INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

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Research Paper

Wound Healing Activity Of 80% Methanol Leaf Extract of Anacyclus. Pyrethrum (L.F) Sond (Asteraceae) In Rats

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ARTICLE INFO

Published: 25 Jan. 2025
Keywords:
Wound Healing Activity, Methanol Leaf Extract of Anacyclus, Pyrethrum (L.F) Sond, (Asteraceae) In Rats.
DOI:
10.5281/zenodo.14737132

ABSTRACT

Anacyclus pyrethrum (L.) is one of the spontaneous plant species of Ethiopia frequently used for external wound treatment in humans. Nevertheless, pharmacological investigations have not been performed to substantiate activity of the plant extract in wound healing. Hence, this study attempted to explore the activity of leaf extract of Anacyclus pyrethrum (L.) in wound healing using a ratmodel. Materials and Methods: The air-dried leaf from Anacyclus pyrethrum (L.) was pulverized and extracted with 80% methanol and prepared with 5% and 10% extract ointments. In order to observe the indications of toxicity, an acute dermal toxicity investigation of the extract was carried out in female rats. After that, 5% and 10% (w/w) extract ointments were administered topically to incision and excision wound models to examine their ability to promote wound healing. Factors including the duration of epithelialization and the constriction of the wound were identified. Results: Upon the application of 10% w/w extract , no signs of dermal toxicity were observed in rat. Both 5% and 10% (w/w) extracts formulations increased percentage wound healing, and contraction, shortened the epithelialization period. Conclusion: The findings of this study collectively showed that the leaf extract of Anacyclus pyrethrum (L.) possessed significant wound healing activity, upholding the folkloric use of the experimental plant

INTRODUCTION

In order to conduct this study, a thorough literature review was conducted in order to compile all available data and create a database on the species and, as a result, the sustainability of our understanding of *A. pyrethrum*. In order to assess

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

the significance of this species in both traditional phytotherapy and contemporary medicine, every attempt was made to present the species' qualities in detail during the compilation of this review, with a focus on the current research, the chemistry of the plant, the chemistry of its essential oils, and its variability in chemical composition. The most recent biological activity supported by scientific research were highlighted. Additionally, research on its safety was published. Furthermore, as plant-derived drugs can be safer and more affordable options, additional study is required to verify their safety and efficacy. Educational institutions should have access to the funding so they may study different animals for upcoming clinical studies and confirm the safer usage of its herbal compounds in biological processes. One of the Asteraceae family's spontaneous plant species, Anacyclus pyrethrum, is native to Morocco and Algeria[1,2]. *A. pyrethrum* var. pyrethrum (L) and *A. pyrethrum* var. depressus (Ball) Maire[3,4]are the two variants that belong to this species. Numerous taxonomists have addressed botanical and systematic descriptions of this species in a variety of flora publications. Research on the chemical and biological activity of the A. prethrum species has gained more attention in recent years. Numerous investigations into the chemical makeup of A. pyrethrum have already been published [5,6], demonstrating the existence of a broad range of phytochemicals, of which around 100 distinct compounds have been identified to date. In traditional medicine, the roots of *A. pyrethrum* are recommended for treating toothaches, salivary secretions, angina, digestive problems, lethargy, female infertility and even paralysis of the tongue and limbs.

Aim And Objectives

- Aim: This study aims to evaluate wound healing, epethelization of 80% methanol crude extract and solvent fractions of *A. pyrethrum* in rat.

Objective:

- This study aimed to evaluate wound healing, contraction and of 80% methanol crude extract and solvent fractions of *A. pyrethrum* in rat.
- To provide an overview of the chemical composition of *A. pyrethrum* .

MATERIALS AND METHODS:

Chemicals, Drugs, and Solvents

Methanol, ketamine hydrochloride (Neon laboratories, India), nitrofurazone USP 0.2% ointment, wool fat, diazepam (Gland Pharma limited, India), white soft paraffin, and hard paraffin, cetostearyl alcohol were used. All chemicals and reagents used were of analytical grade.

Plant Material

Fresh leaves of *Anacyclus. pyrethrum* were obtained from the College of Swami Vivekananda institute of pharmaceutical Sciences,Hyderabad. The plant material has been identified and verified by a taxonomist By JNTU University of Hyderabad.

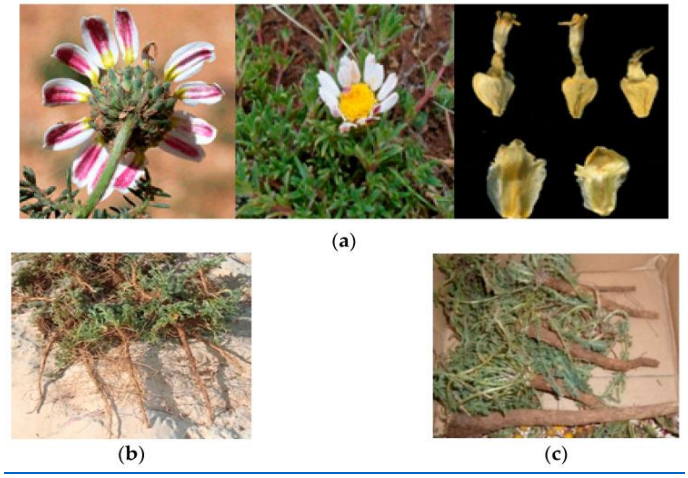


Fig.1. Plant profile

Morphological appearance: Flowers, ray florets and achene of *A. pyrethrum* (**a**), *A. pyrethrum* var. depressus 'Tigendast' roots (**b**) and *A. pyrethrum* var. pyrethrum 'Igendas' roots (**c**). The ethnological surveys conducted in Morocco, on the plant show that it is highly regarded by the local population for its medicinal properties and especially for its social and economic values. Thus, from a therapeutic point of view, the root is the most important and most widely used part of the plant .

Experimental Animals

Adult albino rat of both sexes (25–35 g, and 6–8 weeks of age) were obtained from the animal house of Virchow Biotech Private Limited, Hyderabad. The ratwere randomly kept in groups of 4 and 5 (n=5) in clean cages with a wire mesh top containing a hygienic bed of sawdust (regularly changed every 3 days) and retained in a well-ventilated room (25±1°C with 55±5% humidity) for excision and incision wound models, respectively.

Wound healing Excision Model

The animals were starved for 12 hours prior to wounding. Studies were carried out using ether anaesthetized rats. The rats were divided into five groups (n=6). Animals were depilated at the dorsal thoracic region before wounding. The first Group -I served as control, similar second Group-II served as a positive control, Group III, IV & V served as a Methanolic extract of *Anacyclus pyrethrum* 500 mg/kg (METP), depend upon the body weight by oral route daily for 20 consecutive days[66-68] and on the 20th day the wound contraction was calculated[7,8].

Using a circular seal with a diameter of 2.5 cm, an imprint was formed on the anesthetized rat's dorsal thoracic area, 1 cm from the vertebral column and 5 cm from the ear, creating a wound. To create a wound area with a diameter of around 500 mm2, the entire thickness of the skin in the impressed region was removed. A cotton swab dipped in regular saline was used to blot the wound in order to restore equilibrium.

Wound Contraction Rate

Tracing the incision on a transparent paper was the first step in studying contractions, which aid in wound closure during the two weeks of frost. The degree of wound healing was then determined by taking an impression on a millimeter-scale graph paper, measuring the scar area after full epithelization, and calculating the time in days needed for full epithelization[9,10]. Scare characteristics, epithelization time, and wound closure were the criteria under investigation. On the fourth, eighth, twelfth, sixteenth, and twentieth post-wounding days, the percentage of wound closure was observed, along with the epithelization and the dimensions and form of the scar region.

RESULT:

Wound healing

Table 1 displays the outcomes of the excision wound model. *Anacyclus pyrethrum* at a dosage of 400 mg/kg shown considerable wound healing activity in this parameter, which was determined by calculating the mean percentage of wound area on the fourth, eighth, twelve, fifteen, and twentieth post-wounding days. The 20th day's wound contraction was contrasted with the control. When compared to control, the percentage of wound contraction shows a significant difference at P<0.001. Each group's values are the mean ± SEM of six animals. One-way ANOVA and Dunnett's test were used to evaluate the data.

Table.1. Effect of methanolic extract of *Anacyclus pyrethrum* on Excision wound healing in rats.

Group	4 days	8 days	12 days	15 days	20 days
Control	43.67±1.606	65.83±1.641	73.67±1.43	80.67±1.892	93.50±1.408
Standard	28.83±1.887	32.33±1.687	43.83±1.51	52.33±1.764	63.50±1.258
Meap	35.50±2.156	44.83±1.973	51.17±1.662	62.00±1.844	67.17±2.786

Each value is the mean ± SEM for 6 rat, *P<0.5, **P<0.01, ***P<0.001. Compared with control, data were analysed by using one-way ANOVA followed by Dunnet's test, standard (sulphur ointment mg/kg, of B.W), MEAP- Methanolic extract of *Anacyclus pyrethrum*, dose (400mg/kg of B.W).

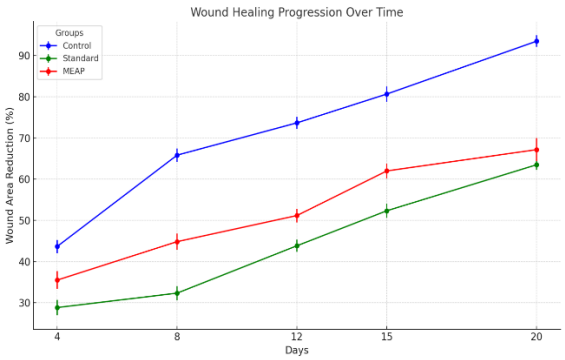


Fig.2. Line graph illustrating the wound healing rates over a 20-day period for all groups.

Wound Contraction Rate

Table.2. Effect of methanolic extract of *Anacyclus pyrethrum* on Wound contraction rate (%) on days 4, 8, 12, and 15 in rats.

Group	4 days	8 days	12 days	15 days
Control	20 ± 2.1	45 ± 3.0	70 ± 3.2	85 ± 2.9
Standard	30 ± 1.8	60 ± 2.5	85 ± 2.8	100 ± 0.0
Meap	28 ± 2.0	58 ± 3.1	82 ± 3.0	95 ± 2.5

Every figure represents the mean ± SEM for six rats, with *P<0.5, **P<0.01, and ***P<0.001 values. Dunnet's test, standard (sulphur ointment mg/kg of B.W.), and MEAP (methanolic extract of *Anacyclus pyrethrum*) dosage (400 mg/kg of B.W.) were used to analyze the findings in comparison to the control.

Fig.3. Line graph illustrating the wound contraction rates over a 15-day period for all groups.



Fig.3. Line graph illustrating the wound contraction rates over a 15-day period for all groups.

Table 3: Time for Complete Epithelialization shows the average number of days needed for full epithelialization.

Group	Time (Days)
Control	16.2 ± 0.8
Standard	11.4 ± 0.6
Meap	12.0 ± 0.5

Control: Showed little re-epithelialization and inadequate granulation tissue. Standard: Displayed full epithelialization, new blood vessel development, and thick collagen fibers. Test: Showed near-complete epithelialization, strong vascularization, and moderate to thick collagen fibers.

Examination of Histopathology Results:

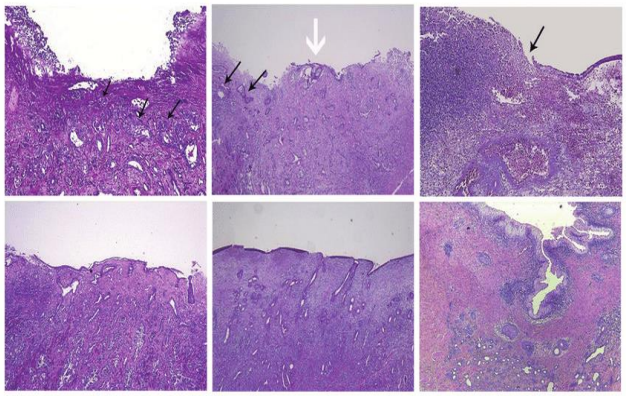


Figure 4: Typical histological pictures of tissues from wounds stained with Masson's trichrome and H&E.

DISCUSSION:

Physical injuries that cause a skin crack or opening are called wounds. The restoration of disturbed anatomical continuity and the functional response of various cell types to harm depend on wounds healing properly. An orderly and measurable series of biological processes, beginning with wound closure and continuing through tissue repair and remodeling, accompany cutaneous wound repair[11]. Despite significant progress in the chemical medicine business, there are currently few compounds available that can promote wound healing[12]. Furthermore, because of the high expense of treatment and the existence of adverse consequences, managing chronic wounds is another significant issue[13]. The natural process of dermal and epidermal tissue regeneration is known as wound healing. A series of overlapping events occurs whenever there is a wound in order to repair the harm. The inflammatory, proliferative, and remodeling phases[14] are among the stages into which these processes have been divided. Bacteria and debris are phagocytosed and eliminated during the inflammatory phase, and cytokines and mediators are generated, which trigger cell migration and division during the proliferative

phase. The proliferative phase[15] is when angiogenesis, collagen deposition, granulation tissue development, epithelization, and wound contraction take place. The epithelial cells cover the wound bed by crawling over it during epithelization[16]. All of these factors work together, along with the process of wound contracture, to finally seal the wound. Myofibroblasts, which grasp the borders of the wound and contract themselves via a process akin to that of smooth muscle cells, reduce the size of the wound during wound contraction. Apoptosis eliminates unnecessary cells during the maturation and remodeling phase, while collagen is reshaped and realigned along tension lines.[17] Up to a dosage level of 2000 mg/kg body weight, Anacyclus pyrethrum ethanolic extract did not cause any hazardous symptoms or death. During the monitoring up to 24 hours for mortality, there was no indication of toxicity or alteration in the behavioral pattern.[18]. Thus the extract was considered to be safe for pharmacological evaluation. Excision wounds heal by contraction and epithelization. The parameters studied were wound closure, time for complete epithelization, scar, size, and shape or complete epithelization[19,20].

CONCLUSION

The present study indicates the potential wound healing property of the plant *Anacyclus pyrethrum* extract. As a result of pharmacological screening the *Anacyclus pyrethrum* extract shows a similar effect on wound healing property on experimentally induced wounds in rats. The literature evidences shows the plant contains crude proteins, crude fibers those were involved in the contraction of tissues. From the results concluded that *Anacyclus pyrethrum* extract possess significant wound healing action which was compared to synthetic anti-inflammatory agent. Further clinical studies are needed to establish its safety and usefulness in wound patients.

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HOW TO CITE: Dr. K. Hemamalini, Dr. B. Rambabu, Wound Healing Activity Of 80% Methanol Leaf Extract of Anacyclus. Pyrethrum (L.F) Sond (Asteraceae) In Rats, Int. J. of Pharm. Sci., 2025, Vol 3, Issue 1, 2098-2104. https://doi.org/10.5281/zenodo.14737132