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Wound healing activity of *Capparis zeylanica* (root)

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ABSTRACT

The methanol extract of *Capparis zeylanica* Linn. root was evaluated for its wound healing potential in two different types of wound models viz., incision and excision. Two dose levels (100, 200 mg/kg b.w.) of methanol extract of *Capparis zeylanica* root were tested by incision wound model. The 100 mg/kg produced mild wound healing activity whereas 200 mg/kg produced pronounced healing properties in the tested parameters, tensile strength and DNA content. Two concentrations, 1% and 2% were tested in the excision wound model. There was better healing property observed in 2% concentration when compared to povidone iodine ointment. It was confirmed by finding the parameters; percentage wound contraction, epithelisation time, collagen content and DNA content.

Keyword: *Capparis zeylanica*, excision & incision wound models, collagen content and DNA content.

INTRODUCTION

A climbing or rambling shrub, 2-10 m in height, armed with 3-6 mm long, recurved thorns, distributed largely east of the line Bombay-Delhi-Dehra Dun and south of the Himalayas and in the Andaman Islands. The fruits are used as a vegetable and sometimes for curry in Konkan(Maharashtra). They are also pickled. The young shoots and leaves are eaten by goats and elephants. The root bark is bitter, stomachic, sedatives, antihidrotic and useful in fever. The paste of the rootbark is reported to contain an alkaloid, a phytosterol, a water soluble acid, and a mucilaginous substance. The paste of the root is used by Mundas of Ranchi district (Bihar) for

body-ache. The leaves contain β -carotene. They are applied as poultice to piles, boils and swelling and also as a counter-irritant. The plant is useful in fever. It contains a saponin and *p*-hydroxybenzoic, syringic, vanillic, ferulic and *p*-coumaric acids [1]. The antimicrobial activity of root of *Capparis zeylanica* has been reported [2]. So the present study is undertaken to evaluate the wound healing activity of root of *Capparis zeylanica*.

EXPERIMENTAL SECTION

The plant *Capparis zeylanica* was collected from the local area Barpali, Bargarh, Odisha in the month of January. The dried root powder of the material was initially extracted successively with petroleum ether, chloroform, ethyl acetate & methanol by Soxhlet apparatus for 18 hrs and solvent was removed by distillation. It was concentrated at 50°C under reduced pressure to a semisolid mass. The percentages of yield of the extract were calculated. The qualitative investigation test performed in the extract to find out various phytoconstituents [3]. Thin layer chromatography studies of the methanol extract was carried out in various solvents at 30°C using silica gel GF 254 plate as adsorbent [4].

Animals

Albino rats of either sex, weighing 180-200g, housed in standard condition of temperature, humidity, and light were used. They were fed with standard rodent diet and water *ad libitum*. All the studies conducted were approved by Institutional Animal Ethical Committee (1376/ac/10/CPCSEA.). The Pharmaceutical College, Barpali, according to prescribed guidelines of the Committee for the purpose of Control and Supervision of Experimental on Animals (CPCSEA), Government of India.

Wound healing activity

Excision wound model

Animals were divided into four groups of six rats each. All animals were kept in separate cages and standard wound of uniform 2 cm diameter was formed with the aid of round seal. Group-I served as control, Group-II and Group-III received (1% & 2 %) external application of extract (*C.zeylanica*) once a day. Group-IV received 5% w/w of povidone iodine ointment topically. The percentage wound contraction, epithelisation time, collagen content and DNA content were evaluated on tenth day [5] [6].

Incision Wound Model

Rats were incised to produce wound under light anesthesia. Dose of the drug was determined as 100 and 200 mg/Kg b.w. from acute toxicity studies (LD₅₀ dose 3000 mg). The animals were divided into four groups of six rats each (n=6). Group-I served as control, received only normal saline (1ml/Kg,p.o) while the Group-II received 100 mg/Kg p.o of *C.zeylanica* root extract daily for ten days. Group-III received 200 mg/Kg body weight p.o of *C.zeylanica* root extract daily for ten days. Group-IV was treated with standard povidone iodine ointment (50 mg/rat) topically [7]. The stitches were removed after 8th day and tensile strength and DNA content of the wound were determined on 10th day [8]. Statistical analysis was made and the P-value was obtained using Student's t-test.

RESULTS AND DISCUSSION

Extraction

The dried root powder of the material was initially extracted successively with petroleum ether, chloroform, ethyl acetate & methanol by soxhlet apparatus for 18 hrs and solvent removed by distillation. The percentages of yield of the extract of root were found to be 1.35 w/w, 2.25w/w, 2.87w/w & 4.16 w/w . The petroleum ether extract of root showed the presence of steroid and alkaloid. The chloroform extract of root showed the presence of carbohydrate, steroid, glycoside & alkaloids. The ethyl acetate extract of root showed the presence of carbohydrate, steroid, glycoside, alkaloids, tannins and phenols. The methanol extract of root revealed the presence of carbohydrate, steroid, glycoside, flavonoid, alkaloids, tannins & phenols (Table-1).

Thin layer chromatographic studies

Thin layer chromatographic studies were carried out in methanol extract. The methanol extract showed maximum seven spots by using solvent system Methanol: Ethyl acetate (7:3) and six spots by using solvent system Chloroform:Methanol (3:2) on TLC plate. Anisaldehyde sulphuric acid was used as detecting agent. Rf values recorded were given in (Table-2).

On excision wound model when 2% solution of extract was applied on the wound area the progress of healing was shown as 80.17% ($15.7 \pm 0.14^{**}$) at 8th day (Table-3)., it reduced the epithelisation time from 22.31 to 19.82 min. The DNA content also increased significantly to 18.2 mg in extract treated groups with that of control. The increased in DNA on eighth post wounding day was comparable with the standard povidone iodine ointment. In addition to this, there was profound increase in the collagen content from 108.3 mg/g tissue to 193.3 mg/g in case of extract treated group. This effect can be comparable with the standard 198.5 mg/g. From this investigation, it is evident that methanol extract of *C.zeylanica* root increases the wound breaking strength, indicating the increase in collagen strength thereby facilitating wound healing. Increase in DNA content indicates the profound cellular growth (Table-4).

On incision wound model the tensile strength was significantly increased from 246 to 353 g/cm² in 200 mg/kg of extract treated group. Whereas 100mg dose level produced less significant increase in tensile strength on tenth day after wounding. DNA content was increased from 9.35mg/g to 11.12 mg/g and 14.41 mg/g in 100 mg and 200 mg dose levels respectively (Table-5).

From this investigation, the healing property of root of *C.zeylanica* may be due to increase in cellular growth and increased wound breaking strength. The presence of components like carbohydrate, steroid, glycoside, flavonoid, alkaloids, tannins & phenols either individually or combined together may exhibit the synergistic effect towards healing of wounds. However, further investigations employing isolation of constituents and screening models are needed for further confirmation of wound potential of root of *C.zeylanica*.

Table-1 Preliminary phytochemical screening of different extracts of root of *Capparis zeylanica*

Test	Petroleum ether	Chloroform	Ethyl acetate	Methanol
TEST FOR CARBOHYDRATE				
Molish test	-	+	+	+++
TEST FOR PROTIEN				
Millon's test	-	-	-	-
TEST FOR STEROID				
Salkowski reaction	+	+	+	+
Liebermann-Burchard reaction	-	+	+	+
TEST FOR GLYCOSIDES				
Baljet test	-	+	+	+
Legal test	-	-	+	+
Saponin glycosides	-	+	+	+
TEST FOR FLAVONOIDS				
Shinoda test	-	-	-	+
Lead acetate test	-	-	-	+
TEST FOR ALKALOIDS				
Dragendorff's test	+	+	+	++
Meyer's test	+	+	+	++
Hager's test	+	+	+	++
Wagner's test	+	+	+	++
TEST FOR TANNINS & PHENOLS				
5% FeCl ₃	-	-	+	++
Leadacetate	-	-	+	++

+ Mild, ++ Moderate, +++ Frequent, - Absent

Table- 2 Thin layer chromatographic studies of *Capparis zeylanica* root extract

S. No	Extract	Solvent systems	Detecting reagent	No. of spots	Colour of spots	Rf values
1	Methanol extract	Methanol: Ethyl acetate (7:3)	Anisaldehyde sulphuric acid	7	Grey	0.25
					Grey	0.33
					Pink	0.5
					Pink	0.62
					Grey	0.69
					Grey	0.8
					Pink	0.93
2	Methanol extract	Chloroform:Methanol (3:2)	Anisaldehyde sulphuric acid	6	Pink	0.27
					Pink	0.3
					Pink	0.38
					Grey	0.6
					Grey	0.83
Pink	0.94					

**Table-3 Effect of *Capparis zeylanica* root extract on excision wound in rats
(Percentage closure of wound area on post wounding day)**

Drug	4 th Day	8 th Day	12 th Day	16 th Day	20 th Day
Control	7.20 ± 0.27	9.9 ± 0.23	15.9 ± 0.36	18.1 ± 0.12	18.92 ± 0.13
Standard	10.2 ± 0.30	14.8 ± 0.27	18.2 ± 0.12	18.8 ± 0.1	19.70 ± 0.04
2% solution	12.3 ± 0.26	15.7 ± 0.14**	18.9 ± 0.07**	19.2 ± 0.45**	20.06 ± 0.02**
1% solution	7.4 ± 0.25	8.6 ± 0.20	14.8 ± 0.29	17.4 ± 0.11*	19.04 ± 0.12*

*P value < 0.05, ** P value < 0.01

Table-4 Other parameters on excision wound of rats

Drugs	Epithelisation (Days)	Collagen content (mg/g tissue)	DNA Content (mg)
Control	22.31 ± 0.24	108.3 ± 0.4	11.3 ± 0.92
Standard	20.13 ± 0.32	198.5 ± 0.11	18.7 ± 0.13
2% solution	19.82 ± 0.07	193.3 ± 0.19*	18.2 ± 0.14**
1% solution	16.31 ± 0.22	162.31 ± 0.3	12.31 ± 0.21

*P value < 0.05, ** P value < 0.01

Table- 5 Tensile strength of restructures incision wound on 10th post wounding day

Treatment	Tensile strength g/cm ²	DNA mg/g
Control	246 ± 08	9.35 ± 0.92
Standard	356 ± 20	15.63 ± 1.13
100 mg extract	254 ± 05*	11.12 ± 0.12*
200 mg extract	353 ± 17**	14.41 ± 0.66**

*P value < 0.05, ** P value < 0.01

CONCLUSION

The result of the present study indicate that the methanol extract of *C.zeylanica* root has significant wound healing property both in excision and incision wound models. The presence of various phytoconstituents may be responsible for wound healing activity.

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