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Antioxidant and wound healing potential of *Pistia stratiotes* L.

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ABSTRACT

Objective: To investigate the effects of *Pistia stratiotes* on wound healing activity in Swiss albino mice by excision wound healing model and its antioxidant study was performed to understand the mechanism of wound healing potency. **Methods:** Mice were topically treated with extract formulated in ointment by using simple ointment vaseline as base. 5% and 10% (w/w) ointment was applied once daily. A standard group was treated with Povidone iodine ointment topically. The area of wound was measured on 4, 6, 8, 10, 12, 14, 16 post-wounding days. The scar area on complete epithelization was measured. The parameters observed were wound contraction (mm²), epithelization period and tensile strength including histopathological studies. Antioxidant activity was determined by in vitro method—H₂O₂ radical scavenging. **Results:** Treatment of wound with ointment containing 5% and 10% (w/w) extract of *Pistia stratiotes* exhibited significant ($P < 0.001$) wound healing activity when compared with control group. All parameters such as wound contraction (mm²), epithelization period and tensile strength and histopathological studies showed significant changes when compared to control. Extracts possess significant antioxidant activity compared to control group. Ascorbic acid was used as reference standard for antioxidant activity ($P < 0.001$ vs Ascorbic acid). **Conclusion:** The results conclude that *Pistia stratiotes* has antioxidant properties, which may be responsible and favorable for faster wound healing and this plant extract may be useful in the management of wounds, it also supports its traditional use.

1. Introduction

The manufacture and clinical evaluation of herbal remedies and/or their constituents have made it possible to transform traditional medicine into a modern industry capable of making a significant contribution to the delivery of healthcare [1]. This revival of interest in plant derived drugs is mainly due to the current widespread belief that “green medicine” is safe, and clinically effective, better tolerated by patients, less expensive and globally competitive [2,3]. Wound healing consists of an orderly progression of events that re-establish the integrity of the damaged tissue: inflammatory, proliferation and remodeling stages [4]. The inflammation stage begins immediately after injury, first with vasoconstriction that favors homeostasis

and releases inflammation mediators. The proliferative phase is characterized by granulation tissue proliferation formed mainly by fibroblast and the angiogenesis process. The remodeling stage is characterized by reformulations and improvement in the components of the collagen fibre that increases the tensile strength [5]. Alterations in any of these phases can lead to delay or inability in dermal wound-healing. Therefore, wound-healing is not a linear process. It is rather a ‘to and fro’ process that depends on various factors. There are many points at which the normal wound-healing process can be disturbed [6,7]. Wounds provide an ideal environment for the growth of microorganisms [8]. An infected wound is less likely to heal, thus removal and prevention of infection is a key to rapid and effective would healing. Much research has been carried out to discover new antioxidant compounds from plant origins that can prevent free radical damage [9–10]. ROS is produced in high amounts at the site of wound as a defense mechanism against invading bacteria [11]. ROS can induce severe tissue damage and even lead to neoplastic transformation

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decreasing the healing process by damages in cellular membranes, DNA, proteins and lipids [12]. The most likely mechanism of antioxidant protection is direct interaction of the extracts (or compounds) and the hydrogen peroxide rather than altering the cell membranes and limiting damage [13]. Nowadays antioxidants have been at the centre of focus in chronic disease prevention research [14]. Plants have been found to synthesize compounds that are useful in the process of wound healing [15]. From the previous research it was found that *Pistia stratiotes* L. contains large amount of two di-C- glycosylflavones of the vicenin and lucenin and lesser amounts of the anthocyanin cyaniding-3-glucoside and a luteolin-7-glycoside, and traces of the mono-C- glycosyl flavones, vitexin and orientin [16]. *P. stratiotes* belongs to the family Araceae used in traditional medicine for its diuretic, antidiabetic, antidermatophytic, antifungal and antimicrobial properties. It is also used traditionally for the treatment of ringworm infection of the scalp, syphilitic eruptions, skin infections, boils, wounds. Sterols are the major constituents of *P. stratiotes* reported in earlier study and there are reports that sterols are responsible for wound healing activity [17].

2. Material and methods

2.1. Preparation of plant material

The *P. stratiotes* leaves were collected from upper lake, Bhopal (M.P), India during the month of October. The specimen was identified by Botanist, Department of Botany, Faculty of Science, Safia College of Science, Bhopal, M.P. (India) through comparison with the voucher specimen (296/Botany/Safia/11) has been deposited at herbarium unit of the department. The collected plant material was dried under shade and then powdered with mechanical grinder. Methanolic extract was prepared by macerating a powder with methanol/water (50/50, v/v) for 48hr with constant stirring. Then it was filtered and the filtrate was evaporated in water bath at low temperature. The concentrated Methanolic extract was then dried at 40°C in an oven and finally weighed.

2.2. Chemicals

EDTA was purchased from Merck Mumbai, FeCl₃ was obtained from S.D. Fine Chemicals, Mumbai, Deoxyribose, Ascorbic acid and TBA were purchased from HiMedia Lab Ltd., Mumbai, H₂O₂ were purchased from Rankem, New Delhi, TCA from Qualigens Fine Chemicals, Phosphate buffer saline from Sigma Chemicals Co. All other chemicals used were obtained commercially and were of analytical grade.

2.3. Hydroxyl radical scavenging assay [18]

The reaction mixture containing dilution series of test sample (10–100 µg/ml) was incubated with 100 µl deoxyribose (3 mM), FeCl₃ (0.1 mM), EDTA (0.1 mM), ascorbic acid (0.1 mM), H₂O₂ (20 mM) in phosphate buffer, pH 7.4.. The

above solution was incubated at 37°C for 1 hr. The reaction was terminated by adding 0.5ml of 1% TBA and 0.5ml of 5% TCA and then heating the tubes in a boiling water bath for 15min. The contents were cooled and absorbance of the mixture was measured at 535nm against reagent blank using spectrophotometer (VIS-260 Shimadzu, Japan). The experiment was carried out in triplicates and results were expressed as mean values ± SD.

2.4. Wound Healing Activity

2.4.1. Preparation of ointment

Methanolic extract of *P. stratiotes* was mixed with Vaseline in a concentration of 5% (50mg extract/gm Vaseline, w/w) and 10% (100mg extract/gm Vaseline, w/w) respectively.

2.4.2. Experimental Animals

Swiss albino mice of either sex, weigh around 24 ± 2 g, were maintained in animal house of the department and were given standard pellet and water ad libitum. All mice were kept at controlled light condition 12h light–dark cycle and temperature 22±1°C. The use of animal as per Institutional Animal Ethical Committee, Dr. Hari Singh Gour Central University, Sagar (M.P) India, (CPCSEA Registration no –379/01/ab/CPCSEA/2010).

2.4.3. Acute dermal toxicity

The study was carried out to determine the therapeutic dose of the methanolic extract. The acute dermal toxicity testing of the methanolic extract was done by applying the Ointments containing extract of the highest concentrations of 10% (w/w) on the shaved back of the rats. The OECD guidelines no. 402 (OECD guidelines, 1987) were followed for the study.

2.4.4. Excision wound model

The animals were grouped into three major group's viz. control, reference standard and treated group (n=6). The control group was treated with simple ointment base (Vaseline 100% pure petroleum jelly) originally produced by Chesebrough–Pond's, USA, and currently by Unilever. The standard group was treated with Betadine (Win Medicare). The test groups were treated with ointments with different concentrations of extracts viz. 5% (w/w) and 10% (w/w) incorporated in simple ointment base. The posterior side of animal was shaved using hair remover Anne French (Geoffery manners). The area of wound to be created was outlined with marker using stainless steel stencil. Then 1 × 1 cm wound was created along the marked area using toothed forceps, a surgical blade no. 18 and anaesthetized using xylocaine. The entire wound left open and wounding day was considered as day 0. The wounds were treated with topical application of the ointments till the wounds were completely healed. Then were monitored and the area of wound was measured on 4, 6, 8, 10, 12, 14, 16 post-wounding days [4].

The period of epithelization was calculated as the number of days required for falling of the dead tissue remnants without any residual raw wound [19]. The tensile strength increment indicates better wound healing stimulation by the

applied drug.

Tensile strength was calculated using the following formula [20]:

$$\text{Tensile strength} = \frac{\text{Breaking strength (g)}}{\text{Cross-sectional area of skin (mm}^2\text{)}}$$

2.5. Histopathological examinations

A specimen sample of skin tissues from control, standard and treated groups were taken out from the healed wounds of the animals in excision and incision wound models for histopathological examinations. The thin sections were cut and stained with haematoxylin and eosin [21] and observed under microscope for the histopathological changes such as fibroblast proliferation, collagen formation, and angiogenesis.

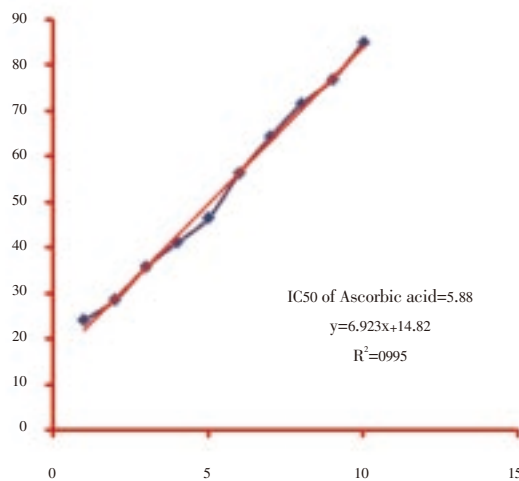
2.6. Statistical analysis

Results obtained from the excision wound models have been expressed as Mean \pm SEM and were compared with the corresponding control group (simple ointment B.P.) by applying ANOVA test.

3. Results

3.1. OH⁻ scavenging effect of *P. stratiotes*

P. stratiotes extract was screened for their free radical



scavenging effect using known antioxidant, ascorbic acid as a positive control with increasing concentrations (10–100 μ g/ml). The percent inhibition of test sample was (18.7–77.6%) against OH⁻ in concentration ranges 10–100 μ g/ml. Reference standard ascorbic acid inhibited (24.0–84.8%) of OH⁻ for the same concentration range (Fig.1). The IC₅₀ values (the concentration of an individual compound leading to 50% inhibition) of *P. stratiotes* extract and ascorbic acid calculated from hydroxyl radical scavenging assay using linear regression analysis was 5.08 μ g/ml (R²=0.995) and 5.88 μ g/ml (R²=0.997). (Fig.2)

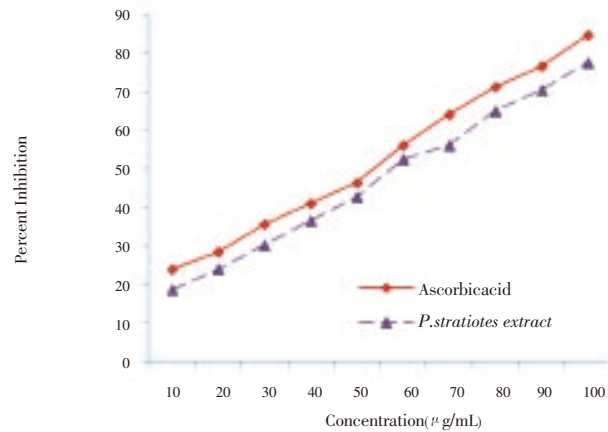


Figure 1. Inhibitory effect of Hydroxyl radical scavenging assay at different concentrations of ascorbic acid and *P. stratiotes* extract

3.2. Healing potential of *P. stratiotes*

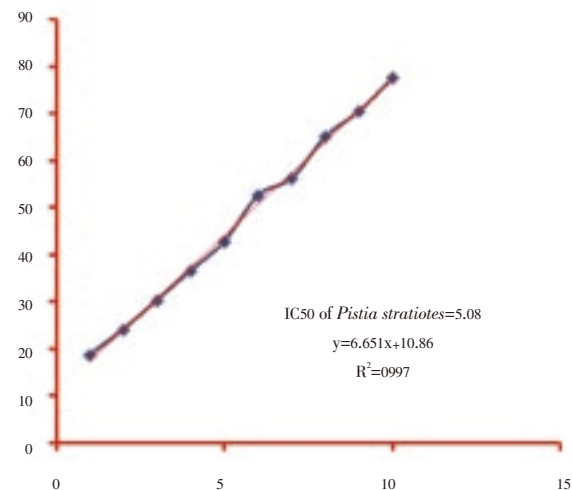


Figure 2. Inhibitory concentration (IC₅₀) of *Ascorbic acid* and *P. stratiotes*

Table 1.

Effect of topical application of Methanolic leaf extract of *P. stratiotes* on wound contraction of excision wound and the period of epithelization.

Treatment group (n=6)	Area of wound mm ² (Mean \pm SEM)				Period of epithelization (Days)
	4th Day	8th Day	12th Day	16th Day	
Control(blank vaseline)	88.77 \pm 2.665	69.95 \pm 2.973	42.4 \pm 3.728	11.52 \pm 4.27	24
Standard (Betadine)	69.5 \pm 6.144*	40.5 \pm 4.05**	17.02 \pm 4.66**	0.50 \pm 0.50*	21
5% Vaseline	75.60 \pm 4.15	54.3 \pm 3.388*	28.30 \pm 6.169	1.5 \pm 0.957*	23
10% Vaseline	73.01 \pm 4.92	48.5 \pm 3.379**	18.3 \pm 4.869*	0.75 \pm 0.75*	22

Values are Mean \pm SEM from six animals in each group. Data is analyzed by one way ANOVA. Results were statistically significant when compared with corresponding control values (Blank Vaseline). ** $P < 0.01$, * $P < 0.05$

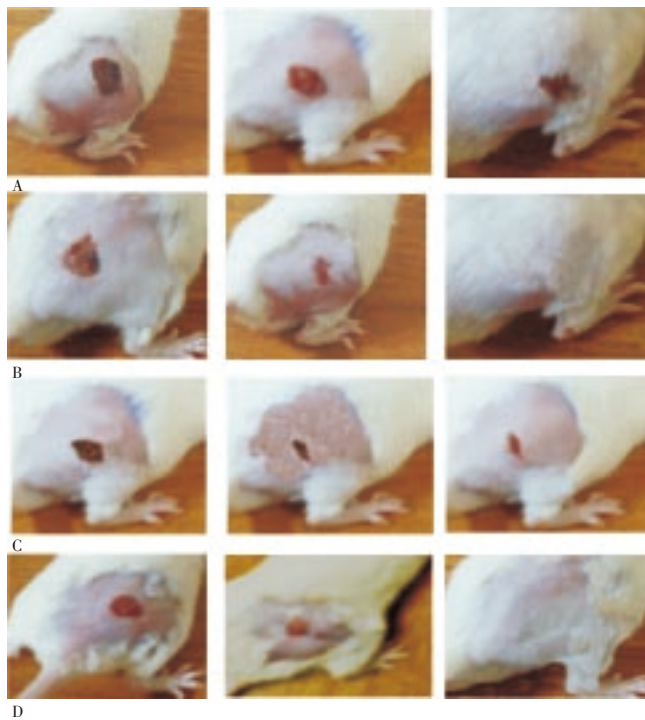


Figure 3. Effect of *P. stratiotes* on wound healing on 0 day, 8th day and 16th day. A) Control Group treated with simple ointment base Vaseline, B) Standard Group treated with topical application of Betadine, C) Treatment Group treated with topical application of 5% Vaseline, D) Treatment Group treated with topical application of 10% Vaseline.

In this study, when compared to control (11.52 ± 4.27) on day 16, 5% vaseline (1.5 ± 0.957), 10% vaseline (0.75 ± 0.75) and standard (0.50 ± 0.50) showed significant improvement $P < 0.05$ in wound contraction. Therefore, the present study showed that topical administration of methanolic extract of *P. stratiotes* at both strengths (5% and 10%) exhibited efficient wound healing activity. However, this effect was found to be concentrated related fashion where 10% ointment promotes significant wound healing activity by increasing cellular proliferation, formation of granulation tissue, synthesis of collagen and by increase in the rate of wound contraction as compared to control animals (Table 1). The study reveals that all the four groups showed decreased wound area from day to day (Fig 3). All readings are found to be statistically significant when compared with corresponding control. The histopathological observation showed increased granulation tissue & epithelial tissue in methanolic leaf extract of *P. stratiotes* test groups as compared to control group (Fig 4). The epithelialization period was measured in as depicted in Table 1. The increase in tensile strength (gm) of wounded skin indicates the promotion of collagen fibers which was found in 5% and 10 % Vaseline (Table 2).

Table 2.

Effect of topical application of ointments containing methanolic extract of *P. stratiotes* on tensile strength of the skin having excision wound.

Treatment Groups (n=6)	Tensile strength in gm (Mean \pm SEM)
Control	326.83 \pm 3.64
Standard (Betadine)	641.00 \pm 4.91
5% Vaseline	671.00 \pm 2.63*
10% Vaseline	737.67 \pm 3.29*

The treated groups are compared by Student t-test with the control group. * $P < 0.01$

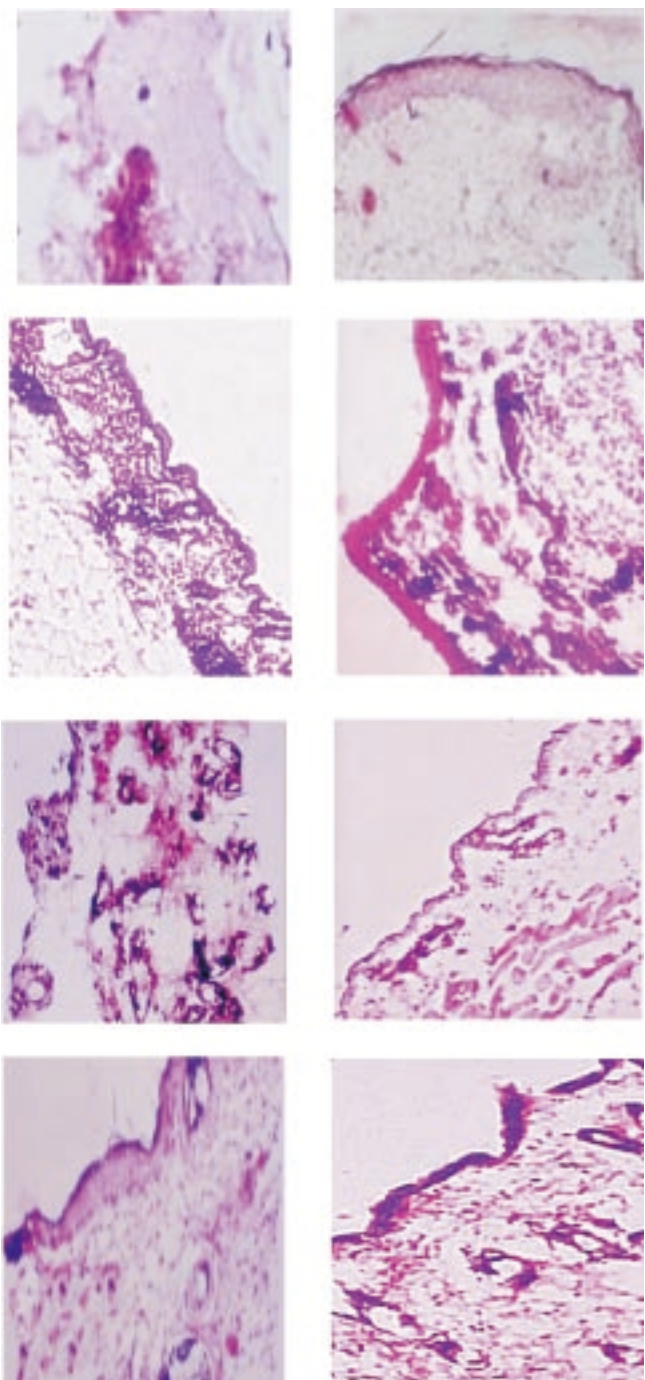


Figure 4. Histopathological characteristics of mice skin was observed on 0 day and 16th day by treatment with a) Vaseline, b) betadine, c) 5% and d) 10% methanolic ointment. Figure shows increased fibroblast cells, blood vessels and collagen fibers.

4. Discussion

Topical application of *Pistia stratiotes* at the wound site produced significant wound healing activity, which may be due to the help for the process of angiogenic and mitogenic potential. A healing tissue synthesizes collagen, which is a constituent of growing cell. Collagen not only confers strength and integrity to the tissue matrix but also plays an important role in homeostasis and in epithelialisation at the latter phase of healing [22]. Better collagenation, seen under

the influence of this plant extract, may be because of the presence of flavonoids and steroids which is responsible for the free radical scavenging activity which is believed to be one of the most important components of wound healing. Sterols may also increase collagen content and degree of collagen–cross linkage within the wound they may also promote cell division and the growth of bone, cartilage and other connective tissues. Free radicals and oxidative reaction products produce tissue damage and are particularly encountered during connective tissue disorders like fibrosis as well as during wound healing. Over production of reactive oxygen species result in oxidative stress thereby causing cytotoxicity and delayed wound healing. Therefore elimination of ROS could be an important strategy in healing of wounds [23]. Hence estimation of antioxidants is also relevant because these antioxidants accelerate the process of wound healing by destroying the free radicals. If any material possess antioxidant activity, it can be concluded that this material also help to promote wound healing and contribute skin regeneration [24]. This study revealed that *P. stratiotes* extract possessed significant antioxidant activity, which would help to prevent oxidative damage and promote wound healing process. The increased tensile strength reveals that the disrupted surfaces are firmly knit by collagen. The wound–healing property of *P. stratiotes* may be attributed to the phytoconstituents present in the plant, and the quicker process of wound healing could be a function of either the individual or the additive effects of the phytoconstituents. Further, phytochemical studies are in progress to isolate, characterize and identify the specific active compounds responsible for wound–healing activity. Hence, we conclude that the waterweeds which are havoc for aquatic fauna can be made as an easy way to save the population against non healing wounds and it will be cost effective and thus will act as a poor man friendly and a drug of choice.

Conflict of interest statement

We declare that we have no conflict of interest.

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