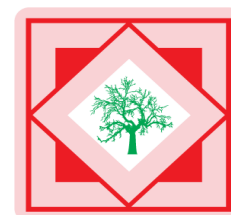




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Der Pharmacia Sinica, 2011, 2 (6):32-38



Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Evaluation of *in vivo* wound healing potential of *Cassia roxburghii* leaves extracts

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ABSTRACT

Cassia roxburghii having the synonym of *Cassia marginata*, *Cathartocarpus marginatus* was found in most parts of central and southern India. *Cassia* species are well known in folk medicine for their laxative and purgative uses. They are also used for treating skin diseases such as ring worm, scabies, eczema and wounds. However there was no scientific evidence justifying the use of *Cassia roxburghii* in wounds, therefore the present study was aimed at evaluation of wound healing activity of leaves of *Cassia roxburghii*. In the present study the leaves of *Cassia roxburghii* were extracted by successive solvent extraction using petroleum ether, Ethyl acetate and Methanol which were studied for wound healing activity by incorporating the extracts in simple ointment base B.P. in concentration of 5% (w/w). Wound healing activity was studied in two types of model in rats viz. excision and incision. In case of the excision wound model wound contraction and period of epithelization was studied while in incision wound model, tensile strength of the wound was measured. Treatment of wound with ointment containing 5% (w/w) methanolic extract showed good wound healing activity.

Key words: *Cassia roxburghii*, wound healing, excision model, incision model, Nitrofurazone.

INTRODUCTION

Medicinal plants have the curative properties due to the presence of various phytoconstituents called as alkaloids, glycosides, corticosteroids, essential oils etc. These botanical remedies were given to folks suffering from illness without regard to prior investigation [1]. *Cassia roxburghii* having the synonym of *Cassia marginata* and *Cathartocarpus marginatus* was found in most parts of central and southern India, often along the roads for shade. *Cassia roxburghii* leaves are used for ring worm infections. The presence of Anthraquinine glycosides and Chrysophanol, Physcion, Rhein, Roxburghinol has been reported earlier [2]. *Cassia* species are well known in

folk medicine for their laxative and purgative uses [3]. They are also used for treating skin diseases such as ring worm, scabies, eczema and wounds [4, 5]. However there was no scientific evidence justifying the folklore use of *Cassia roxburghii* in wounds, therefore the present study was aimed at evaluation of wound healing activity of three different extracts of *Cassia roxburghii* leaves.

MATERIALS AND METHODS

Collection of Plant Material

The leaves of *Cassia roxburghii* were collected in October from Warangal district, India and botanically identified and authenticated by Dr. Raju S Vastavya, Taxonomist, Department of Botany, Kakatiya University, Warangal and a voucher specimen (KSR/03/2011) was stored in Dept. Of Pharmacognosy & Phytochemistry, Vaagdevi College of Pharmacy, Hanamkonda, A.P., India for further reference. The leaves were made free from all the earthy and foreign matter, shade dried and then milled in to powder by a mechanical grinder. The powder was used for further studies.

Preparation of the extracts

500gm powder of *Cassia roxburghii* leaves were successively extracted by soxhlation procedure (8 hr) using petroleum ether, Ethyl acetate and Methanol. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator and stored in desiccators, which were designated as PECR, EACR and MECR whose yield were found to be 1.5, 1.2 and 1.8% respectively [6, 7]. 10gm of 5 % w/w ointment for all the three extracts were prepared using simple ointment base B.P. [8, 9].

Pharmacological activity

Animals used

Male Wistar albino rats (150–180 g) were selected for the experiment. Six rats were taken for each group. The rats were used after acclimatization to the laboratory environment for a 7- day period. They were kept in the departmental animal house at 26 ± 2 °C and light dark cycles of 10 and 14 h, respectively. Animals were provided with rodent diet and water *ad libitum*. All the experimental procedures were approved by Institutional animal ethical committee of Vaagdevi College of Pharmacy, Hanamkonda, Andhra Pradesh, India vide approval No. 1047/AC/09/CPCSEA. In this experiment, the rats were divided into five groups ($n = 6$): group I was the control Group which received simple ointment base, Group II was treated with reference standard (0.2%, w/w Nitrofurazone ointment), Group III received PECR ointment 5% (w/w), Group IV received EACR ointment 5% (w/w) and Group V received MECR ointment 5% (w/w) topically on wound created.

Excision wound model

Animals in each group were anaesthetized by with anesthetic ether. The rats were depilated on the back. One excision wound was inflicted by cutting away a 500 mm² full thickness of skin from a predetermined area. The wound was left undressed to the open environment. Then the extracts and standard were administered topically for 16 days. Contractions, which contribute for wound closure, were studied by tracing the raw wound. Wound area was measured by retracing the wound on a milli meter scale graph paper every alternate day. The degree of wound healing

was calculated. Wound contraction was calculated as percent reduction in wound area using following formula:

$$\% \text{ of wound closure} = \frac{\text{wound area on day 0} - \text{wound area on day N}}{\text{Wound area on day 0}} \times 100$$

Where N =number of days 2nd, 4th, 8th, 12th, and 16th day.

Period of epithelization was also calculated and compared with that of control group [10, 11].

Incision wound model

Animals in each group were anaesthetized and one paravertebral-long incision was made through the skin and cutaneous muscles at a distance of about 1.5 cm from the midline on each side of the depilated back of the rat. Full aseptic measures were not taken and no local or systemic antimicrobials were used throughout the experiment. All the groups were treated in the same manner as that of excision wound model. No ligature was used for stitching. After the incision was made, the parted skin was kept together and stitched with black silk at 0.5cm intervals; surgical thread (No. 000) and a curved needle (No. 11) were used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. The wound was left undressed. Sample extract along with simple ointment (control) and standard drug were topically administered once daily for 9 days; when wounds were cured thoroughly the sutures were removed on the 10th day and tensile strength was measured with a local made Tensiometer [12, 13].

Tensile strength

The tensile strength of a wound represents the degree of wound healing. Usually wound-healing agents promote a gain in tensile strength. The sutures were removed on the 9th day after wounding and the tensile strength was measured on the 10th day. The mean tensile strength on the two paravertebral incisions on both sides of the animals was taken as the measures of the tensile strength of the wound for an individual animal. The tensile strength different extracts ointment-treated wounds were compared with control groups. The tensile strength increment indicates better wound healing stimulation by the applied drug. Tensile strength was calculated using the following formula: [14, 15].

$$\text{Tensile strength} = \frac{\text{Breaking strength (g)}}{\text{Cross-sectional area of skin (mm}^2\text{)}}$$

Statistical analysis

Results obtained from both the wound models have been expressed as Mean±S.D. and were compared with the corresponding control (simple ointment) values. The percentage of Wound Contraction was calculated as a percentage of the corresponding 0 day's (original) wound area (mm²).

Table No. 1 Effect of *C. roxburghii* leaf extracts and Nitrofurazone ointment on wound healing by excision wound method in rats

Post wound day	Wound area in mm ² (percentage of wound contraction)				
	Control (Simple ointment)	Standard (Nitrofurazone ointment)	5% PECCR ointment	5% EACR ointment	5% MECCR ointment
0	500.7±20.8 (0)	517.4±24.6 (0)	510.5±15.9 (0)	505.6±18.7 (0)	515.1±19 (0)
2	455.6±14.24 (9)	406.8± 24.32 (21.3)	397.6±14.49 (22.1)	406.5±10.3 (19.59)	397.7±20.4 (22.7)
4	395.08±28.23 (18.5)	216.3±19.04 (58.2)	350.6±15.76 (31.3)	363.07±13.7 (28.2)	338.2±9.4 (34.3)
6	261.6±21.95 (47.7)	133±14.42 (74.2)	268.7±11.63 (47.3)	276.2±8.5 (45.3)	237.1±6.7 (53.9)
8	240.6±11.95 (51.9)	77.8±14.81 (84.9)	125.0±4.7 (75.5)	132.8±8.3 (73.7)	117.9±5.5 (73.1)
10	229.3±10.06 (54.2)	43.4±6.71 (91.6)	76.7±7.5 (84.9)	86.6±6.7 (82.8)	63.6±5.7 (87.6)
12	207.5±13.37 (58.5)	14.3±3.01 (97.2)	47.1±3.56 (90.7)	56.8±5.4 (88.7)	31.9±4.9 (93.8)
14	176.8±12.68 (64.6)	5.0±2.83 (99)	16.8±1.85 (96.7)	21.6±2.3 (95.7)	14.2±1.96 (97.2)
16	147.5±7.68 (70.5)	2.7±1.59 (99.4)	8.3±1.50 (98.3)	12.9±3.9 (97.4)	1.5±1.78 (99.6)

All values were expressed as Mean±S.D.

Table. No. 2 Period of epithelization in excision wound model

Group	Period of epithelization (Days)
Control (Simple ointment)	25.3±0.81
Standard (Nitrofurazone ointment)	16.25±0.5
5% PECCR Ointment	18.5±0.57
5% EACR Ointment	20.25±0.95
5% MECCR Ointment	16.75±0.95

All values were expressed as Mean±S.D.

Table No. 3 Tensile strength by incision wound model

Group	Tensile strength (gm)
Control (Simple ointment)	349.5±0.57
Standard (Nitrofurazone ointment)	719.75±0.5
5% PECCR Ointment	552±2.03
5% EACR Ointment	513.7±1.1
5% MECCR Ointment	575.7±0.5

All values were expressed as Mean±S.D.

RESULTS AND DISCUSSION

Excision wound study

The progress of the wound healing induced by *Cassia roxburghii* leaf extract ointments (5% w/w) treated groups, simple ointment (control) treated group and Nitrofurazone (standard drug) treated group of animals are shown in Table 1. It is observed that the wound contracting ability

of the extract ointments was significantly greater than that of the control (i.e. simple ointment treated group). The 5% (w:w) extract ointment treated groups showed significant wound healing from the second day onwards, which was comparable to that of the standard drug, i.e. Nitrofurazone ointment treated group of animals (figure no. 1). The wound closure time was lesser, as well as the percentage of wound contraction and epithelization was found to be significant (figure no. 2) when compared to that of control treated groups.

Incision wound model

The measurement of the effect of the extract and standard drug on the tensile strength of the incision wound is shown in Table 2. The tensile strength of the 5% extract ointment treated group was found to be significant. Thus ointment of extract as well as the standard drug showed a significant increase in tensile strength in the 10 days old wound (figure no. 3).

Fig. No. 1 Effect of *C. roxburghii* leaf extracts and Standard on wound healing

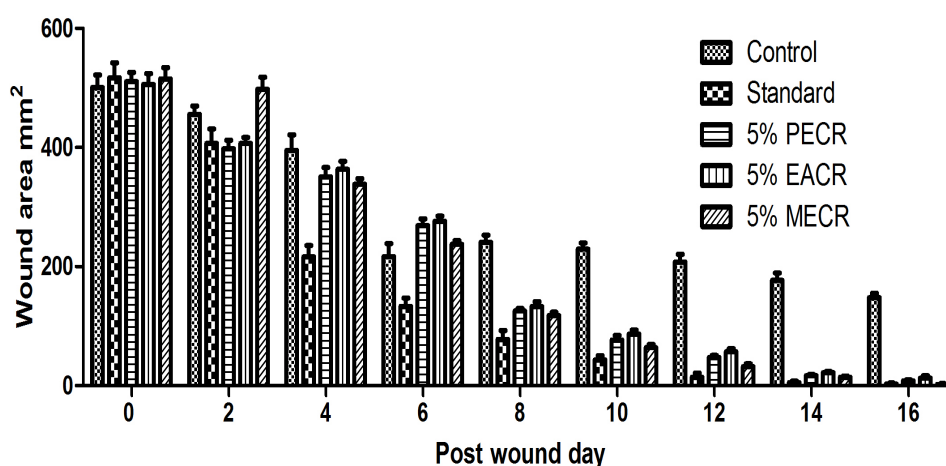


Fig. No. 2 Period of epithelization in excision wound model

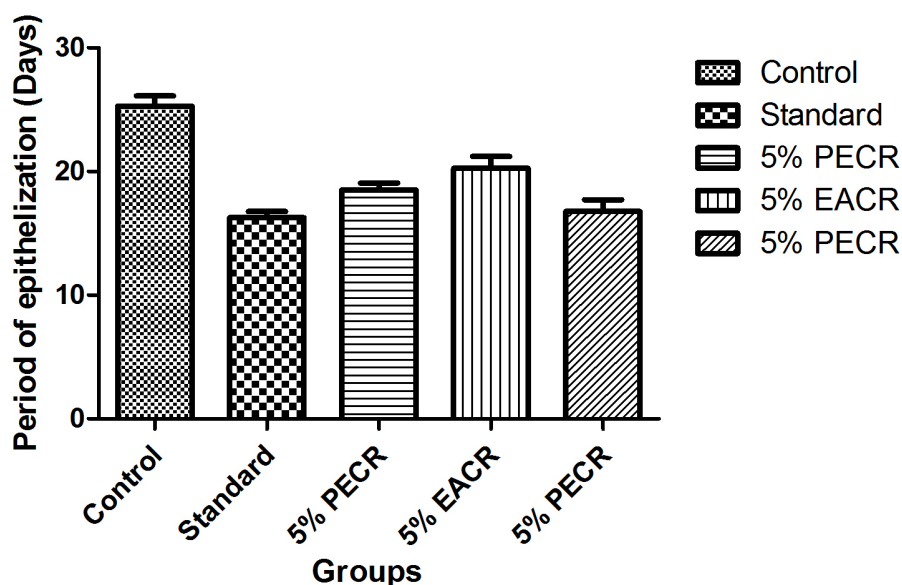
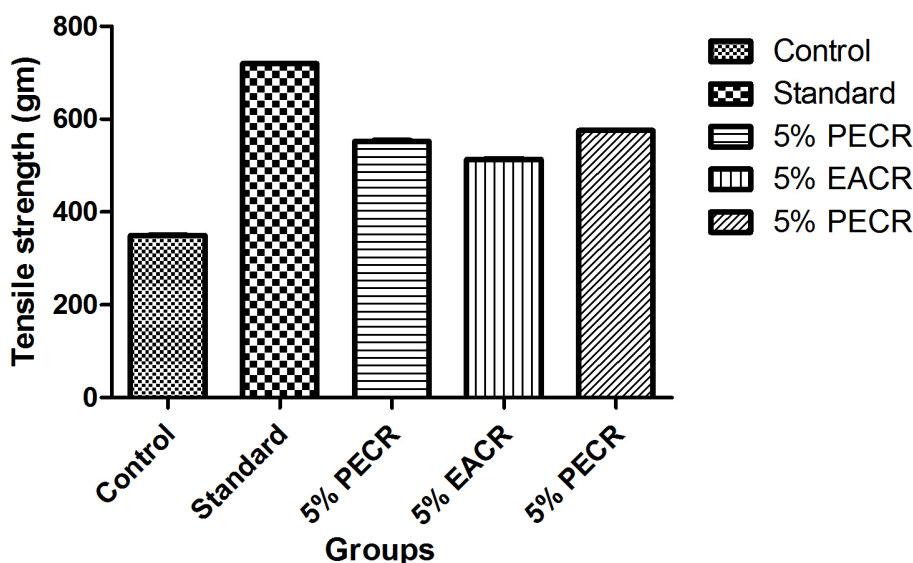


Fig. No. 3 Tensile strength by incision wound model



CONCLUSION

The repair of wounds involves different phases including contraction, the formation of epithelialisation and fibrosis [11]. The biological response regulating the body's own cellular defense mechanisms contributes to the wound and its repair. The leaf extracts of *Cassia roxburghii* has been shown to possess saponins and phenolic compounds [12]. The methanolic extract of *Cassia roxburghii* leaves shown good wound healing property when compared to the remaining two extracts in all the tested models. The wound healing potential of the *Cassia roxburghii* extracts may probably be as a result of the presence of a mixture of phytoconstituents including flavonoids, steroids, etc. [15], the isolation of which is under way in our laboratory. Thus from this study it can be concluded that the *Cassia roxburghii* methanolic leaf extract has a reproducible wound healing potential and thereby justifies its use in folklore medicine in India.

REFERENCES

- [1] J. Swarbrick, J.C. Boylan, (2nd edition), Plants as drugs-Encyclopaedia of Pharmaceutical Technology (Marcel Dekker Inc., New York, **2002**) 2214-2216.
- [2] J.K. Duggal, K. Misra, *Planta med*, **1982**, 45, 48-50.
- [3] P. Rastogi, B.N. Mehrotra, (volume 5), Compendium of Indian medicinal plants, (**2002**)178.
- [4] S. Mohanty, A.B. Das, *J of Bio sci*, **2006**, 4, 664-670.
- [5] K. Madhava Chetty, K. Sivaji, K. Tulsi Rao, (1st edition), Flowering plants of Chittoor district, Andhra Pradesh, India (Students offset printers, Tirupati, **2008**) 110.
- [6] D.V. Rangari, (1st edition), Pharmacognosy and Phytochemistry (Career publications, Nashik, India, **2004**) 129-50.
- [7] K.R. Khandelwal, (10th edition), Practical Pharmacognosy (Nirali Prakashan, Pune, India, **2003**) 38-161.

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- [8] B.M. Methal, (6th edition), A Text Book of Pharmaceutical Formulations (Vallabh Prakashan, New Delhi, **2004**) 236-243.
- [9] Cooper and Gunn, (11th edition), Dispensing for pharmaceutical studies (CBS Publishers and distributors, New Delhi, **1985**) 133.
- [10] D.K. Dnyaneshwar, Y. Rahul, B.K. Mandar, N. Minakshi, R. Prachi, C. Mehendale, H. Chhaya, *J of Ethnopharmacol*, **2009**, 124, 311–315.
- [11] K.D. Rao, B. Shashidhar, *Asian J Plant Sci. and Res*, **2011**, 1 (2), 26-34.
- [12] M. Rajeet, W. Anu, S. Vipin, G. Sumeet, *Eur J Of Exp Biol*, **2011**, 1 (2), 33-40.
- [13] S. Yogesh, S.K. Pradeep, U.K. Patil, R.S. Pawar, *J of Ethnopharmacol*, **2010**, 127, 614–619.
- [14] G. Nilesh, J. Umesh, *Der Pharm Sinica*, **2011**, 2 (2), 256-262.
- [15] S.P. Pattanayaka, P. Sunita, *J of Ethnopharmacol*, **2008**, 120, 241–247.