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Extraction, Isolation and Wound Healing Activity of Flavonoid from *Coscinium Fenestratum*

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ABSTRACT

The Plant *Coscinium fenestratum* belonging to the family *stercularaceae* contains flavonoids as the chief constituent. In the present study an attempt was made to isolate flavonoids and to evaluate wound healing activity. The plant was collected from the region of Kerala and was authentified by Department of Botany in Kerala University. The whole plant was air dried and ground into fine powders and extracted with hexane for defatting. The defatted material was extracted twice with ethanol and after careful removal of the solvent a dark green syrupy mass was obtained. The extract responded to shinoda test for flavonoids and molisch's test for glycoside type compounds. The extract was subjected to acid hydrolysis by treating with 25 ml alcohol and 4% hydrochloric acid and refluxing for 2 hours to separate the aglycon. The extract was chromatographed over silica gel using ethyl acetate: benzene (1:4). The mixture yielded a yellow coloured compound having melting point 276^0C. The thin layer chromatography was carried out using methylene chloride : ethyl acetate (6:1). The compound showed a single spot at the R_f value of 0.43. The compound obtained was formulated into O/W cream and used for wound healing activity by excision and incision wound model. The flavonoids which were responsible for the free radical scavenging activity were believed to be one of the important components in wound healing. The mean period of epithelization in the control group was 15.46 ± 0.45 days and it was significantly reduced to 10.15 ± 0.50 days in test group and 10.08 ± 0.04 in the standard group. The percentage of wound contraction on 16^{th} day in control group was 59.50 ± 1.14 and it was significantly increased in test group to 75.1 ± 1.25 and 76.25 ± 1.26 in the standard group. This revealed the wound healing activity of flavonoids isolated from *Coscinium fenestratum.*

Keywords: *Coscinium fenestratum, wound healing, isolation, hydroxyproline*

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INTRODUCTION

The plant was collected from the region of Kerala and was authentified by Department of Botany in the University of Kerala. The whole plant was air dried and ground into powders and extracted with hexane for defatting. The flavonoids were isolated using column chromatography and identified by thin layer chromatography. The flavonoids were used to formulate into an ointment which was used for the evaluation of wound healing activity by incision and excision wound models. Hence here an attempt was made to isolate the flavonoids and to evaluate its activity. The percentage of wound contraction and the mean period of epithelization were studied to evaluate the activity.

Objectives

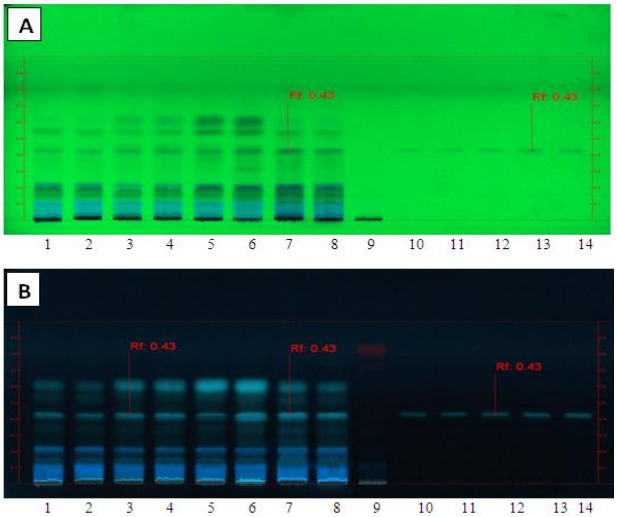
Collection and authentification of the sample
Isolation, Identification and separation of flavonoids from *Coscinium fnestratum*
Preparation of ointment using flavonoids.
Evaluation of Wound healing Activity.

MATERIALS AND METHODS

Extraction, isolation and purification of flavonoids

The whole plant was air dried and ground into powders and extracted with hexane for defatting, the defatted material was extracted with ethanol, after careful removal of the solvent, a dark green syrupy mass was obtained.

The extract was chromatographed over silica gel using ethyl acetate : benzene (1:4), mixture yielded a yellow coloured compound having melting point 276^{0}C, on thin layer chromatography using methylene chloride : ethyl acetate (6:1). The R_f value was found to be 0.43.



HPTLC of flavonoid from *Coscinium fenestratum*

Preparation of Ointment

Simple IP Ointment formula

Wool fat	10gm
Hard Paraffin	10gm
Cetosteryl alcohol	10gm
White soft Paraffin	170 gm

The flavonoid of *Coscinium fenestratum* was mixed with simple IP ointment 5 %(w/w) and 10% (w/w). Framycetin 0.1 %(w/w) were used as reference standard. Hard paraffin & Cetosteryl alcohol was melt on water bath. To this incorporate wool fat & white soft paraffin.Examine the contents for any foreign particles.

Evaluation of Wound healing activity

Excision wound model

The wound healing activity was studied using albino rats of wistar strain of either sex with the weight of 120-150g. The animals were divided into four groups like control, standard, test formulation A and test formulation B with six animals in each group.

The animals in the control group was applied with the simple ointment base, the animals in the standard group was applied with Framycetin 0.1 %(w/w), the test group 1 was applied with 5% of test formulation and the test group B was applied with the 10% of test formulation. The wound contraction was studied by tracing the raw wound area on a transparent paper on 4^{th}, 8^{th}, 12^{th} and 16^{th} day. The wound area was measured plan metrically with the help sq mm scale paper. The degree of wound healing was calculated as percentage wound closure area with the original wound area using the formula given below.

The Percentage closure was calculated using the formula:

$$\text{Percentage closure} = (1-Ad/Ao) \times 100$$

Where Ao = Wound area on day zero.
Ad= Wound area on corresponding days.

The scar shape and area were traced and measured. The result was subjected to statistical analysis.

Incision wound model

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Incision wound model was used for determination of tensile strength of skin. The rats were anesthetized by inhalation of diethyl ether for 2-10 minutes based on the body weight & observed for eye refluxes to confirm unconsciousness. One incision wound of six centimeter was created with surgical scalpel on the back of rats with one and half centimeter away from the spinal cord. Aseptic condition was maintained. Incised wound was sutured with suture (Mersilk) for good adaption of wound. The sutures were removed on 8^{th} day and the tensile strength was determined on 10^{th} day after wounding using constant water flow method.

Determination of hydroxyproline during wound healing

At the end of the 10^{th} day, collagen tissue of excision wound was removed and dried at 108^{0} c for 16 hours & weight was determined. Collagen tissue was placed in volumetric flask and 6N HCL was added and autoclaved for *3 hours at 50pounds pressure* after sealing in order to hydrolyze the proteins then filtered to remove cell debris. From the above solution 1ml was pipetted to each tube. To each tube 1ml of 0.01M $CuSO_4$, 1ml 2.5N NAOH, 6% H_2O_2 solution was added. Shaken vigorously for every 5 minutes, on water bath at 80^{o}c for 5 minutes. Tubes were chilled in ice water bath. Added 4ml of 3N H_2SO_4 with vigorous shaking. Orange color was obtained with 1ml of p-dimethylamino benzaldehyde measured at 559 nm in spectrophotometer [1-9].

RESULTS & DISCUSSION

Effect of the *flavonoid (Coscinium fenstratum)* ointment on percentage wound contraction in Excision wound model (sq.mm)

Sl no	Control (simple ointment)				Standard (Framycetin ointment)				Test drug							
									Low dose(5%w/w)				High dose(10%w/w)			
	4^{th} day	8^{th} day	12^{th} day	16^{th} day	4^{th} day	8^{th} day	12^{th} day	16^{th} day	4^{th} day	8^{th} day	12^{th} day	16^{th} day	4^{th} day	8^{th} day	12^{th} day	16^{th} day
1	20.00	24.7	56.85	85.78	23.66	38.00	66.00	95	15.88	29.55	75.29	100	9.75	47.50	62.5	100
2	15.76	25	43.03	85.65	19.63	32.29	65.00	88.4	11.84	39.23	69.78	97.35	7.14	41.87	69.8	100
3	18.74	24.88	58.85	90.00	20.75	37.20	65.84	94.64	14.28	25.33	74.58	95.26	8.75	46.66	68.44	98.88
4	15.48	24.96	46.88	65.9	16.95	35.34	64.20	91.66	6.84	36.36	72.56	90.22	7.24	42.84	67.6	100
5	19.15	24.88	60.45	90.78	19.15	38.00	65.76	92.51	8.44	34.44	70.66	96.42	9.64	45.64	69.48	100
6	13.33	24.94	65.00	76.46	18.95	37.48	55.96	86.55	10.75	28.89	74.94	91.54	7.54	47.5	65.34	96.65
7	17.07	24.7	56.85	85.78	20.43*	38.00	66.00	95	11.33	29.55	75.29	100	8.343	47.50	62.5	100

Effect of the *flavonoid (Coscinium fenstratum)* Ointment on percentage wound contraction in Excision wound model (sq.mm)

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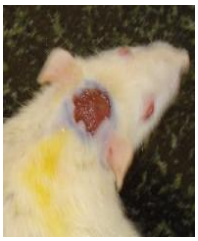
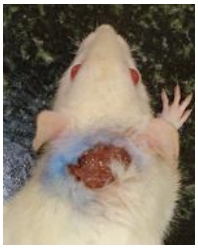
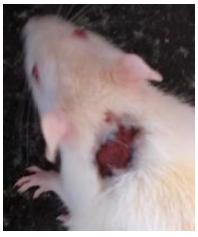
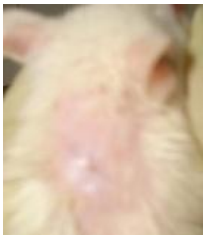
Day 0	Day 8	Day 16
		
Control	Control	Control
		
Standard	Standard	Standard
		
Test A [5%]	Test A [5%]	Test A [5 %]
		
Test B [10%]	Test B [10%]	Test B [10%]

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CONCLUSIONS

- The flavonoids were responsible for the free radical scavenging activity were believed to be one of the important components in wound healing.
- The flavonoids isolated from the plant showed a significant activity when compared with the standard drug Framycetin ointment.
- This revealed the wound healing activity of flavonoids isolated from *Coscinium fenestratum.*
- Hydroxyproline concentration since hydroxyproline is a amino acid which is present in larger amount but along with this amino acid other amino acids are in combination form the collagen tissue which is offering better tensile strength.

REFERENCES

[1] Altan VM. Curr Med Chem 2003;10: 1317–1327.
[2] Baron AD Diabetes Res Clin Pract 1998;40(suppl): S51-S555.
[3] Campbell RK, White JR Jr, Saulie BA. Clin Ther 1996;18: 360-371.
[4] Nair GM, Narasimhan S, Shiburaj S, Abraham TK. Fitoterapia 2005; 76: 585-587.
[5] Pinho PMM Paulo, Pinto MMM Madalena, Kijjoa A, Pharadai K, Diaz JG, Herz W Phytochem 1992; 31:1403-1407.
[6] Punitha IS, Rajendran K, Shirwaikar A, Shirwaikar A Evid Based Complement Alternat Med 2005;2(3): 375-381.
[7] Reuser AJ, Wisselaar HA. Eur J Clin Invest 1994; 24 (suppl):19-24.
[8] Shirwaikar A, Rajendran K, Punitha IS. J Ethnopharmacol 2005; 97: 369-374.
[9] Strojek K. Acta Diabetol 2003;40 (Suppl 2): S334–S337 Varier PS (1994). Indian Medicinal Plants Compendium of 500 Species vol. 2, Orient Longmann Ltd., Hyderabad pp. 191–193.