrophages and fibroblasts. [Figure 3] Compared to the control group of animals, methanol leaf extract treated animals showed significant increase in dry weight of granulation tissue ( $184.46 \pm 0.49$ ) and breaking strength ( $387.72 \pm 3.25$ ) followed by aqueous leaf extract treated group of animals. [Table 2] Estimation of hydroxyproline content in the granulation tissue revealed that the animal groups treated with methanol leaf extract had high hydroxyproline content ( $2250.00 \pm 0.57$ ) followed by the aqueous leaf extract treated group ( $1979.33 \pm 0.80$ ). However, the control group showed less hydroxyproline content ( $1398.66 \pm 1.02$ ).

## Discussion

Wound healing is a fundamental response to tissue injury

that results in restoration of tissue integrity, which is due to the synthesis of the connective tissue matrix. Collagen is a major protein of the extracellular matrix and is the component that ultimately contributes to wound strength. Breakdown of collagen liberates free hydroxyproline and its peptides. Measurement of the hydroxyproline could be used as an index

for collagen turnover. The data depicted in Table 2 reveal that the hydroxyproline content of the granulation tissue of the animals treated with methanol and aqueous leaf extract was significantly increased when compared to the control group, indicating increased collagen turnover. Increase in breaking strength of granulation tissue of methanol and aqueous leaf

**Table 1**

**Effect of topical application of aqueous and methanol leaf extracts of *Vernonia arborea* on healing of excision wound model**

Group	Post wounding days							Period of epithelialisation
	0-day	3rd day	6th day	9th day	12th day	15th day	18th day	
Control	511.91 ± 0.46 (0.00)	484.53 ± 1.49 (5.34)	404.21 ± 1.14 (21.03)	357.62 ± 0.58 (30.14)	277.85 ± 0.72 (45.72)	191.16 ± 0.54 (62.65)	88.32 ± 0.50 (82.74)	24.29 ± 0.23
Standard	510.00 ± 1.48* (0.00)	407.30 ± 0.61* (20.13)	323.59 ± 1.31* (36.35)	252.37 ± 0.53* (50.51)	136.35 ± 0.47* (73.26)	9.32 ± 0.44* (98.17)	0* (100)	16.15 ± 0.21*
Aqueous extract	506.68 ± 2.12* (0.00)	463.69 ± 1.49* (8.48)	343.40 ± 0.54* (32.22)	271.26 ± 0.54* (46.46)	148.46 ± 0.57* (70.69)	66.22 ± 0.60* (86.93)	7.50 ± 0.43* (98.51)	19.03 ± 0.59*
Methanol extract	508.81 ± 1.51* (0.00)	443.20 ± 1.18* (12.89)	331.64 ± 0.58* (34.82)	268.25 ± 0.55* (47.27)	141.40 ± 0.43* (72.28)	18.50 ± 0.43* (96.36)	0* (100)	17.86 ± 0.19*
One-way F	12.87	25.19	15.54	12.70	10.52	12.31	15.43	11.59
ANOVA P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Values are expressed as mean ± SEM; df = 3, 20; n = 6 animals in each group; Numbers in parenthesis indicate percentage of wound contraction; \*P ≤ 0.001 when compared to control.

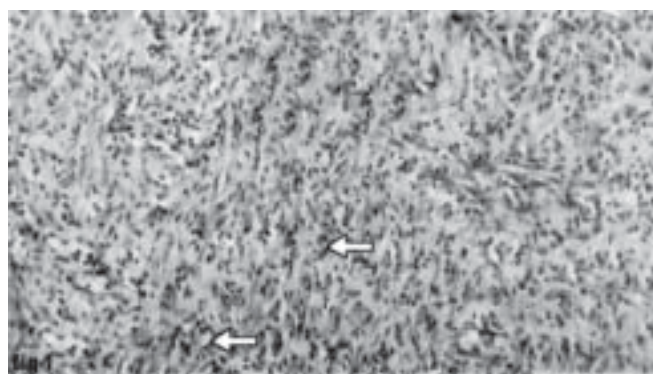
**Table 2**

**Effect of aqueous and methanol leaf extracts of *Vernonia arborea* on healing of dead space wound model**

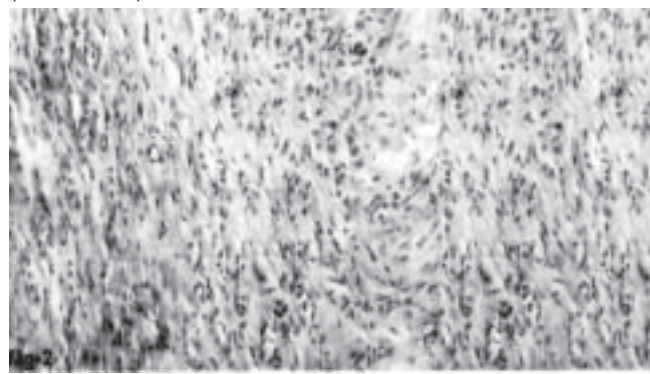
Group	Granulation tissue dry weight (mg/100 g)	Breaking strength (g)	Hydroxyproline (mg/100 g)
Control(1ml of 1% gum tragacanth/kg, b.w.)	87.94 ± 0.61	230.46 ± 2.57	1398.66 ± 1.02
Aqueous extract	146.34 ± 0.61*	347.12 ± 3.53*	1979.33 ± 0.80*
Methanol extract	184.46 ± 0.49*	387.72 ± 3.25*	2250.00 ± 0.57*
One-way F	11.02	10.89	15.03
ANOVA P	<0.001	<0.001	<0.001

Values are expressed as mean ± SEM; df = 2, 15; n = 6 animals in each group; \*P ≤ 0.001 when compared to control.

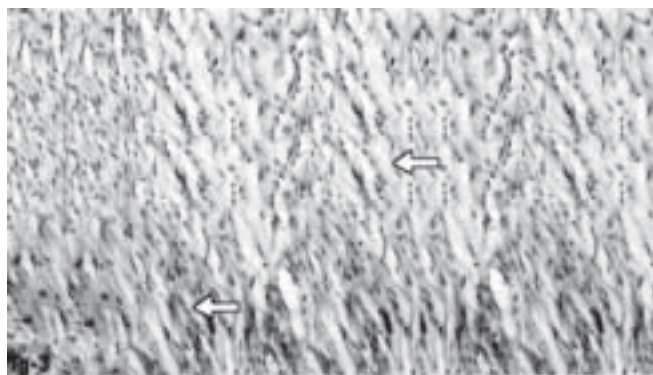
**Figure 1.** Histological section of granulation tissue of control animal showing more macrophages (arrows). (H & E, 100X)



**Figure 2.** Histological section of granulation tissue of the aqueous leaf extract treated animal showing moderate collagen deposition. (H & E, 100X)



**Figure 3.** Histological section of granulation tissue of the animal treated with methanol leaf extract of *Vernonia arborea* showing increased collagenation (arrows) and lesser macrophages and fibroblasts. (H & E, 100X).



extract treated animals indicated the enhanced collagen maturation by increased crosslinking. In addition, increase in dry granulation tissue weight also indicated the presence of higher protein content.<sup>[11]</sup> In the present investigation, preliminary phytochemical analysis of aqueous leaf extract revealed the presence of flavonoids, saponins, tannins and glycosides whereas methanol extract showed positive test to flavonoids, saponins, tannins, glycosides, sesquiterpenes and triterpenoids. Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Hence, any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibres, increasing the circulation, preventing the cell damage and by promoting the DNA synthesis.<sup>[12]</sup> Tannins,<sup>[13]</sup> flavonoids,<sup>[14]</sup> triterpenoids<sup>[15]</sup> and sesquiterpenes<sup>[16]</sup> 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. The sesquiterpene lactones are known to possess antioxidant property,<sup>[17, 18]</sup> which may also contribute to the wound-healing process. Thus, wound-healing potency of *Vernonia arborea* may be attributed to the phytoconstituents present in it, which may be either due to their individual or additive effect that fastens the process of wound healing. Between the two extracts studied, the methanol leaf extract was found to possess better wound-healing property. Which component(s) of the extract is responsible for this effect, however, was not investigated. Further phytochemical studies are in progress where the methanol extract will be subjected to further fractionation and purification to identify and to isolate

the active compound(s) responsible for these pharmacological activities. The present findings provide scientific evidence to some of the ethnomedicinal properties of *Vernonia arborea*.

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