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Original Research Article

Assessment of Wound Healing Potential of *Cleome viscosa* Seeds Extracts

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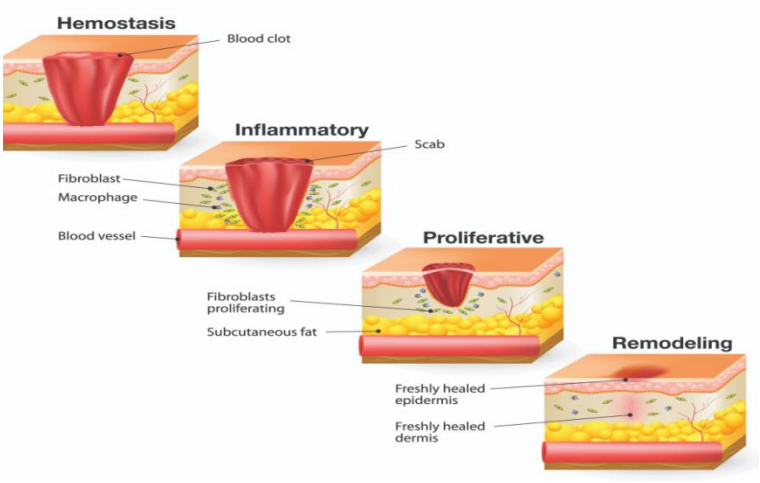
Abstract: In a natural way, wound healing is slow phenomenon and sometimes may become long lasting with a long clinical course there by causing constant liberation of inflammatory modulators that cause pain and redness. Wound healing begins at the time of injury and can vary in length depending on the severity of the wound. The wound healing process can be divided into three stages: the inflammatory stage, the growth stage, and finally the remodeling stage, which determines the strength and appearance of the healed tissue. In the present work an attempt had been made to evaluate wound healing potential of Simple ointment base B.P was applied on control group rats. Cipladine (Povidone-Iodine IP 5% w/w, Batch no. UZ300) ointment was applied on standard group rats. Ointments with different extracts concentrations i.e. 0.5% (w/w), 1% (w/w) and 2% (w/w) assimilated in simple ointment base were applied on test group rats, in each model.

Keywords: Wound healing**,** ointment base B.P, *Cleome viscosa* & Period of epithelization.

INTRODUCTION

Herbalism is very important, especially in the healthcare system of developing countries. In ancient Indian literature, medicinal plants are defined by a very broad logic as a possible source of therapeutic ingredients [1]. The number of patients looking for alternative or herbal remedies is growing rapidly. Chinese herbal medicine has been the integration of generations of practitioner treatment experience in the indigenous health system for hundreds of years. Not only are Chinese herbs cheap, they are culturally acceptable, they are highly resistant to the human body, they have few side effects in case of injury, and they vary, so the demand for primary health care is high in poor countries [2]. It lasts for a variety of times, depending on the severity of the wound. The wound healing process can be divided into three stages: the inflammatory stage, the growth stage, and the remodeling stage, which determines the strength and appearance of the tissue that has finally healed [3].

Phase of wound healing [4]



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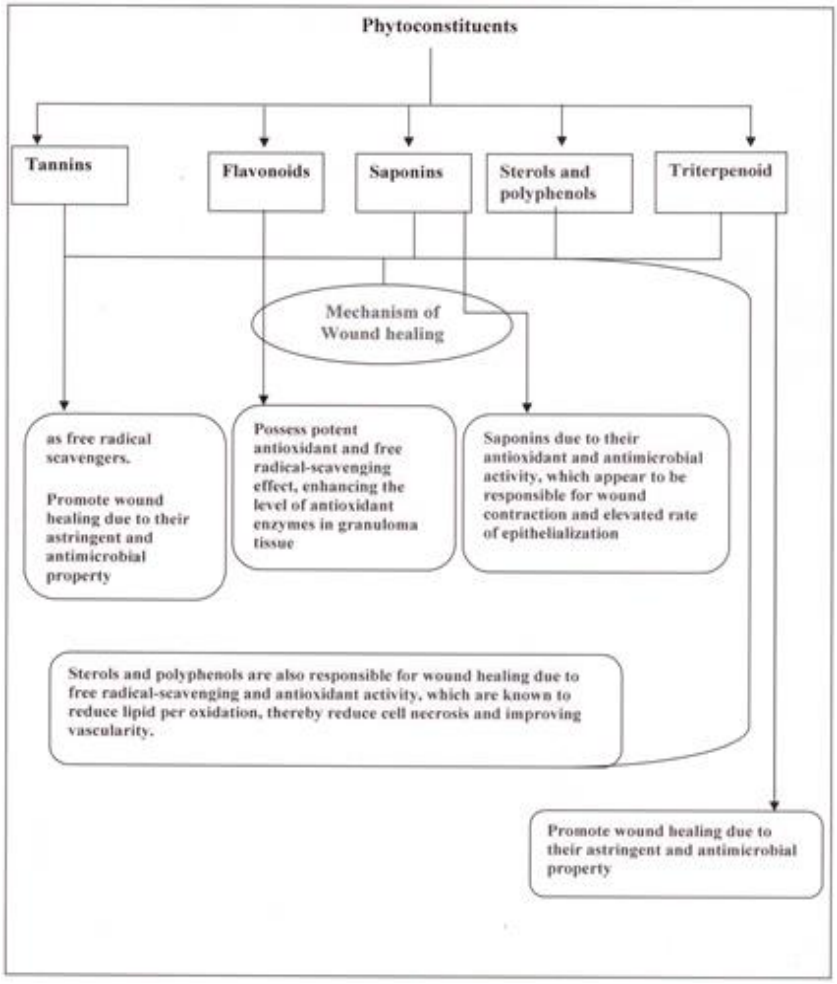
Alternative reasserts of drug treatments like natural drug treatments may also quickly come to be key additives within side the healthcare provision enterprise for each people and animals mainly in growing countries. These natural drug treatments will fill an opening resulting from a lower with inside the quantity of latest cutting-edge drug treatments being evolved within side the previous few decades (mainly with the case with anti-infective), growing costs, drug resistance, and aspect outcomes of cutting-edge pharmaceuticals[5]. Most herbs or components thereof incorporate Phytoconstituent which have huge ranging medicinal value like antimicrobial, anti-inflammatory, antioxidant, antipruritic, hypotensive, proliferative, hypoglycemic, and analgesic which are regularly key role in wound control or healing [6].



Role of Phytocontituent in wound healing

Cleome viscosa Linn. (wild or dog mustard), belonging to the family (Capparaceae), is a common weed found in the plains of India and other tropical regions of the world [7] and is called ―hurhur in India.

Fig-1: *Cleome viscosa* plant & seeds

Biological description	Phytochemistry	Distribution
Cleome viscosa Linn. is an annual erect, 30 - 90 cm high plant. Its stem is grooved and densely clothed with glandular and simple hairs. Leaves of the plant are 3 - 5 foliate. The petioles are longer, (2.5 - 5 cm) in lower parts of the plant and become shorter in the upper parts. The bracts are sub sessile. Leaflets are elliptical – oblong or obovate, acute or obtuse. Petioles are short and hairy. Flowers are yellow, axillary, growing out into a lax raceme. Pedicels are slender, terete and hairy. Sepals are 4.5 cm long oblong –lanceolate, glandular – pubescent outside. Petals are oblong – obovate, about 12 mm long, veined. There are more than 20 stamens. Capsules 5-6.3 by 0.4 cm, erect, hairy, obliquely striate, compressed, and tapering towards both ends, terminated by a style 3 mm. long. Seeds are brown – black in colour when ripe, finely transversely striate, subglobose [8].	Cleome viscosa Linn. is found throughout the tropical regions of world including India, Pakistan, Sri Lanka, China, Africa and America[7].	Various parts of the plant such as the root, stem, leaf and seed, have been subjected to phytochemical work to isolate and identify the compounds. Preliminary phytochemical screening of the extracts was performed and it was reported that terpenes, flavonoids, phenol carboxylic acid, polyphenols were present [9]. Other products reported to be isolated from the leaves, seeds and root extract include monoterpenes hydrocarbons, sesquiterpenoids, and oxygenated derivatives.

Traditional uses	Pharmacological activity
Traditionally, *Cleome viscosa* Linn. Plant is used as an antimalarial and is useful in other conditions such as disorders of the blood, diseases of the uterus [10].	Analgesic, antiemetic, antidiarrhoeal, Heptoprotective, antifibrotic and antitumor activity [10-14].

In the present work an attempt had been made to evaluate wound healing potential of simple ointment base B.P was applied on control group rats. Cipladine (Povidone-Iodine IP 5% w/w, Batch no. UZ300) ointment was applied on standard group rats. Ointments with different extracts concentrations i.e. 0.5% (w/w), 1% (w/w) and 2% (w/w) assimilated in simple ointment base were applied on test group rats, in each model.

MATERIAL AND METHODS

Selection of plants

The plants were selected on the basis of their anti inflammatory activities, chemical constituent and wide medicinal uses in the traditional literatures. The ease of availability of plant is also taken into consideration during selection.

Collection and Authentication

The mature seeds of *Cleome viscosa* L. were collected from Jammu and nearby areas and authenticated.

Extraction

Preparation of extracts

The powdered plant material was extracted successively with redistilled, analytical grade petroleum ether (PEE), Ethyl acetate (EAE), methanol (MEE) and water (AEE) by soxhelation.

Fig-2: Extraction of crude plant seeds

Evaluation of wound Healing Potential

Animals were divided into three major groups i.e. control group, test group and standard group. Six animals in each group were taken. Simple ointment base B.P was applied on control group rats. Cipladine (Povidone-Iodine IP 5% w/w, Batch no. UZ300)

ointment was applied on standard group rats. Ointments with different extracts concentrations i.e. 0.5% (w/w), 1% (w/w) and 2% (w/w) assimilated in simple ointment base were applied on test group rats, in each model.

Excision woulnd model

Ketamine (0.5 mL/kg b.w.) was administered to animals by intraperitonial route. Back of rats were then shaved. After 30 min, circular excision wound of about 500 mm2and 1.5 mm depth was made on shaved back. The day when circular excision wound was made was considered as 0th day. Ointment was applied topically as described above until complete healing of wounds was achieved. Wounds were observed daily and wound area was measured on 4, 8, 12, 16, 20 24, 28 post-wounding days and % wound closure (mean). By noting days required falling of eschar, the period of epithelization was calculated [15].

% of wound closure = Wound area on day 0 – wound area on day n X100
Wound area on day 0
Where n = number of days [4th, 8th, 12th and 16th day].

Incision wound model

Ketamine (0.5 mL/kg b.w.) was administered by intraperitoneal route in rats. Back of rats were shaved. After 30 min, incision wounds of about 5.5 cm in length and 2.1 mm in depth were made on the shaved back by sterile scalp. Black silk was used to stich the parted skin at about 0.4 cm interval (Fig. 6.2). For stiching purpose, surgical thread (No. 30) and a curved needle (No. 8) were used. On both wound edges, thread was tightened for complete closure of the wounds. Then for about 10 days ointments of different concentrations were applied topically on incision wound of different group of rats as described above. Day 0 i.e. the day when incision wound was made was considered as wounding day. On the 8th post-wounding day i.e. when complete healing of incision wound was achieved, sutures were removed (Fig. 6.5). On the 10th post wounding day, tensile strength of the skin was measured using tensiometer. Tensile strength is the weight (in g) needed to break open the wound/skin [15].

Extimation of hydroxyproline as biochemical marker

As described in excision wound model circular wound of (500 mm2) approx. was created. For time period of 19 days, ointments were applied topically on excision wounds. On 20th day, eschar was removed and dried in oven at about 110 °C. It was weighed (10 mg) and kept in glass stoppered test tubes. To each tube containing 10 mg of the dried eschar, 1 mL of 6 N HCl was added. The tubes were then kept on boiling water bath for 24 h (12 h each day for two days) for hydrolysis. The hydrolysate was then cooled and excess of acid was neutralized by 10 N NaOH using phenolphthalein as indicator. The volume of neutral hydrolysate was diluted to a concentration of 20 mg/mL with distilled water. The final hydrolysate was used for the estimation of hydroxyproline.Hydroxyproline (HPR). To each tube, 1 mL each of hydrolysate, 2.5 NNaOH, 0.01M CuSO4, and 6% H2O2 were added. Tubes were shaken vigorously and placed immediately in water bath at 80°C. After 15 min, tubes were removed and cooled for 5 min in cold water. 0.6 mL of freshly prepared 5% solution of paradimethyl aminobenzaldehyde in n-Propanol and 1.2 mL of 3 N H2SO4 were added. The test tubes were once again placed in a hot water bath at 75°C for 15 min and then cooled for 5 min under running stream of water. Color intensity was measured at 540 nm against the blank, using spectrophotometer. Hydroxyproline content in the tissue was estimated as per standard curve prepared with standard 4-Hydroxy-L-proline (CDH Laboratories Pvt. Ltd., New Delhi, India), from 10 to 100 #g/mL.[16].

RESULT AND DISCUSSION

In this work evaluate wound healing potential of simple ointment base B.P was applied on control group rats. Cipladine (Povidone-Iodine IP 5% w/w, Batch no. UZ300) ointment was applied on standard group rats. Ointments with different extracts concentrations i.e. 0.5% (w/w), 1% (w/w) and 2% (w/w) assimilated in simple ointment base were applied on test group rats, in each model. Rats group treated with petroleum ether extract ointment (2% w/w), ethyl acetate extract ointment (2% w/w), methanol extract ointment (2% w/w), and aqueous extract ointment (2% w/w) showed potent wound contraction ability ($P < 0.001$) on 12th day and 16th in comparison to control group rats. In case of rats treated with petroleum ether extract ointment (2% w/w), period of epithelization observed was found to be 20 days only. In the results reported in excision wound model and incision wound model it was observed that rats group treated with petroleum ether extract ointment (2% w/w), ethyl acetate extract ointment (2% w/w), methanol extract ointment (2% w/w), aqueous extract ointment (2% w/w) show potent wound healing potential in comparison to control. Thus on 20th day, the content of hydroxyproline (biochemical marker) in the eschar of excision wound created in rats treated with above mentioned in table 1 & graph.1. The tensile strength of the incision wound was measured for various treatment options, which included control (simple ointment B.P. treated group.), standard (cipladine treated group) and the test viz. petroleum ether extract ointment treated group (0.5%, w/w; 1%, w/w and 2% w/w), ethyl acetate extract ointment treated group (0.5%, w/w; 1%, w/w and 2%, w/w), methanol extract ointment treated group (0.5%, w/w; 1%, w/w and 2%, w/w) and aqueous extract ointment treated group (0.5%, w/w; 1%, w/w and 2%, w/w).The results are shown as mean weight in gram+SEM required to pierce open the resutured wound Fig. 4 (Table 2).In incision wound model on the 10th day the tensile strength of the tissues in various

groups were control group was 188.93 + 3.84 and the tissues in cipladine treated group was 727.10 +5.01 while in 2% SO.(PEE) treated tissue it was 726.13 +2.15. It is observed that there was no significant difference in tensile strength of 2% SO. (PEE) treated group and standard drug treated group. The increase in tensile strength of treated wounds may be due to the increase in collagen concentration, Hexosamine, Uronic acid and stabilization of the fibers by increase in protein content [16]. The synthesis of new extracellular matrix was improved by the matrix molecules Hexosamine and Uronic acid which act as ground substratum. The collagen synthesized was laid down at the wound site, cross linked to form fibers. Collagen not only deliberates the strength and integrity to the tissue matrix but also plays a vital role in homeostasis and in epithelialization at the later phase of healing. Collagen is the predominant extracellular protein in the eschar of a healing wound and there is a rapid increase in the synthesis of this protein in the wound area soon after an injury. Free hydroxyproline and its peptides are liberated upon breakdown of collagen. Assessment of hydroxyproline serves as an index of collagen turnover. Higher content of hydroxyproline in the group of rats treated with petroleum ether extract ointment (2% w/w), ethyl acetate extract ointment (2% w/w), methanol extract ointment (2% w/w), aqueous extract ointment (2% w/w) has indicated faster collagen turnover leading to rapid healing with concurrent increase in the breaking strength of the treated wounds. Rats treated with petroleum ether extract ointment (2% w/w), ethyl acetate extract ointment (2% w/w), methanol extract ointment (2% w/w), aqueous extract ointment (2% w/w) showed significantly high ($P < 0.001$) levels of hydroxyproline content (43.58 µg/500 mg, 39.45 µg/500 mg, 37.86 µg/500 mg, 40.09 µg/500 mg respectively) as compared to control (29.52 µg/500mg). Out of these four extracts, the petroleum ether extract in the concentration of 2% (w/w) is found to be the most effective (table.3).

Table-1: Effect of different extracts on circular excision wound

Group Mean	(%) of wound contraction ±SEM				Period of Epithelization (days)
	4th day	8th day	12th day	16th day	
Gr I(Control)	18.65 +1.98	37.99 +1.45	55.95 +1.58	74.51 +1.55	28
Gr II(Standard)	31.15 +1.18	57.88 +1.85	87.69 +1.00	99.15 +0.58	20
Gr III (0.5% SO.(PEE))	24.30 +1.45*	41.67 +1.79	73.39 +1.78*	86.07 +1.46**	24
Gr IV (1% SO.(PEE))	25.96 +1.95*	40.89 +2.63	77.97 +1.14*	94.39 +1.59**	22
Gr V (2% SO.(PEE))	29.52 +1.40*	57.16 +0.25*	87.98 +1.88**	98.99***	20
Gr VI (0.5% SO.(EAE))	18.68 +2.00	38.00 +2.10	69.25 +1.76*	79.05+1.61**	25
Gr VII(1% SO.(EAE))	20.72 +1.57	39.89 +3.85	73.88 +1.53*	84.56+1.49**	24
Gr VIII(2% SO.(EAE))	23.82 +1.85*	44.75 +2.96*	79.78 +1.59*	91.96+0.76**	22
Gr IX (0.5% SO.(MEE))	19.30 +1.76	38.78 +3.99	69.18 +1.65*	82.12+1.59**	25
Gr X (1% SO.(MEE))	22.52 +1.38	40.56 +3.63	72.68 +1.84*	84.39+1.59**	24
Gr XI (2% SO.(MEE))	24.92 +1.96*	43.87 +3.86	78.98 +1.95*	90.89+0.69**	23
Gr XII (0.5% SO.(AEE))	15.51 +1.09	41.61 +2.83	68.88 +1.98*	84.00+0.79*	24
Gr XIII(1% SO.(AEE))	17.59 +1.99	44.69 +3.86*	72.79 +0.96*	87.96+0.80**	23
Gr XIV (2% SO.(AEE))	19.56 +0.69	49.83 +0.86*	79.88 +0.45*	89.49+0.54**	23

SO: simple ointment base; n = 6 animals in every group.

The treated group of rats is compared by Student t test with the control group rats. *** $P < 0.001$, ** $P < 0.01$,* $P < 0.05$.

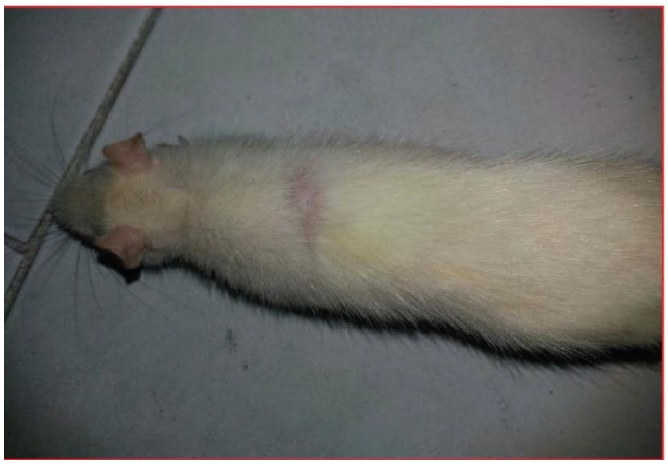


Fig-3: Healed excision wound on 20

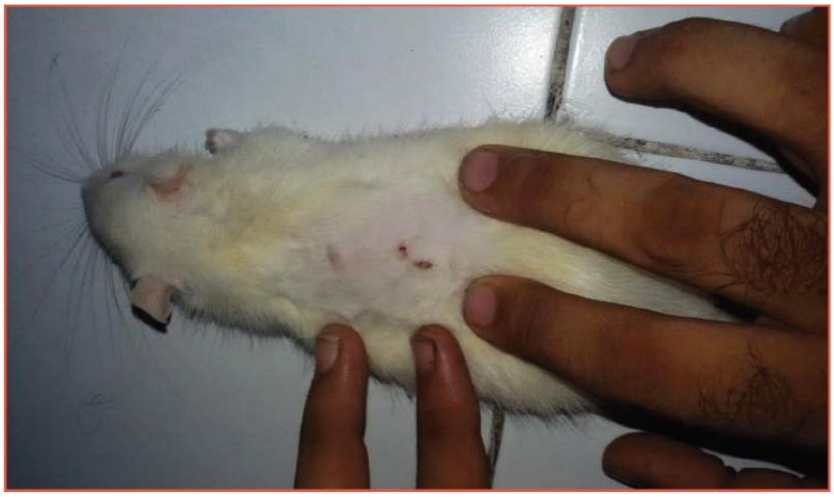
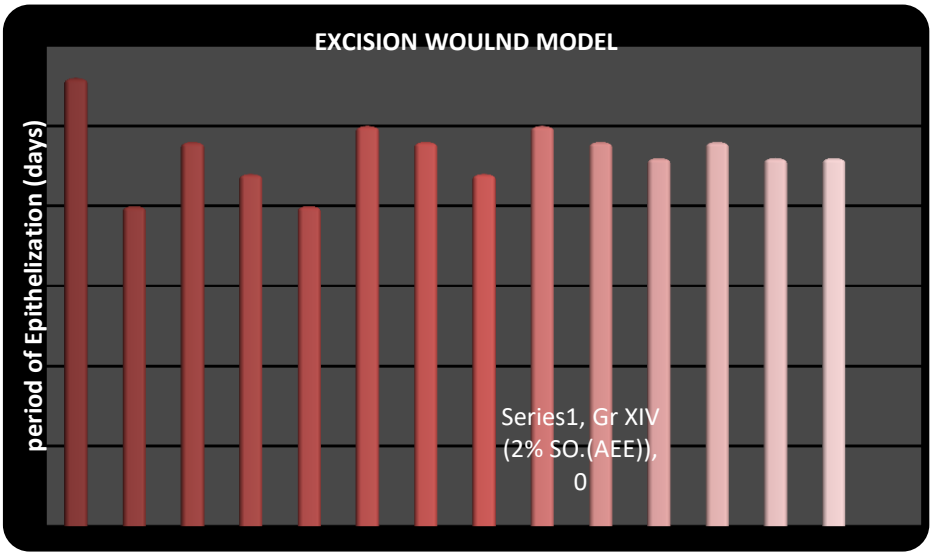


Fig-4: Healed incision wound after 8 days treatment



Graph-1: Excision wound model (Period of epithelization)

Table-2: Effect of ointments of different extracts of the seeds of Cleome viscosa on the tensile strength of skin having an incision wound

Group (N= 6)	Tensile strength in gram (mean±SEM)
Gr I (Control)	188.93 + 3.84
Gr II(Standard)	727.10 +5.01
Gr III (0.5% SO.(PEE))	596.00 +2.75**
Gr IV(1% SO.(PEE)).	638.78 +3.56**
Gr V (2% SO.(PEE))	726.13 +2.15***
Gr VI (0.5% SO.(EAE))	503.59 +5.26**
Gr VII (1% SO.(EAE))	585.86 +6.01**
Gr VIII (2% SO.(EAE))	610.55 +5.35**
Gr IX (0.5% SO.(MEE))	480.30 +6.03**
Gr X (1% SO.(MEE))	575.56 +3.59**
Gr XI (2% SO.(MEE))	673.25 +2.53**
Gr XII (0.5% SO.(AEE))	595.86 +2.90**
Gr XIII (1% SO.(AEE))	639.77 +2.21**
Gr XIV (2% SO.(AEE))	660.94 +3.21**

SO: simple ointment base. n = 6 animals in each group.
The treated groups are compared by Student t-test with the control group.
** P < 0.01, *** P < 0.00

Table-3: Effect of applying different extracts ointments of Cleome viscosa seeds on content of hydroxyproline in the eschar of excision wound

Group (N= 6)	Hydroxyproline (µg/ 500 mg)
Gr I(Control)	29.52 +0.67
Gr II(Standard)	44.02 +0.70
Gr V (2% SO.(PEE))	43.58 +0.45***
Gr VIII (2% SO.(EAE))	39.45+0.15**
Gr XI (2% SO.(MEE))	37.86+0.90**
Gr XIV (2% SO.(AEE))	40.09 +0.49**

SO, simple ointment base; n = 6 animals in each group.
The treated groups are compared by Student t-test with the control group.
** P < 0.01, *** P < 0.001

CONCLUSION

On the basis of the results obtained in the present investigation, it is possible to conclude that the petroleum ether extract ointment (2% w/w) extract has significant wound healing potential by synergestic effect of phenolic and flavonoids present in the extract. The above findings justify the wound healing properties of the plants.

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