ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of Wound Healing Activity of Leaf and Stem Extract of Kedrostis *Foetidissima (Jacq.) Cogn*.

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ABSTRACT

The wound healing process is a complex series of events that begins at the moment of injury and may continue for months together. The aim of our present study is to investigate wound healing activity of the leaf and stem pet ether extract of *Kedrostis foetidissima (Jacq.) Cogn.* (*Cucurbitaceae*), on healthy young adult Albino Wistar rats weighing 180-220g using incision wound model. Pet ether leaf and stem extracts of *Kedrostis foetidissima (Jacq.)Cogn* was evaluated for its wound healing activity and compared with control. The present investigation reveals that the stem extract of *Kedrostis foetidissima (Jacq.)Cogn* shows excellent wound healing activity and the leaf extract also shows significant wound healing activity due to the presence of active constituents, there by justifying its use in indigenous system of medicine.
Keywords: *Kedrostis foetidissima (Jacq.)Cog;* Wound healing; Incision wound

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ISSN: 0975-8585

INTRODUCTION

Wound healing is an important biological process involving tissue repair and regeneration. A wound is described as "a break in the continuity of tissue, from violence or trauma" and is regarded as healing if there is a restoration of the wounded or inflamed tissue to normal condition [1].Certain factors that influence wound healing include bacterial infection, nutritional deficiency, drugs, sterility, obesity, movement of wound edges, site of wound, and wasting diseases [2].Several drug classes have been used in the management of wounds. Among these are the antibiotics, penicillin and streptomycin have been widely employed in combating post operative infections in man and animals[3].The antibiotics are chosen based on their ability to destroy or inhibit the growth of pathogenic organisms, while the tissues is left unharmed[4].

Kedrostis foetidissima (Jacq.) Cogn. (*Cucurbitaceae*), locally named as Appakovai, occurs in India, Africa, Sri Lanka and Western Malesia [5], which is very effective in the treatment of skin diseases, Measles [6], Chest pain, asthma and urinary tract infection [7], pasture bloat and frothy bloat in cattle [8], smallpox [9], recommended for opportunistic infections like HIV[10] and Snake bite [11].The leaves, stem and tubers of *Kedrostis foetidissima (Jacq.) Cogn.* have also been Pharmacologically documented to posses antibacterial [12], anti fungal[3] and antioxidant activities[14].

We are unable to find any information on the wound healing properties of this plant. The present study is therefore an attempt to assess the efficacy of the pet ether extract of leaf and the stem of *Kedrostis foetidissima (Jacq.) Cogn.* On wound healing in rats.

MATERIALS AND METHODS

Plant Materials

Leaf and stem of ***Kedrostis*** *foetidissima (jacq.) cogn.,* were collected during October 2010 –February 2011, from Aliyar hills, near Pollachi, Coimbatore District, Tamilnadu. The voucher specimen was submitted to Botanical Survey Of India, Southern Regional Centre, Tamilnadu Agri. University, Coimbatore, Tamilnadu and the Specimen was identified as *Kedrostis foetidissima (jacq.) cogn (=Trichosanthes foetidissima Jacq.) Cucurbitaceae* family. Collected plant materials were washed thoroughly to remove mud particles, separated and then shade dried. The leaves were powdered; the stems were crushed and stored in a tightly closed container for further use.

Extraction of Plant Materials

Crushed leaves and stem (100 g) of Kedrostis *foetidissima (jacq.) cogn.,* were first defatted with petroleum ether. Petroleum Ether (PE) extract was obtained by Reflux (6h) using 100g of powdered leaves and crushed stem with 1 liter of pet ether. The extracts were concentrated to dryness and the residues were refrigerated for performing various assays.

Phytochemical Screening

The dry pet ether extracts were subjected to various chemical tests in order to detect the presence of different phytoconstituent. (Table-1).

Animals and Experimental Groups

Healthy young adult Albino Wistar rats weighing 180-220g were randomly divided into five groups of four animals. Before commencing the experiment each animal was assigned a unique identification marking with paint like head, tail, and body and unmark.

Group I (G1) – Control
Group II (G2) – Wound + Standard (Betadine)
Group III (G3) – Wound + Vehicle
Group IV (G4) – Wound + Pet Ether leaf extract.
Group V (G5) – Wound + Pet Ether stem extract

The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee, and was cleared by the same before beginning the experiment.(NO.685/PO/02/a/CPCSEA KMCRET/PhD/08/2012-13).

Housing and Feeding

Animals were facilitated with standard temperature (23 ± 2ºC) controlled environment (12 h: 12h light: dark cycle) and have a humidity of 40%. The standard laboratory animal food pellets with water and labium feed was supplied to animals during the study period.

Incision Wound Model

Incisions wound models were made through the skin at a distance of about 5cm length with sterile scalpel blade. The parted skin was sutured with surgical thread at 1 cm intervals using a curved needle (no: 42). The continuous thread on both wound edges was tightened for good closure of the wounds and the wounds were left undressed. The ointments were administered topically to the animals of respective groups until 11^{th} day. The animals were sacrificed on 11^{th} day and the skin breaking strength of the healed wound was measured with INSTRON universal tensile testing system.

Ointment Base Preparation

Ingredients

Yellow soft paraffin -17 g, Hard paraffin -1g, Cetosteryl alcohol -1g, Wool fat-1g.

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Procedure

Vehicle was prepared by mixing wool fat, hard paraffin, yellow soft paraffin and Cetosteryl alcohol and the contents were warmed in a water bath until it melted. To that vehicle slowly added Pet Ether leaf extract, slightly warmed in the flame and kept in a proper container. The same procedure was adopted for Pet Ether stem extract.

Wound Contraction

The progressive reduction in the wound area was monitored by graph paper and the percentage of wound healing was computed at the beginning of experiments and the next 4, 7 and 9 days.

Skin Tensile Strength

The animals were sacrificed on the 14th day and the healed tissue along the normal skin strips of 70mm length were cut from the animal. This was preserved by normal saline, then loaded between the upper and lower holder of the ISTRON universal tensile testing machine 5500R/6021. The total breaking strength was measured in gram force.

Histopathological Studies

The removed tissues from the animals were preserved separately in 10% formalin and dehydrated through alcohol, cleaned in xylene and embedded in parasin mixed paraffin wax (melting point 55-57 ^{0} C). Serial section of 5 µm were cut and stained with Hematoxylin and Eosin stains. The section was examined under light microscope (LABOMED, Germany) and photomicrographs were taken.

RESULTS AND DISCUSSION

The preliminary Phytochemical screening of pet ether leaf and stem extracts of *Kedrostis foetidissima (jacq.)Cogn.* Shows the presence of Alkaloids, Flavonoids, Tannins, Triterpenoids and Steroids.(Table-1).

Table-1.

Phytoconstituent	Leaf	Stem
Alkaloids	++	+
Flavonoids	+	+
Tannins	+	+
Triterpenoids	+++	+++
Steroids	+++	+++

Note:(+) - Less Precipitation, (++) - Moderate Precipitation, (+++) - Higher Precipitation

Table-2 shows the result of wound healing activity of the leaf and stem pet ether extracts of *Kedrostis foetidissima (jacq.)cogn* by incision method. In this model, animals

ISSN: 0975-8585

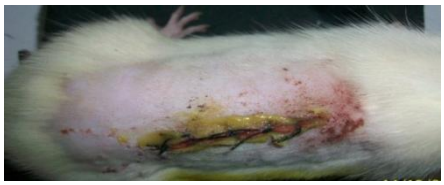
treated with stem pet ether extract shows significant percentage of wound closure up to 99.20 ± 0.76 when compared to control animals (74.60 ± 0.76). The animals treated with the leaf pet ether extract also revealed significant percentage of wound closure (96.52 ± 1.09) but somewhat less than that of the standard(Betadine ointment) (97.26 ± 3.01).Hence we can infer that the leaf and stem pet ether extracts of *Kedrostis foetidissima (jacq.)cogn* not only is actively promoting faster wound contraction, but also acting as potent agent in aiding the process of tissue granulation and remodeling in the first week of the healing process.(Figure. 1-5)

Table-2

Group	Treatment	Percentage (%) Wound Closure		
		4^{th} day	7^{th} day	9^{th} day
I	Control	20.11±1.22	49.54±1.30	74.60 ± 0.76
II	Standard-Betadine	26.32±2.08*	73.68±1.21**	97.26 ± 3.01**
III	Vehicle	22.43±1.59	51.03±0.87	75.44 ±2.12
IV	Pet Ether leaf extract	25.21±0.34	80.43±1.71**	96.52 ±1.09**
V	Pet Ether stem extract	24.62±1.04	90.44±0.89**	99.20 ±0.76**

Values are expressed as mean ± S.E. (n=4). All columns are significant using ANOVA,*P<0.05,**P<0.01 when compared to control; Dunnet's t-test.

Figure-1



Day-1 pet ether stem extract

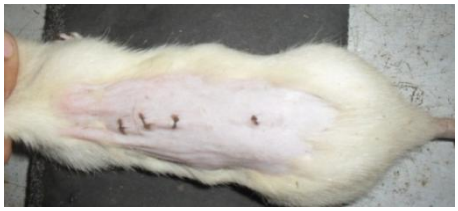
Figure-2

Day-4 pet ether stem extract

Figure-3

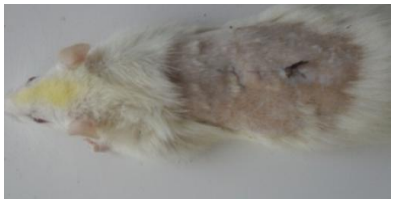
Day-7 pet ether stem extract

Figure-4



Day-9 pet ether leaf extract

Figure-5



Day-11 pet ether stem extract

ISSN: 0975-8585

Table-3 shows the percentage tensile strength at maximum load. The results were expressed as mean ± standard error. Tensile strength of incision wound area. In this investigation, the group treated with the leaf pet ether and stem pet ether extract shows significant percentage of tensile strain at maximum load when compared to control. It is interesting to note that the extracts show significant tensile strength than the standard ointment base.

Table-3

Group	Treatment	Tensile strain at Maximum Load (%)
I	Control	121.165 ± 18.1196
II	Standard-Betadine	93.73 ± 6.027
III	Vehicle	76.45 ± 2.4512
IV	Pet Ether leaf extract	121.925 ± 3.9724
V	Pet Ether stem extract	121.69 ± 4.3133

Values are expressed as mean ± S.E. (n=4)

Wound healing involves various phases which include granulation, collagenation, collagen maturation and scar maturation which are concurrent but independent to each other. Histological studies of tissues obtained from animals treated with pet ether stem extract shows normal epidermis, significant increase in collagen deposition and the upper dermis showing fibrosis with mild inflammation indicating a healed wound (Figure-6). The lower dermis, skin appendages and the skeletal muscle are normal. Similarly the animal treated with leaf pet ether extract, the skin shows normal epidermis. The dermis show focal area of fibrosis with fibroblasts indicating a healed wound, minimal inflammation with occasional inflammatory cell seen. The skin appendages are normal and subcutaneous fat, skeletal muscles are unremarkable (Figure-7). The animals treated with the standard, the skin shows normal epidermis, dermis with appendages, subcutaneous fat and skeletal muscle. There is no inflammation, no vascular and fibroblast proliferation or granulation tissue (Figure-8). The skin of the animals without any treatment shows a small wound with ulcerated epidermis. Vascular and fibroblast proliferation indicating inflammatory granulation tissue (Figure -9 and 10).

Figure-6

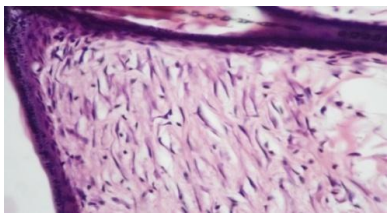


Figure-7

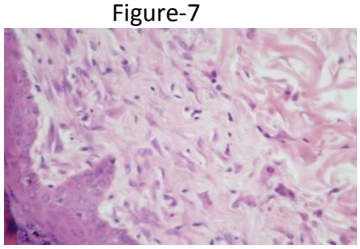


Figure-8

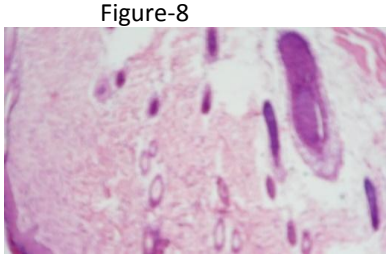


Figure-9

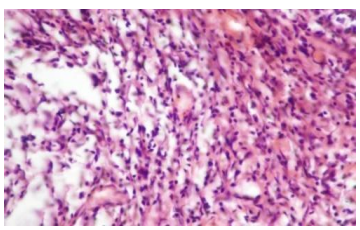


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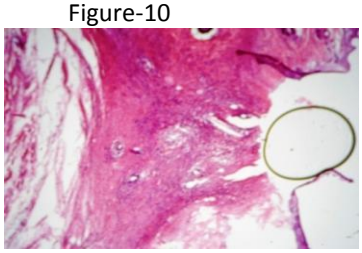
Figure-10



CONCLUSION

It may be concluded that, the wound healing activity of stem and leaf pet ether extracts may be attributed to the phytoconstituent(s) they contain, which may be either due to their individual or additive effect that accelerates the process of wound healing. The methanol, ethanol and aqueous extracts of major plants were found to possess better wound healing property over other extracts, also reported in the literature. But it is interesting to note that the pet ether extracts of *Kedrostis foetidissima (jacq.)cogn* shows significant wound healing activity. At this stage it is difficult to say which component(s) of the extracts are responsible for the wound healing activity. However, further Phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities.

ACKNOWLEDGEMENT

The authors thank the concerned authorities of the Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore for providing necessary facilities to carry out this work.

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