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Wound healing activity of leaf extracts of *Cordia rothii* Roem & Schult.

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Abstract

The plant *Cordia rothii* is a small herb or tree, grows particularly well along rocks and in the dry mountainous areas of Gujarat. The leaf paste is applied on an open wound and inflamed areas by the local people of this region. The present study was carried out to evaluate the traditional claim of the plant in treatment of wounds. The powder of dried leaves of *Cordia rothii* was subjected to continuous extraction with soxhlet extractor using various organic solvents. The preliminary phytochemical screening with the various qualitative chemical tests revealed the presence of carbohydrates, glycosides, saponins, steroids, triterpenes, proteins, amino acids and mucilage were present in the leaf extracts of *Cordia rothii*. All the test extracts at different dose level and standard were administered topically once daily from day 0 to the day of complete healing or the 21st postoperative day, whichever occurred earlier. A significant increase in the wound-healing activity was observed in the animals treated with the alcohol and water extracts of *Cordia rothii* leaves at a dose of 100, 200 and 400 mg/kg of body weight, when compared with the control treatments. Both the alcoholic and water extracts of *Cordia rothii* (100, 200 and 400 mg/kg body weight) showed dose dependent increase in percent wound contraction. The alcoholic extract of *Cordia rothii* at a dose of 400 mg/kg of body weight (AESO 400) shown increase in percent wound contraction from 31.46 % to 91.80 % from day 4th to 16th, while epitheliazation period and scar area were 18.90 days and 19.55 mm^2 respectively. The warer extract of *Cordia rothii* at a dose of 400 mg/kg of body weight (WESO 400) shown increase in percent wound contraction from 33.61 % to 94.45 % from day 4th to 16th, while epitheliazation period and scar area were 17.59 days and 16.84 mm^2 respectively.

Key-Words: *Cordia rothii*, Leaf extracts, Excision wound, Epitheliazation period and Scar area

Introduction

The plant *Cordia rothii* Roem. & Schult, is an evergreen tree found in India. The plant possesses good medicinal value and is used by the people for the treatment of various diseases. The leaf paste was applied on an open wound and also useful in inflammation of legs[1]. The plant possess antidote activity, fruit pulp has astringent, antidiarrhoeal and antiseptic activities and reduces burning sensation of urinary tract, root has abortifacient and anti-inflammatory activities, while the whole plant has antidiabetic and antileprotic activities[2-5]. It has been reported that leaf extracts of certain species of *Cordia* such as *Cordia myxa*, *Cordia francisci*, and *Cordia serratifolia* have significant analgesic, antiinflammatory, and antiarthritic activity in the rat[6].

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The aerial parts of *Cordia verbenacea* plant are used in folk medicine for their anti-rheumatic, antiinflammatory, analgesic and healing properties in the form of alcoholic extracts, decoctions and infusions[7]. The antibacterial properties of the ethanol, petroleum ether, ethyl acetate, methanol and water extracts of *Cordia sinensis* stem bark, at concentration 100 mg/ml were tested against six standard bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Ethanol extract of *Cordia sinensis* stem bark showed promising result against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*[8]. The alcoholic extract of *Cordia rothii* bark was found to possess significant hypoglycaemic activity[9].

A survey of literature revealed that scientific study on the anti-inflammatory and wound healing activity of the plant *Cordia rothii* has been not been previously

reported to validate the folklore claims. The present study was carried out to evaluate the traditional claim of the plant in treatment of wounds. The main objectives of the present research work are biological screening of various extracts for wound healing activity using excision wound model of rat to compare the therapeutic safety and efficacy of the plant.

Material and Methods

Collection and Identification of Plant

Leaves of *Cordia rothii* Roem & Schult, were freshly collected from the Kachchh district of Gujarat (India) in the month of July. The plant material was identified and authenticated by the Botany Department, University School of Sciences, Gujarat University, Ahmedabad, Gujarat (India). The voucher specimen **CRZAH-01** was also preserved for future reference. The collected leaves were shade dried for 15 days and size reduced by mechanical grinder into coarse powder. It was then stored in a well closed container free from environmental climatic changes till usage.

Method of extraction[10]

The powder of dried leaves of *Cordia rothii* was subjected to continuous extraction with soxhlet extractor using various organic solvents such as petroleum ether (60-80 °C), chloroform, ethyl acetate, ethanol and water respectively. After concentration and drying of each extract, identification of phytoconstituents was carried out by performing different qualitative chemical tests.

Preliminary phytochemical screening of various extracts[10-13]

The leaf extracts of *Cordia rothii* obtained during the extraction process were subjected to preliminary phytochemical screening to determine the presence of various phytoconstituents by using reported methods.

Preparation of the plant extracts

Fresh leaves of *Cordia rothii* Roem. & Schult. were collected and dried under shade and powdered by a mechanical grinder. 1 Kg of the pulverized plant material was extracted with different solvents like petroleum ether, chloroform, ethyl acetate, ethanol and water using a soxhlet apparatus. After concentration and drying of each extract, chloroform, ethyl acetate, ethanol and water extracts were selected for the biological screening in various animal models. All the plant extracts were suspended in simple ointment base and used for the topical application to the animals for wound healing activity.

Vehicle

All the plant extracts and standard drug indomethacin were suspended in normal saline and administered orally to animals. Carrageenan were diluted separately in normal saline and injected.

Experimental animals

Albino rats (Wistar strain) of either sex weighing between 150-200 g were used for experimental purpose. The animals were kept in polypropylene cages at room temperature and under 12:12 hours light/ dark cycle. The animals had free access to standard rat pellet and water under strict hygienic conditions. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize, if any non-specific stress. The animals were divided into groups of six animals each and fasted for 12 hours before the experiment. The study was carried out as per the guidelines of Institutional Animal Ethical Committee.

Acute toxicity studies[14]

An acute toxicity study was conducted for all the extract by the stair-case method. The healthy Wistar rats of either sex were fed with plant extracts in increasing doses of 50, 100, 500, 1000, 2000 and 4000 mg/kg body weight respectively. The toxicity was assessed by mortality and behavior changes of the animals.

Selection of dose[15]

Safety of the medicinal plants is equally important when they are used clinically. The doses up to 4000 mg/kg body weight did not produce any signs of toxicity and mortality. The animals were physically active and were consuming food and water in a regular way. A 2.5, 5 and 10 % of the maximum tolerated dose i.e. 100, 200 and 400 mg/kg body weight was selected for the study. A substance is considered safe if it produces no adverse effect in 10 times of the therapeutic dose. These findings support the observation of safety of the plant extracts.

Wound healing studies in rat: Excision wound model[16]

The animals were randomly allocated into six groups comprising six animals each (n=6) for the study **(Table 1)**. Animals were anesthetized prior to and during creation of the wounds, with light ether anesthesia. The rats were inflicted with excision wounds. 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. A full thickness of the excision wound of 2.5 cm in width was created along the markings using toothed forceps, scalpel and pointed scissors. The negative control group was applied with simple iodine ointment. The positive control group was applied with 5% povidone iodine ointment. The remaining twelve groups applied with the chloroform, ethyl acetate, alcoholic and water extracts of *Cordia rothii* at the three different dose levels of 100, 200 and 400 mg/kg body weight. All the test extracts at different dose level

and standard were administered topically once daily from day 0 to the day of complete healing or the 21st postoperative day, whichever occurred earlier. The following parameters were evaluated such as wound area measurement, percentage wound contraction, period of epithelialization and scar area. The rate of wound closure was assessed by tracing the wound on days 0, 4, 8, 12, 16 and 21 post-wounding using transparency papers and a permanent marker. The wound areas recorded were measured using graph paper. The day of eschar falling, after wounding, without any residual raw wound was considered as the period of epithelialization. The percentage wound contraction was calculated by using following formula:

Percentage Closure = [(Wound area on corresponding day - Wound area on zero day)/ Wound area on zero day]× 100

Statistical analysis

All data were expressed as standard error of mean (±SEM) and one-way analysis of variance (ANOVA) was applied to determine the significance difference between the rats treated with test extracts, controls and standards. P value <0.05, <0.01 and <0.001 were considered statistically significant.

Results and Discussion

Preliminary phytochemical screening of *Cordia rothii* leaves

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, tannins, triterpenes, sterols *etc*. Thus the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds.

The preliminary phytochemical screening with the various qualitative chemical tests revealed the presence of carbohydrates, glycosides, saponins, steroids, triterpenes, proteins, amino acids and mucilage were present in the leaf extracts of *Cordia rothii*.

Wound healing activity: Excision wound model

A significant increase in the wound-healing activity was observed in the animals treated with the alcohol and water extracts of *Cordia rothii* leaves at a dose of 100, 200 and 400 mg/kg of body weight, when compared with the control treatments. **Table 2** shows the effect of the varoius extracts of *Cordia rothii* on wound-healing activity in rats inflicted with excision wound. In this model, the extract treated animals showed a more rapid decrease in wound size and a decreased time to epithelialization compared with the control rats which received distilled water. The rate of wound contraction was less in control group of animals, whereas the percentage of wound closure was high in water extract treated group followed by alcohol extract treated animal group, indicating the effect of the plant on promoting healing of excision wound.

Control rats showed a time dependent increase in percent wound contraction from 17.34 % to 42.38 % from day 4th to day 21st, while complete wound closure and epitheliazation was observed on 31st day of wound induction compared with day 0 which was taken as 0%. The mean epitheliazation period and scar area were 31.57 days and 28.15 mm^2 respectively in control rats. Standard drug (Povidone iodine ointment) treated rats shown increase in percent wound contraction from 37.40 % to 100 % from day 4th to 16th **(Figure 1)**. The mean epitheliazation period and scar area were 13.94 days and 18.50 mm^2 respectively **Table 3**.

The chloroform extract of *Cordia rothii* at a dose of 400 mg/kg of body weight (AESO 400) shown increase in percent wound contraction from 20.04 % to 56.32 % from day 4th to 21st, while epitheliazation period and scar area were 26.13 days and 20.39 mm^2 respectively. The ethyl acetate extract of *Cordia rothii* at a dose of 400 mg/kg of body weight (AESO 400) shown increase in percent wound contraction from 19.11 % to 52.30 % from day 4th to 21st, while epitheliazation period and scar area were 27.08 days and 21.55 mm^2 respectively.

Both the alcoholic and water extracts of *Cordia rothii* (100, 200 and 400 mg/kg body weight) showed dose dependent increase in percent wound contraction. The alcoholic extract of *Cordia rothii* at a dose of 400 mg/kg of body weight (AESO 400) shown increase in percent wound contraction from 31.46 % to 91.80 % from day 4th to 16th, while epitheliazation period and scar area were 18.90 days and 19.55 mm^2 respectively. The water extract of *Cordia rothii* at a dose of 400 mg/kg of body weight (WESO 400) shown increase in percent wound contraction from 33.61 % to 94.45 % from day 4th to 16th, while epitheliazation period and scar area were 17.59 days and 16.84 mm^2 respectively.

Conclusion

Wound represents a major health problem, both in terms of morbidity and mortality. Wound healing is a fundamental response to tissue integrity. It mainly depends on the repairing ability of the tissue, type and extent of damage and general state of the health of the tissue[17]. A therapeutic agent selected for the treatment of wounds should ideally improve one or more phases of healing without producing deleterious side effects[18]. Traditional Indian system of medicine has many plants with versatile medicinal properties, which require detailed investigation for effective drug development.

Plant products are potential agents for wound healing and largely preferred because of their widespread availability, non-toxicity, absence of unwanted side effects and their effectiveness as crude preparations[19]. In continuation of the development of drugs from plants to medicine, the extracts of *Cordia rothii* leaves were selected for the wound healing effect.

The preliminary phytochemical analysis of the *Cordia rothii* revealed the presence of carbohydrates, glycosides, saponins, steroids, triterpenes, proteins, amino acids and mucilages. Several studies, including our earlier work with other plant materials demonstrated the presence of similar phytochemical constituents which were responsible for promoting wound healing activity in rats[20-21]. Thus wound healing property of *Cordia rothii* 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. Since *Cordia rothii* are abundantly grown, it could be a fairly economical therapeutic agent for wound management as a pro-healer, as well as to control abnormal healing.

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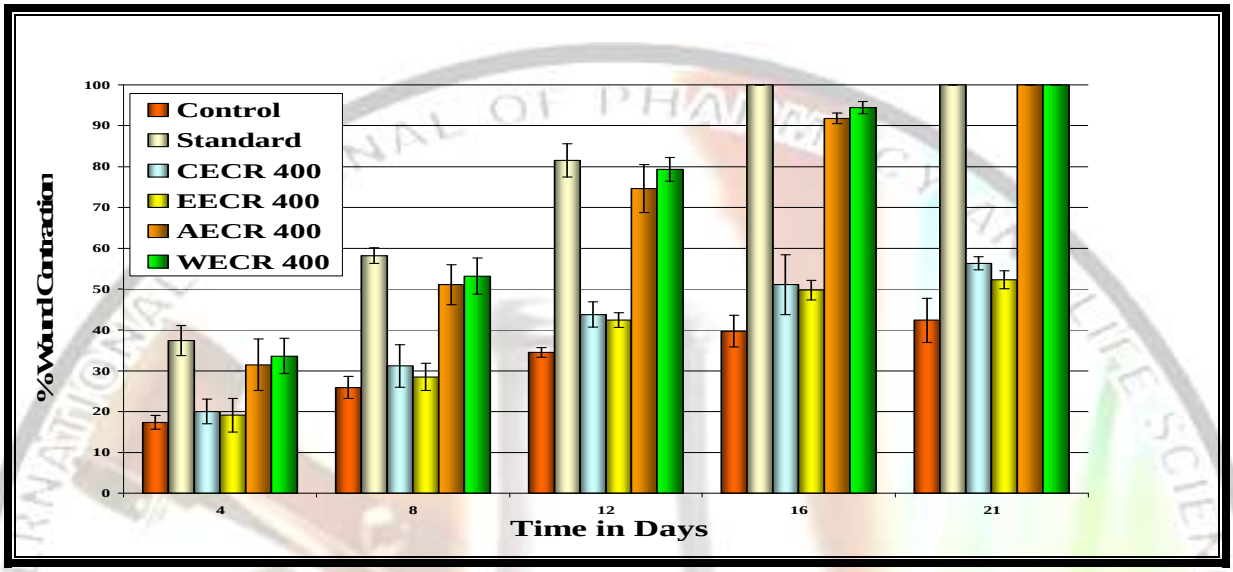

Fig. 1: Percentage wound contraction by various extracts of *Cordia rothii* at the dose level of 400 mg/kg of body weight, in comparison with control and standard

Table 1: Experimental design for wound healing studies in rat

Group	Treatment	Dose
I –Control	Simple ointment base	-
II-Standard	5 % w/w Povidone iodine ointment	-
III-Test Extract CECR-100	Chloroform extract of *Cordia rothii* (prepared with simple ointment base)	100 mg/kg; topically
IV-Test Extract CECR-200	Chloroform extract of *Cordia rothii* (prepared with simple ointment base)	200 mg/kg; topically
V-Test Extract CECR-400	Chloroform extract of *Cordia rothii* (prepared with simple ointment base)	400 mg/kg; topically
VI-Test Extract EECR-100	Ethyl acetate extract of *Cordia rothii* (prepared with simple ointment base)	100 mg/kg; topically
VII-Test Extract EECR-200	Ethyl acetate extract of *Cordia rothii* (prepared with simple ointment base)	200 mg/kg; topically
VIII-Test Extract EECR-400	Ethyl acetate extract of *Cordia rothii* (prepared with simple ointment base)	400 mg/kg; topically
IX-Test Extract AECR-100	Alcohol extract of *Cordia rothii* (prepared with simple ointment base)	100 mg/kg; topically
X-Test Extract AECR-200	Alcohol extract of *Cordia rothii* (prepared with simple ointment base)	200 mg/kg; topically
XI-Test Extract AECR-400	Alcohol extract of *Cordia rothii* (prepared with simple ointment base)	400 mg/kg; topically
XII-Test Extract WECR-100	Water extract of *Cordia rothii* (prepared with simple ointment base)	100 mg/kg; topically
XIII-Test Extract WECR-200	Water extract of *Cordia rothii* (prepared with simple ointment base)	200 mg/kg; topically
XIV-Test Extract WECR-400	Water extract of *Cordia rothii* (prepared with simple ointment base)	400 mg/kg; topically

Table 2: Percentage wound contraction in excision wound model

Group/ Treatments	Wound Area in mm^2 (% Wound Contraction)						
	Dose	Day 0	Day 4	Day 8	Day 12	Day 16	Day 21
Control (Distilled water)	-	267.43± 1.4	221.06 ±1.7 (17.34)	198.09 ±2.7 (25.93)	175.14 ±1.2 (34.51)	161.21 ±3.9 (39.72)	154.09 ±5.4 (42.38)
Standard	-	249.57± 4.8	156.23 ±3.7* (37.40)	104.25 ±1.9** (58.23)	46.07 ±4.1*** (81.54)	00.00 (100)	00.00 (100)
CECR 100	100	273.21± 1.7	225.18 ±2.3 (17.58)	200.18 ±1.2 (26.73)	172.72 ±1.9 (36.78)	158.05 ±3.1 (42.15)	153.05 ±1.6 (43.98)
CECR 200	200	280.37± 2.3	228.14 ±2.9 (18.63)	201.39 ±1.7 (28.17)	168.36 ±1.8 (39.95)	151.09 ±2.3 (46.11)	146.27 ±1.9 (47.83)
CECR 400	400	276.62± 1.2	221.19 ±3.0 (20.04)	190.34 ±5.2* (31.19)	155.52 ±3.1** (43.78)	135.32 ±7.3** (51.08)	120.83 ±1.6** (56.32)
EECR 100	100	292.17± 3.2	239.78 ±2.6 (17.93)	212.17 ±4.3 (27.38)	187.19 ±2.7 (35.93)	169.81 ±1.9 (41.88)	166.07 ±3.2 (43.16)
EECR 200	200	276.93± 2.3	227.86 ±1.4 (17.72)	286.71 ±1.9 (32.58)	165.91 ±2.2 (40.09)	150.90 ±3.1 (45.51)	144.95 ±1.5 (47.66)
EECR 400	400	289.26± 5.0	233.98 ±4.1 (19.11)	206.85 ±3.3 (28.49)	166.58 ±1.8 (42.41)	145.27 ±2.4* (49.78)	137.98 ±2.2** (52.30)
AECR 100	100	291.33± 1.5	234.23 ±1.9 (19.60)	189.86 ±2.4 (34.83)	153.44 ±1.6 (47.33)	101.82 ±3.6 (65.05)	69.39 ±4.1 (76.18)
AECR 200	200	276.62± 5.0	200.88 ±4.1 (27.38)	165.56 ±3.3 (40.15)	114.80 ±1.8 (58.50)	57.48 ±2.4* (79.22)	27.19 ±3.5* (90.17)
AECR 400	400	287.81± 1.5	197.26 ±6.3 (31.46)	140.83 ±4.9* (51.07)	73.05 ±5.9* (74.62)	23.60 ±1.3** (91.80)	00.00 (100)
WECR 100	100	279.47± 1.4	218.32 ±3.3 (21.88)	175.26 ±1.2 (37.29)	137.97 ±3.6 (50.63)	86.64 ±4.3 (69.00)	57.26 ±3.6* (79.51)
WECR 200	200	277.09± 3.0	197.45 ±2.1 (28.74)	155.39 ±1.7 (43.92)	105.60 ±3.2 (61.89)	48.35 ±2.5* (82.55)	15.90 ±4.2* (94.26)
WECR 400	400	280.72± 4.6	186.37 ±1.3 (33.61)	131.38 ±4.9* (53.20)	58.05 ±2.7** (79.32)	15.58 ±3.2** (94.45)	00.00 (100)

*p<0.05, **p<0.01, ***p<0.001 as compared to control, as per one way analysis of variance (ANOVA). Values are expressed as mean ± SEM of n=6 animals in each group.

Table 3: Period of epithelialization and scar area in extract treated excision wound model of rat

Group/Treatments	Dose	Period of Epithelialization (days)	Scar Area (mm^2)
Control (Dist. water)	-	31.57±0.36	28.15±1.9
Standard (3% Povidone Iodine Ointment)	-	13.94±0.16	18.50±1.4
CECR 100	100	31.22±0.28	27.35±2.6
CECR 200	200	28.40±0.11	24.06±1.8
CECR 400	400	26.13±0.23	20.39±1.2
EECR 100	100	30.82±0.20	26.95±1.3
EECR 200	200	29.33±0.16	24.72±2.9
EECR 400	400	27.08±0.15	21.55±1.4
AECR 100	100	25.33±0.14	21.43±1.5
AECR 200	200	23.56±0.10	20.03±2.7
AECR 400	400	18.90±0.29	19.55±1.3
WECR 100	100	24.62±0.24	20.16±2.0
WECR 200	200	22.41±0.28	19.06±1.1
WECR 400	400	17.59±0.20	16.84±2.3