

## ANTI-OXIDANT AND WOUND HEALING ACTIVITY OF THE BENZENE EXTRACT OF *DERRIS BENTHAMII*

N. S. NAGARAJAN\*, P. GNANASOUNDARI  
and P. TIRUPATHY KUMARESANA<sup>a</sup>

\*Department of Chemistry, Gandhigram Rural Institute – Deemed University,  
GANDHIGRAM– 624 302. Dindigul District, (T.N.) INDIA

<sup>a</sup>Department of Pharmacology, Arulmigu Kalasilangam College of Pharmacy,  
KRISHNAN KOVIL–626 196, (T. N.) INDIA

### ABSTRACT

In the present study, the vacuum dried benzene extract of *Derris benthamii* leaves was evaluated for its wound healing property by incision and excision wound models. The extract was also investigated for its anti-oxidant activity by thin layer chromatography using  $\beta$ -carotene linoleate oxidation method. The extract exhibited significant wound healing properties and also possessed anti-oxidant activity. The wound healing property may be attributed to the anti-oxidant activity of the extract.

**Keywords :** Wound healing, *Derris benthamii*

*Derris benthamii* (family Leguminosae, sub-family Papilionaceae) is a slender glabrous climber with rusty inflorescence axis and brown silky pods. *Derris* species are reported to have diuretic and anti-bacterial activities and also the rich sources of flavanoids<sup>1</sup>. So far, no literature has been evidenced in favour of pharmacological and phytochemical examination of *Derris benthamii*.

In the present study, the benzene extract of leaves of *Derris benthamii* was investigated for its wound healing properties. The parameters evaluated were wound contraction, period of epithelization and tensile strength in the wound models. Qualitative chemical tests were also performed to identify the presence of flavonoids.

The anti-oxidant property of the extract was evaluated quantitatively by thin layer chromatography on  $\beta$ -carotene linoleate oxidation model.

### MATERIALS AND METHOD

#### Animals

Wistar albino rats of either sex weighing each about 180–200 g were obtained from King Institute, Chennai. They were kept in colony cages at  $25 \pm 2^{\circ}$  C, relative humidity of 45–55%

was maintained under 12 hour light and dark cycles. They were fed with standard animal feed. All the animals were acclimatized for a week before the study.

### Extraction of the leaves

*Derris benthamii* collected from the forests of Kerala, was identified by Tropical Botanical Garden and Research Institute, Palode, Kerala.

About 500 g of the shade dried leaves of *Derris benthamii* were extracted successively with petroleum ether (60–80°C) and benzene for 6 x 6 hours each under hot condition. The crude, concentrated extracts were refrigerated and the benzene extracts was taken up for the study.

The vacuum dried crude extract was formulated as an ointment in simple ointment base for excision wound healing model and suspended in 0.5% simple ointment for incision wound healing model.

### Preliminary identification of chemical constituents

Preliminary chemical tests like ammonia fuming test, zirconium oxychloride test, p-benzoquinone test, Gibb's test, boric acid test, lead acetate test and neutral ferric chloride test and Shinoda test<sup>2</sup> for flavonoids were performed to detect the presence of flavonoids in the benzene extract.

### Anti-oxidant property by thin layer chromatography (TLC) method<sup>3</sup>

Benzene extract of leaves of *Derris benthamii* was solublized in methanol and subjected to TLC on 20 x 20 cm glass plates coated with silica gel-G.  $\text{CHCl}_3$  : MeOH (9 : 1, v/v and  $\text{CHCl}_3$  : EtOAc : HCOOH (5:4:1, v/v) were used as developing solvents.

The spots in the chromatogram were located under UV light.  $\beta$ -carotene linoleate (a mixture of  $\beta$ -carotene in 30 mL of  $\text{CHCl}_3$  and 2 mL of purified linoleic acid in 60 mL of 95% ethanol) was sprayed uniformly on the plates which were exposed to day light for about 4 hours. The background of the spots was bleached and the spots, which contained the flavonoids and phenolic compounds, retained the yellow colour indicating the anti-oxidant activity.

### Excision wound healing model<sup>4</sup>

Albino rats, numbering six were used for the study. A round seal of 2.5 cm in diameter was impressed on the hair removed dorsal thoracic region (5 cm away from the ears) of the pentobarbitone sodium (30mg/kg) anesthetised rats. Fully thickened skin from the demarked area was incised to produce a wound measuring around 500 mm<sup>2</sup>. The wound was washed with cotton swabs soaked in warm saline. The animals were maintained individually in separate cages. The extract (10%) was formulated as an ointment in simple ointment base. 0.5 g of formulated ointment was applied on the wound once daily for 21 days starting from the days of

wounding .Wound contraction (4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup> and 16<sup>th</sup> day of wounding) and period of epithelization were observed. The data are presented in Table 1.

### Incision wound healing model<sup>5</sup>

Rats, numbering six were wounded to bear two vertebral incision wound of 6 cm each on either side of hair removed dorsal portion of the animal. The wounded edges were closed by interrupted silk sutures (non-adsorbable). The sutures were removed on 7<sup>th</sup> day of wounding and the tensile strength was measured on the 10<sup>th</sup> day. 1g/kg and 500 mg/kg of benzene extract of leaves of *Derris benthamii* and  $\alpha$ -tocopherol were administered orally once a day by intra-gastric tube.  $\alpha$ -tocopherol served as the standard. Unpaired student t-test was performed to ascertain the significance of pharmacological parameters. The data are presented in Table 2.

## RESULTS AND DISCUSSION

Qualitative chemical tests confirmed the presence of flavonoids in the extract. Anti-oxidant property of the extract was confirmed qualitatively by  $\beta$ -carotene linoleate oxidation method by TLC. Contraction of excision wounds was promoted from 4<sup>th</sup> day of treatment till 16<sup>th</sup> day. A significant reduction in the period of epithelization was observed when compared to the control.

A significant increase in the tensile strength of incision suggests that the extract promoted collagen formation equipotent to  $\beta$ -tocopherol. The wound healing property may be attributed to the anti-oxidant activity of the extract, which may be due to the flavonoids present in it.

**Table 1. Effect of the benzene extract of leaves of *Derris benthamii* on the excision wound healing model**

S.No.	Treatment	Mean period of epithelization (days) $\pm$ SEM	Days			
			4 <sup>th</sup> day	8 <sup>th</sup> day	12 <sup>th</sup> day	16 <sup>th</sup> day
1.	Control	20.5 $\pm$ 0.21	9.33 $\pm$ 3.5	35.2 $\pm$ 7.8	65.3 $\pm$ 7.9	86.4 $\pm$ 2.6
2.	Extract	16.6 $\pm$ 0.3	16.2 $\pm$ 3.8*	57.8 $\pm$ 0.01*	89.6 $\pm$ 2.7*	99.9 $\pm$ 0.08*

\*p < 0.001

**Table 2. Effect of the benzene extract of leaves of *Derris benthamii* on the tensile strength of incision wounds**

S.No.	Drug	Dose	Mean period of epithelization (days) $\pm$ SEM after 10 days
1.	Control	-	275.00 $\pm$ 26.2
2.	Extract	1g/kg	371.60 $\pm$ 25.8*
3.	$\alpha$ -Tocopherol	500 mg/kg	387.90 $\pm$ 33.2

\*p < 0.001

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