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WOUND HEALING ACTIVITIES OF *FICUS RACEMOSA* LEAVES ETHANOLIC EXTRACT ON EXCISION WOUND MODEL OF WISTAR ALBINO RATS

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ABSTRACT: Present study describes wound healing activities of *Ficus racemosa* (Linn.) leaves extract on excision wound model of wistar albino rats. During the study, fresh plant materials after shade drying was used for the isolation of extract using soxhlet apparatus and percentage yield was obtained (3.50% in ethanol). Then, preliminary phytochemical screening of the extract was done and certain secondary metabolites viz. alkaloids, glycosides, tannin and flavonoid were confirmed in the *Ficus racemosa* extract. Then, thin layer and column chromatography of the extract was done and Rf value 0.12 and 0.84 were calculated and obtained fractions (FR-1 to FR-5) were tested on Wistar albino rats for wound healing activities. In the results of the present study, complete wound healing activity was found to be maximum 84.36% on day 17 when treated with mupirocin 5% ointment. Similarly, complete wound healing activity was found 81.30% on day 18th, by applying ethanolic extract of *Ficus racemosa* as compared to the control group i.e. 62.22% on day 24th.

INTRODUCTION: Naturally, wound healing starts from the moment of injury and can continue for varying periods of time depending on the extent of wounding and the process can be broadly categorized into three stages viz. inflammatory phase, proliferative phase and finally the remodeling phase which ultimately determines the strength and appearance of the healed tissue.[1] The inflammatory phase prepares the area for healing and immobilizes the wound by causing it to swell and become painful, so that movement becomes restricted.

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The fibroblastic phase rebuilds the structure and then the remodeling phase provides the final form.[2] Plants are more potent healers because they promote the repair mechanism in the natural way.[2] Medicinal plants have been shown to possess wound healing activities in animal studies.[3, 4] Sharma et al.[5] have reviewed about Wound healing potential of plants, which are helpful for researcher to develop new Wound healing formulation for human use. Sanjay et al.[6] have also evaluated wound healing potential of poly herbal formulation. Therefore, in the present study, wound healing activities of *Ficus racemosa* leaves extract were tested on Wistar albino rats.

MATERIALS AND METHODS:

In the present study, plant *Ficus racemosa* was identified and authenticated by Taxonomist Dr. P. G. Diwakar, Joint Director, Botanical Survey of India, Pune and was procured in the Herbarium

Record at Se. No. Rajesh2. About 1 kg fresh leaves were collected and brought into the laboratory for shade drying at room temperature and pulverized to powdered at 40-60 mesh size and used for the isolation of crude extract by Soxhletion applying different solvents in increasing order of polarity. The obtained crude extracts were filtered using Whatman's filter paper No.1 and extract evaporated under reduced pressure by using rotary vacuum evaporator (RE 100 Model) to get semisolid crude. The percentage yield of crude extracts was also noticed as shown (**Table 1**). Preliminary phytochemical screening of the plant extract (**Table 2)** was carried out as per the standard methods and presence of different phytoconstituents was noticed by applying the various tests.

The presence of different constituent and their separation in extract of the plant was confirmed by TLC and Column chromatography and Rf value with active fractions were obtained (**Table 3, 4**). In the present study, experimental bioassay was performed at Pest Control and Ayurvedic Drug Research Laboratory and approval from CPCSEA was sought with the approval No. 804/03/CPCSEA and 24 Wistar albino rats of either sex weighing about 100-200 g were housed under the standard environmental conditions of temperature and humidity (25±0.5°C) and 12 h light/dark cycle with proper acclimatization and were utilized for the studies. The animals were fed with standard pellet diet and water *ad-libitum* (**Table 3, 4**).

A full thickness of excision wound of circular area (Approx. 250 mm^2 and 2 mm depth with deep steel stencil was made on the shaved back of the rats by electric clipper and 30 min later the administration of ketamine injection. The wounding day was considered as initial day. The wounds were treated with topical application of the extracts of the plant separately with *Acacia* gum as described above till the wounds were completely healed (**Fig. 1-3**). The wounds were monitored and the area of wound was measured for test and standard on day 0, 4, 8, 12, 16 post-wounding days, respectively and the mean % wound closure were reported in and the period of epithelization was calculated mentioned in **Table 5** as the number of days required for falling of the dead tissue remnants without any residual raw wound. Percentage of wound healing was measured by the formula:

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

Where; n = number of days 0, 4, 8, 12, 16 day.

RESULTS AND DISCUSSION:

In the present study, folklore information was gathered from the tribal peoples for wound healing properties of the plant *Ficus racemosa* and leaves of this plant were shade dried and extracted in ethanol by using Soxhlet apparatus and percentage yield of extract was obtained 3.50% in ethanol (**Table 1**). Similarly, Ganatra et al.[7] have described phytochemical analysis of *Ficus racemosa* leaves extract which showed the presence of phenols, flavonoids, quinones, saponins, cardinolites, steroids, tannins and terpenoids in various extracts (**Table 2**).

Very recently, phytochemical screening of the prepared *Ficus racemosa* leaves extracts was conducted by Rai et al [8] with various qualitative tests to identify the presence of chemical constituents and to perform the tests. Thin layer chromatography have been also been carried out on different extracts of leaves of *Ficus racemosa*, which show different Rf values viz. 0.85 of ethanol extract, 0.57 of methanol extract, 0.54, 0.73, 0.88 of ethyl acetate, 0.79, 0.84, 0.97 of acetone extract, 0.74 of n-hexane extract and possible combination of chromatography solvents i.e. Toluene: Ethyl acetate: Formic Acid having a ratio of 5: 1.5: 0.5 for separation of these phytochemicals (**Table 3**). In the present study, HPTLC was also done for *Ficus racemosa* leaves extract also by using Benzene (45): Methanol (35): Formic acid (20) solvent system and two spots were obtained with Rf value 0.12 and 0.84. John et al.[9] have also performed HPTLC on 10×10 cm HPTLC plates coated with 0.25 mm layer of silica gel 60 F254 (Merck, Germany).

The column chromatography of the *Ficus racemosa* extract was also done by using Benzene (45): Methanol (35): Formic acid (20) solvent systems, respectively and 5 fractions of the plant extract were obtained. In the same manner, Rai et al.[8] have

also obtained the crude extract of *Ficus racemosa* leaves by soxhletion and further fractionation was done with alcohol and acetone. Then, the insoluble fraction of alcohol and acetone was dried and passed through column chromatography (**Table 4**). The mobile phase was consisting of chloroform and the stationary phase was consisting of silica gel (200-400 mesh) and the eluent was collected and dried to obtain whitish powder (FRE) of the fraction. Purified fraction of *Ficus racemosa* done by thin layer and column chromatography was used for wound healing activities.

In the present study, progression of wound healing activities was done by applying ethanolic extract of *Ficus racemosa* and one standard drug Mupirocin ointment applied on created excision wound in albino rats and size of wound was measured by tracing the wound on transparent paper in every 2 days during the process. The measured surface area was then applied to calculate the wound contraction percentage by taking initial size of the wound as 100% and by applying the formula of wound contraction i.e. wound size of initial day subtracted by wound size of final day then multiplied by hundred and finally divided by wound size of initial day.

In the present study, healing patterns of complete wound was found to be maximum 84.36% on day 17 on treatment with mupirocin 5% ointment. Similarly, complete wound healing was found 81.30% on day 18, by applying ethanolic extract of *Ficus racemosa* as compared to the control group i.e. 62.22% (24 days).

In excision wound model, the epithelization period was also measured starting from the first day which was found to be significantly ($P<0.001$) reduced in group II i.e. *Ficus racemosa* ethanolic extract treated animals which showed 81.30±1 contraction on day 16 which was very nearer to the contraction value 84.36% of the reference standard drug mupirocin 5% ointments (**Fig.1-3**).

The epithelization period was found to be highest (17 days) in standard group followed by group II (18 days) and finally maximum epithelization period was taken by the control group which was 24 days (**Table 5**). Very recently, Londhe et al.[10] have discussed wound healing activity of ethanolic extract of *Ficus racemosa* leaves extract. They have reported excision and incision both wound models and used to study wound healing activity in rats. For both models, extract was given in the form of ointment (5% and 10 % w/w). In the excision model, all drug treated animals showed significant ($P<0.01$) increase in percentage wound contraction and incision wound model showed significant ($P<0.01$) increase in breaking strength when compared to control. They have noticed that in biochemical parameter, hydroxyl proline level was significantly ($P<0.01$) increased in all drugs treated groups as compared to control in excision wound model.

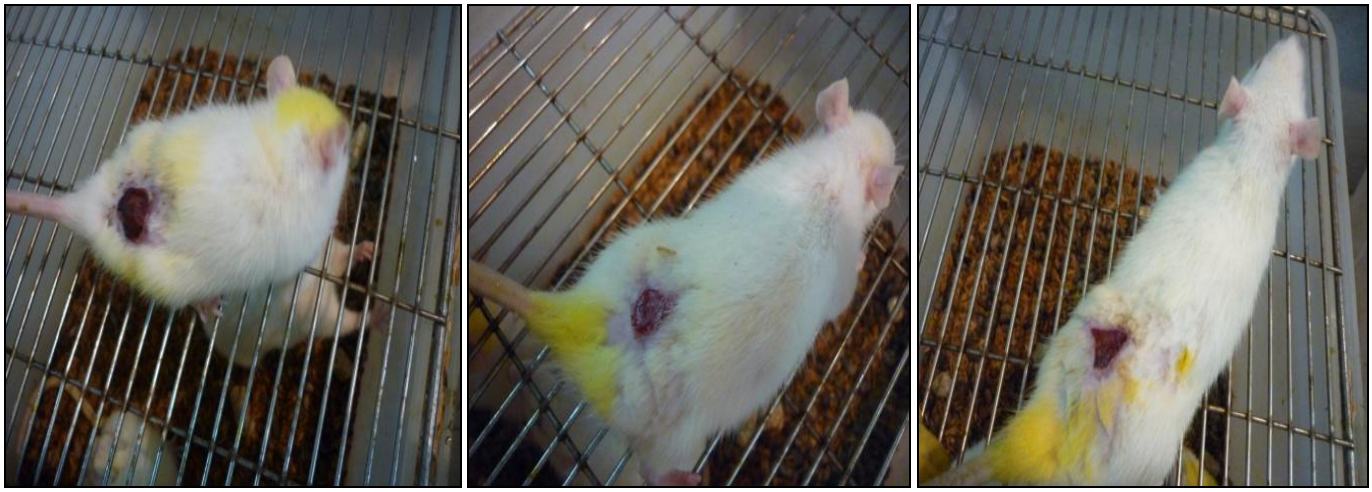

FIG. 1: CONTROL ALBINO RATS SHOWING CREATED EXCISION WOUND, FIG.2: ALBINO RATS TREATED WITH *FICUS RACEMOSA* EXTRACT SHOWING REDUCTION IN EXCISION WOUND, FIG. 3: ALBINO RATS TREATED WITH *MUPIROCIN* SHOWING REDUCTION IN EXCISION WOUND.

TABLE 1: ISOLATION OF EXTRACT FROM POWDERED MATERIAL BY SOXHLETION

Weight of Powdered materials (gm.)*	Solvent Used in Extraction	Extract obtained in (gm.)	% yield of crude extract
Ficus racemosa **Leaves 150 gm**	Ethanol 900 ml	5.27	3.50%

*Powdered material defatted with in n-Hexane.

TABLE 2: PRELIMINARY PHYTOCHEMICAL SCREENING OF *FICUS RACEMOSA* EXTRACTS.

Constituents of plants	Ethanolic Extract
Alkaloids	+
Glycosides	+
Saponins	-
Triterpenoids	-
Tannin	+
Flavonoids	+

TABLE 3: THIN LAYER CHROMATOGRAPHY OF *FICUS RACEMOSA* LEAVES EXTRACTS.

Plant Extract	Solvent System Used	Spots	Rf Value	Color characterization		
				Visual light	Uv-light	Iodine chamber
Ficus racemosa Leaves	Benzene: Methanol: Formic acid (45: 35: 20)	1	0.12	Light brown	Brown	Dark yellow
		2	0.84	Light green	Green	Dark green

TABLE 4: COLUMN CHROMATOGRAPHY OF *FICUS RACEMOSA* PLANT EXTRACTS.

Plant extract	Solvent system used	Fractions obtained	Fractions obtained (gm)	Color characterization
Ficus racemosa Leaves	Benzene: Methanol: Formic acid (45:35:20)	FR-1	0.98	Brown
		FR-2	0.84	Light brown
		FR-3	0.76	Brownish green
		FR-4	0.54	Light green
		FR-5	0.40	Dark green

TABLE 5: PERCENTAGE OF WOUND CLOSURE AND EPITHELIZATION PERIOD IN EXCISION WOUND MODEL.

Experimental Animal Groups	Body wt. (gm)	Wound size(mm^2)	Percentage of wound healing (% in days)				Period of epithelization
		0 days	4 days	8 days	12 days	16 days	Days
Control	109	501.52	15.95±0.071	33.57±0.169	49.28±0.041	62.22±0.293	24
Ficus racemosa Ethanolic extract	129	500.45	32.01± 0.208	50.57±0.441	62.32±0.141	81.30±0.040	18
Standard drug (Mupirocin 5%)	142	500.93	34.35±0.193	52.66±0.229	64.61±0.145	84.36±0.062	17

P<0.001 as calculated by ANOVA test

CONCLUSION: Wound healing activities of *Ficus racemosa* (Linn.) leaves extract purified fraction was found to be more potent on excision wound model of wistar albino rats and complete wound healing was found 81.30% on day 18, by applying ethanolic extract of *Ficus racemosa* as compared to the control group i.e. 62.22% on day 24.

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