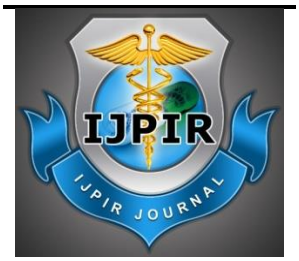
Review Article



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Wound healing activity of methanolic extract of leaves of *pedalium murex* in various models

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ABSTRACT

The plant P.Murex belonging to the family *pedaliaceae* were proposed according to the literature for its wound healing property with Methanolic extract by using excision and incision wound healing model. The plant extracts were applied topically three times a day for the estimation of wound healing and wound contractions. The plant extract showed significant wound healing potential in excision and incision models.

Keywords: Wound healing, Pedalium murex leaves, Excision model, Incision model.

INTRODUCTION

The medicine obtained from herbs has the still the main importance in the developing countries for the primary health. About 75-80% world population is using the herbal medicine [1]. It is because of the fact and belief that herbal medicine doesn't have any adverse effects and are obtained cheaply and easy availability [2]. Many of the conventional medicines are obtained from plant and their sources. In past decades most of the effective medicines are obtained from plants. For example, digoxin from fox glove, morphine from the opium poppy and quinine from cinchona bark. In different nations, including India and china, a huge number of tribal groups still utilize plants for the cure of different infections. The extraordinary enthusiasm towards the utilization and essentialness of restorative plants in numerous creating nations has prompted increased exertions on the documentation of ethno therapeutic information of therapeutic plants. Asia is one of the biggest biodiversity districts on the planet, containing a portion of the wealthiest nations in plant assets. India is a boundless archive of restorative plants that are utilized within conventional therapeutic medicines, which additionally structures a rich wellspring of learning. India is the biggest maker of therapeutic herb sand and so it is botanical garden of the world.

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WHO brought up that more than 80% of population in the world relies on upon plants to meet their essential medicinal services needed. Over abuse of the chosen restorative plant species lead to the diminishment in number of plants in the wild and incorporation of their name in the red information book [3-7].

Herbal medications constitute a real impart of all formally perceived frameworks of wellbeing in India. Today evaluated that around 80% of individuals in developing nations still depends on traditional drugs which are largely depended upon species of different plants and animals for their health. Herbal drugs are very popular and their prevalence is expanding every day. About plants with therapeutic utilization are specified in the literature of ancient and around 800 plants have been utilized as a part of indigenous frameworks of drug. India is a boundless storehouse of restorative plants that are utilized as a part of conventional medicinal treatments [8].

Ayurveda, siddha, unani and society solutions are the significant frameworks of indigenous pharmaceuticals. Among these frameworks, Ayurveda is most created and generally rehearsed in India. Ayurveda going once more to 500-800 BC has been a vital piece of Indian society. The term originates from the Sanskrit root and Veda. As the name infers it is not just the exploration of treatment of the sick however covers the entire range of upbeat human life including the physical, mystical and profound aspects [9].

An expansive extent of world population particularly in the developing nations relies on upon the conventional frameworks of pharmaceutical for a mixture of ailments. A few hundred plant genera are utilized restoratively primarily as a part of the manifestation of natural arrangement in indigenous arrangement of drugs in different nations. Products of plants are extremely powerful and compelling medications that have stood the test of times and present day science has not possessed the capacity to supplant the greater part of them. Naturally active compounds from common sources have dependably been of incredible enthusiasm to researcher taking a shot at irresistible ailments. Plant kingdom speaks to a tremendous emporium of undiscovered restorative potential and a portion of the conventional therapeutic are still included as a component of the periodic treatment of different diseases. Investigative enthusiasm toward therapeutic plant has expanded lately because of expanded effectiveness of new plant determined medications and climbing worries about the reactions of current medicine [10].

MATERIALS & METHODS

Pedalium is a genus of pant in the pedaliacae family comprising one species. Distributed in India, Srilanka and tropical Africa.

A diffuse yearly, tremendously expanded, spreading, succulent, glandular, up to 60 cm tall. Roots are like turmeric in shade. Leaves are basic, inverse, praise or elliptical obovate, 1-4.5 cm long,

0.5-3 cm wide, truncate or unfeeling, unpredictably and coarsely crenate-serrate, glabrous above, minutely layered underneath, petiole-l-4 cm [11]. Blossoms 1.5-2 cm over; pedicel 1-2 mm long, expanding up to 4 mm in tree grown foods. Calyx c.2 mm long, teeth straight, flaky outside, tenacious. Petals are connate into a wide tube, 1-3 cm long; projections harsh. Stamens incorporated, 0.5-1 cm long; fibers expanded, glandular bristly at the base; kidney shaped anthers. Soil grown foods indehiscent, sharply contracted at the base and with a patent spreading spine at every basal corner of the more extensive section, 1-1.8 cm long, 0.5-1 cm expansive, spine 2-4 mm long. Seeds are 2 or 1 for every locule, oblong [12].

Qualitative phytochemical investigation of entire plant concentrates of P. Murex demonstrated pretty much comparable attributes. Vicinity of alkaloids, glycosides, saponins, proteins, altered oils, tannins and phenols, flavonoids and gums and adhesive are available in both species. Phytosterol is available just in P. murex and more of alkaloids and gums and adhesive are available in P. murex [13-19].

Collection of Plant Materials and its Authentication

The leaf of Pedalium murex were collected from Sri Venkateshwara University, Tirupati in the month of Jan, 2014 and wasauthentified by a botanist named Madhavachetty.

Extracting Solvents

Petroleum ether, Methanol.

Extraction Process

About 200 g of powder of *P.Murex* was packed into a Soxhlet apparatus and extracted with 2 – 2.5 liters of petroleum ether at 40°C by continuous hot percolation for defatting. The extracts were subjected to distillation and it was stored on desiccators and the % yield value was determined. The same method was continued with methanol. Percentage yield of extracts were determined [20].

Phytochemical Screening

Alkaloids

A little of the dissolvable free concentrate was mixed independently with a couple of drops of dilute HCl and separated. The filtrate may be tested by using mayer's reagent.

Mayer's test: Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Yellow precipitate formation indicates the alkaloids presence.

Carbohydrates

Little amount of extract was dissolved separately in 5ml distilled water which is the filtered and the filtrate should be used for testing the presence of carbohydrates.

Molisch's test

The filtrate was treated with few drops of alcoholic α- naphthol solution in the test tube. Occurrence of violet ring near the junctions tells the presence of carbohydrates.

Glycosides

The Extracts were hydrolysed with dil. HCl, and then the extract should be tested for glycosides. Modified Borntrager's test: Extracts should be treated with the solution of Ferric Chloride and it should be immersed in boiling water for around 5 minutes. The mixture was then cooled and it should be extracted with equivalent volumes of benzene. The benzene layer should be separated and treated with the solution of ammonia. The formation of rose-pink color in the ammonical layer demonstrates the presence of anthranol glycosides.

Phytosterols

Salkowski's test: Extracts should be treated with chloroform and then filtered. The filtrate was treated with few drops of Conc. H_2SO_4, shaken and permitted to stand. Appearance of brilliant yellow color shows the triterpenes presence.

Saponins

Froth test: The extract should be diluted with distilled water to 20ml and it was then shaken in a graduated cylinder for about 15minutes. 1cm layer of foam formation indicates the saponins presence.

Tannins

Gelatin test: 1% gelatin solution which contains sodium chloride was added to the extract. White precipitate formation indicates the tannins presence.

Flavonoids

Alkaline reagent test

Extracts should be treated with few drops of the solution of sodium hydroxide. Intense yellow color formation, which then becomes colorless upon addition of the dilute acid, indicates the flavonoids presence.

Phenols

Ferric chloride test

Extracts should be treated with 3-4 drops of solution of ferric chloride. Bluish black color formation indicates the presence of phenols.

Procurement of animals for the Experiment

Healthy inbred Swiss albino rats of either sex of 2 months age, weighing 200±5 gms, were utilized for the present study and they were obtained from the suppliers of NIN, Hyderabad, India. The rats were brought from the stock in breed province. They were housed at room temperature of 23±1°c, relative humidity of about 55±55% under 12 hr light/12 hr dark cycles in the animal house. Rats were fed pellet diet and water ad libitum. The animals were shifted to the research center not less than 1 hr before the experiment starts. All animaltests were performed after getting the approval from the IAEC (institution of animal ethical committee).

The protocol of experiments was approved by institutional animal ethics committee (Reg. No. 1447/PO/a/11/CPCSEA).

Acute oral toxicity studies were performed as per OECD-423 guidelines, with the methanolic extract of *P.Murex*using albino rat (n=6) of either sex, which was supposed to be selected by random sampling for studiesof acute toxicity. Animals should be fasted before the dosing (e.g. with the rat, food should be withheld over-night but not water and with the mouse, food should be withheld for 3-4 hr but not water). Following the fasting period, the animals must be weighed and the test substance should be administered. After administering substance, food has to be withheld for further 3-4 hr in rats or 1-2 hr in mice. For each step six animals should be used. The dose level hasto be used as the starting dose has to be selected from one of the four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. As the available information suggests that mortality is unlikely at the highest starting dose level (2000 mg/kg body weight) then, exceptionally, and only when it is justified by specific regulatory needs, additional use of upper dose level of 3500 mg/kg and 5000 mg/kg body weight may be considered. Individually all the animals has to be observed after dosing, once during the first 30 minutes after giving the dose, and periodically during the first 24 hours after the dosing period, with special care given during the first 4 hours, except where they have to be removed from the study and humanely killed for animal welfare reasons are found dead. If mortality was observed for two out of three animals, then the dose administered is known as toxic dose. If mortality has been observed for one animal after the dosing, then the same dose should be repeated again to confirm the toxic level dose, if mortality was not at all observed, the procedure was repeated for its higher doses [21,22].

Wound Healing Activity

Wound healing activity was observed by Excision and Incision models.

Excision model

The animals should be anesthetized and an impression is made on the dorsal thoracic region 1 cm away from vertebral column and 5 cms away from ear. The skin area of particular area has to be shaved one day before the experiment. The skin of shaved area is excised to the full thickness to form a wound area of about 500mm^2. Haemostasis will be achieved by blotting of the wound with the cotton swab which has to be soaked in normal saline. The animals should then be grouped and treated as follows,

Group I - Solvent control (normal saline,)
Group II - Standard Povidine iodine 5 % v/v
Group III - with leaves extracts of P.Murex. 500mg/kg

Wound contractions, which contribute for closure of wound in the first two weeks, were studied by the tracing of wound on a transparent paper initially. Then an impression should be taken on a graph paper containing millimeter scale, area of scar after complete epithelization and the time

taken for complete epithelization in days has been evaluated to calculate the degree of wound healing. The parameters that have to be studied were wound closure, scar features and epithelization time. The percentage of wound closure observation were then recorded on 4th , 8th, 12th, 16th and 20th post wounding day and also for epithelization and size and shape of scar area.

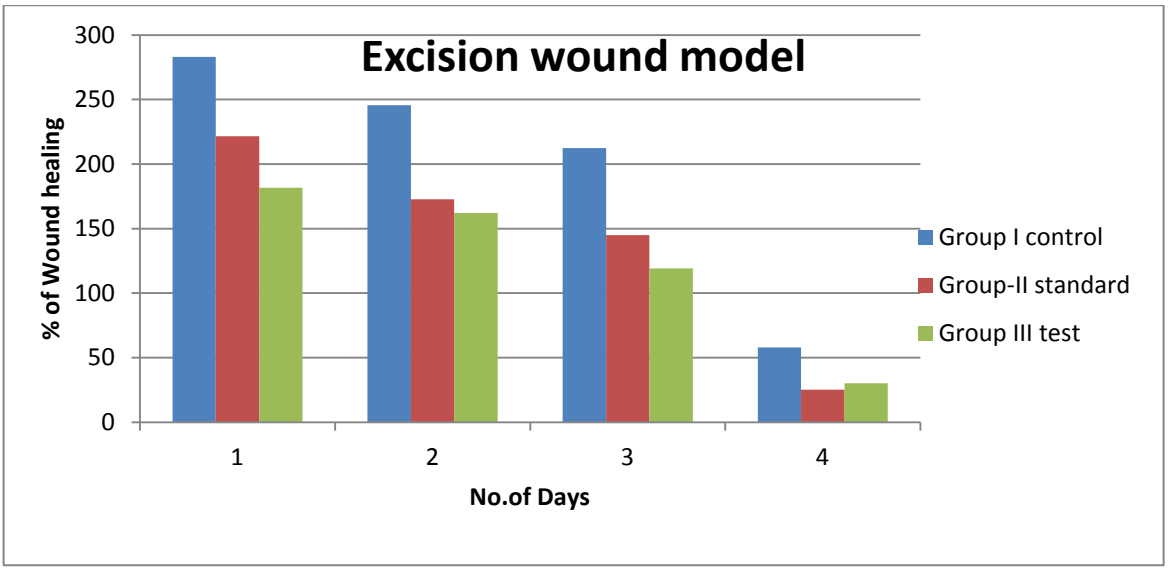
Incision model

The animal were anaesthetized under light either and back of the animal was shaved and washed with sprit.6 cm paravertebral parallel incision was made through the entire thickness of the skin on the right side of the vertebral column with the help of a sharp blade. The wounds were closed with interrupted sutures 1 cm apart. Male albino wistar rats weighing 110 -150 g were divided in to three groups containing 6 animal each. Group I served as control, Group II served as reference standard and group III served with Methanolic extract of of P.Murex. the wounds were closed with interrupted sutures which were removed on the eighth day of wounding wound breaking strength was measured on 10 day by adopting continuous water flow technique. The breaking strength was expressed as the minimum weight of water necessary to bring about gaping of the area.

RESULTS

During the preliminary phytochemical investigation on Pedalium murex it reveals that there are Alkaloids, Carbohydrates, Glycosides, Phytosterols, Saponins, Tannins, Flavonoids, Phenols. According to the above toxic studies the Methanolic extract of Pedalium murex with a 500mg/kg body weight /oral route are regarded as safe or low toxicity.

The result of wound contraction studies are shown in table 1 in excision model the extract showed significant wound healing activity and the percentage of wound contraction on the day 16 was maximum significant when compared to the control



The effect of wound contraction on excision model of *P.murex* on different days

Table:1 The effect of wound contraction on excision model of *P.Murex* on different days

Days	Group I control	Group-II standard	Group III test
0	283±2.68	221.66±2.65	181.66±1.93
4	245.33±3.60**	172.66±2.90**	162.16±2.648**
8	212.33±3.096**	144.83±1.89**	119.16±2.014**
16	58±1.99**	25.16±2.014**	30.166±1.332**

Values are mean ±SEM from 6 animals in each group, data was analyzed by one way ANOVA which was followed by Dunnett's test, and P<0.01* was considered as significant with comparison of control, **P<0.001.

Incision wound model

The result of wound contraction studies are shown in table incision model the extract showed significant wound healing activity and the percentage of wound contraction on the day 24 was maximum significant when compared to the control.

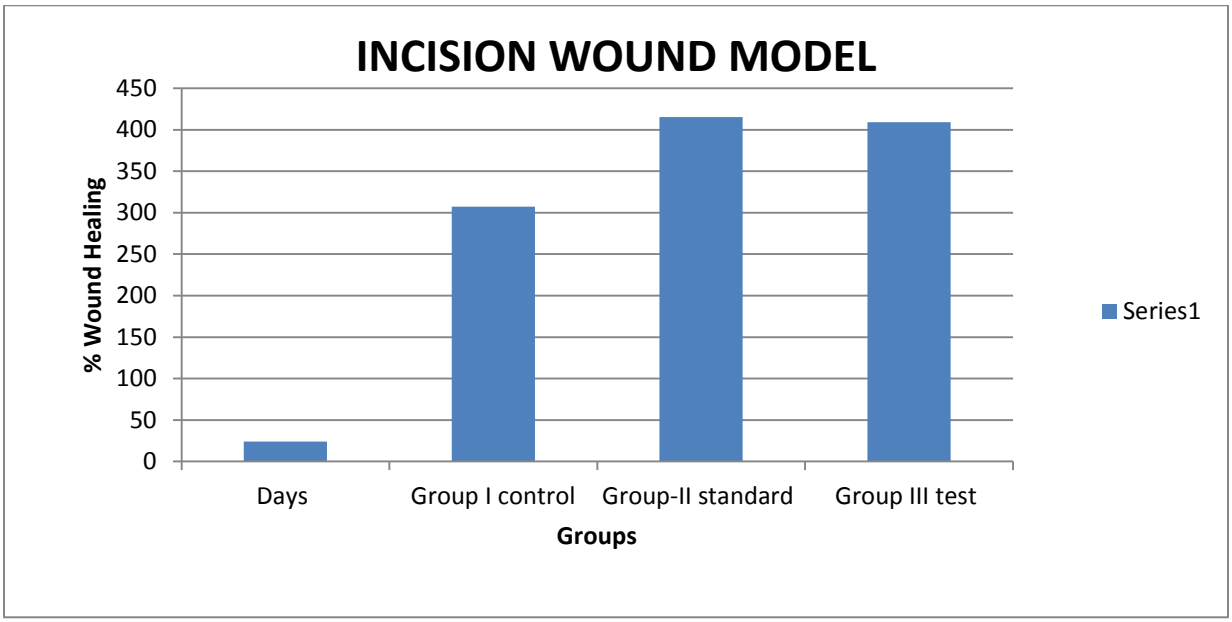


Table:2 Effect of *P.Murex* leaves on wound breaking strength (Incision Wound)

Days	Group I control	Group-II standard	Group III test
24	307.33±12.33	415.33±15.22**	409.22±0.5**

Values are mean ±SEM from 6 animals in each group, data was analyzed by one way ANOVA which was followed by Dunnett's test, and P<0.01* was considered as significant with comparison of control, **P<0.001.

DISCUSSION

Wound contraction is a factor which indicates the rates of reduction of unhealed area during the course of treatment. This centripetal movement of wound margin is due to the activity of presence of myofibroblast [22,23]. The increase in the activity of wound contraction in the treated group may be due to the enhancement of activity of macrophages and fibroblasts. The slow rate of wound closure in the control group may be attributed due to the presence of microorganisms and metabolites of microorganisms, which would inhibit wound contraction and then delay the process of wound healing [24, 25]. The leaves showed that there is significant reduction of wound area in the different groups for over a period of 16 days. In the study of excision wound it is possible to conclude that the plant extract showed that there was a significant wound contraction due to the presence of phytoconstituent's. In the incision model the mean showed the significant breaking strength when compared to that of the standard ointment.

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