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Wound healing activity of *Cleome rutidosperma* DC. roots

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ABSTRACT

The petroleum ether, chloroform, methanol and aqueous extracts of *Cleome rutidosperma* DC (Family: Capparidaceae) roots 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. Nitrofurazone (0.2% w/w) in simple ointment I.P. was used as reference standard for the activity comparison. The results of the study revealed that the animals treated with methanol and aqueous extracts of *C. rutidosperma* showed faster rate of wound healing 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 methanol and aqueous extracts. The petroleum ether extract did not produce significant results. The present work justifies the use of *C. rutidosperma* roots for wound healing activity as claimed in the folklore literature.

Key Words: *Cleome rutidosperma* DC, wound healing activity, nitrofurazone, excision wound, incision wound.

INTRODUCTION

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 skin (Karodi *et al.*, 2009). 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 potential (Kumar *et al.*, 2007).

Cleome rutidosperma DC (Family: Capparidaceae) is a low-growing herb, up to 70 cm tall with trifoliate roots and small violet-blue flowers, which turn pink as they age, found in waste grounds and grassy

places. The plant is native to West Africa and has become naturalized in various parts of tropical America as well as South East Asia (Widespread, 1972; Waterhouse, 1998). Traditionally, the roots, leaves and seeds of the plants of *Cleome* genus are used as stimulant, antiscorbutic, anthelmintic, rubifacient, vesicant and carminative (Kiritikar and Basu, 1991). In Malaysia, planting of *C. rutidosperma* around field edges may be considered as part of an insect control programme (Burkill, 2004). The antiplasmodial activity of the chloroform-methanol (1:1) extract of the leaves was reported by Bidla *et al.* (2004), similarly diuretic, laxative, analgesic, anti-inflammatory, antipyretic, antimicrobial, antioxidant and free radical scavenging activities of the aerial parts of *C. rutidosperma* have been reported earlier (Bose *et al.*, 2008). The roots are also reported to have hypoglycaemic effect (Mondal *et al.*, 2009) and anthelmintic activity (Mondal *et al.*, 2009).

As per the folklore information the tribal people apply the juice of roots over severe open wounds and claim for its promising effectiveness towards healing of wounds, so in the present study was undertaken to provide on wound healing activity of the roots using all possible models and provide a scientific support to its use in the folklore medicines for treating wound infection.

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MATERIALS AND METHODS

Plant Material

The plant material (roots) were collected from the rural belt of Vishakhapatnam district of Andhra Pradesh during November 2011 and authenticated. The roots were washed, shade dried and pulverized to coarse powder. The powdered roots (500g) were successively extracted with petroleum ether (40-60°C), chloroform, methanol and water for 48 h in a Soxhlet extractor. Following extraction, the liquid extracts were concentrated under vacuum to yield dry extracts. Standard methods (Harborne, 1984; Trease and Evans, 1989) 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). Six 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 (Normal wounds)

Animals were anesthetized prior to and during creation of the wounds, with 1ml of intravenous ketamine hydrochloride (10mg/kg). The rats were inflicted with excision wounds as described by

Morton and Malone (1972) and suggested by Kamath *et al.* (2003). An impression was made on the dorsal thoracic region 1cm away from vertebral column and 5cm 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 500mm² and 2mm 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 (James and Friday, 2010). All surgical procedures were performed under aseptic conditions.

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 nitrofurazone in simple ointment I.P. Other groups of animals were treated with the following: petroleum ether, chloroform, methanol or aqueous extracts of *C. rutidosperma* 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 1, 4, 7, 10, 13 and 16 post wounding days using transparent paper and a permanent marker. The wound areas recorded were measured using graph paper (Fulzele *et al.*, 2003). The percentage of wound healing was calculated of original wound size for each animal of group on predetermined days i.e. 1, 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 contraction (Dash *et al.*, 2009). The results are tabulated in **Table 1**.

Table 1: Effect of various extracts of *C. rutidosperma* roots on percentage (%) wound closure (Excision Wound Model).

Group Treatment	Concentration	Percentage (%) wound closure.					Epithelialization Period (days)
		4 th days	7 th days	10 th days	13 th days	16 th days	
I Control	-	23.52±1.21	51.92±1.71	67.28±2.23	75.24±1.18	80.56±1.03	24.06±0.62
II Nitrofurazone	0.2%	48.53±2.87*	84.8±1.26**	96.54±1.29**	100±00**	-	13.18 ± 1.47 **
III Pet ether extract	10% w/w	22.47±1.49	56.25±3.08	72.56±1.23	81.79±1.36	83.26±1.4	21.16±0.99
IV Chloroform extract	10% w/w	24.75±1.32	58.82±2.57	73.54±2.77	84.51±1.77*	88.36±0.97*	19.16±1.25*
V Methanol extract	10% w/w	29.88±1.86	82.08±1.6**	92.96±1.14**	97.59±1.39**	100±00**	17.16±1.04**
VI Aqueous Extract	10% w/w	27.69±1.57	63.24±1.39*	82.35±2.22**	92.35±1.4**	94.9±1.09**	18.83±1.02*

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; Dunnett's t-test.

Table 2: Effect of various extracts of *C. rutidosperma* roots on wound breaking strength (Incision Wound Model).

Group	Treatment	Breaking strength (g)
I	Control	325.23±26.08
II	Nitrofurazone (0.2% w/w)	488.51±18.36**
III	Pet ether extract (10% w/w)	332.26±10.23
IV	Chloroform extract (10% w/w)	378.63±18.02*
V	Methanol extract (10% w/w)	457.20±12.58**
VI	Aqueous extract (10% w/w)	383.83±10.82*

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.

Incision Wound Model

The rats were anaesthetized prior to and during creation of the wounds, with 1ml of intravenous ketamine hydrochloride (10mg/kg). The dorsal fur of the animals was shaved with an electric clipper. A longitudinal paravertebral incision of 6cm long was made through the skin and cutaneous tissue on the back as described by Dash *et al.* (2009). After the incision, the parted skin was sutured 1cm 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 application (Reddy *et al.*, 2002). 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 by using GraphPad InStat® software. 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 ± SEM.

RESULTS

The preliminary phytochemical screening of *C. rutidosperma* roots 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 *C. rutidosperma* showed significant promotion of wound healing activity with both aqueous and methanol extracts in the excision and incision wound models. In excision wound model, the mean percentage closure of wound area was calculated on the 1, 4, 7, 10, 13 and 16 post wounding days as shown in Table 1. The methanol extract treated animals showed faster epithelialisation of wound than the animals treated with aqueous roots extract. The percentage of wound closure was 100±00 in the case of standard drug nitrofurazone on 13th day of treatment, where as the methanol extract demonstrated similar effects on 16th day. But the petroleum ether extracts did not reveal significant activity. The period of epithelialization was 17.16±1.04 days for the methanol extract treated group of animals as against 13.18±1.47 for the standards drug treated group.

In incision wound model (**Table 2**), the chloroform, methanol and aqueous extract treated animals showed significant increase in breaking strength (378.63±18.02, 457.2±12.58 and 383.83±10.82 respectively), when compared to the control (325.23±26.08). The mean breaking strength was also significant in animals treated with standard drug nitrofurazone (488.51±18.36) whereas the Pet-ether extract failed to produce significant effects. The methanol 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 methanol and aqueous extracts of *C. rutidosperma* 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 methanol and aqueous extracts. The petroleum ether extract of *C. rutidosperma* did not produce significant results. The wound healing effects of the chloroform, methanol 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 (Karodi *et al.*, 2009; James and Friday, 2010).

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 vascularity (Getie *et al.*, 2002).

Thus, wound-healing property of the methanol 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 methanol extract of *C. rutidosperma* roots 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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