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Research Article

Preliminary Phytochemical Investigation and Wound Healing Activity of *Jasminum sambac (*linn) ait. (Oleaceae) Leaves

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ABSTRACT
In the Present study, aqueous and ethanol extracts of *Jasminum sambac* leaves were evaluated for its wound healing activity in the ointment dosage form in excision wound model using albino mice. The leaves of *Jasminum sambac* L. (Family Oleaceae) were subjected to continuous extraction with solvents; ethanol and water. The extracts were tested for various preliminary phytoconstituents and screened for wound healing activity at two dose level (200 mg/kg B.W. and 400mg/kg B.W) by dermal route. Aqueous extract had shown significant increase in wound contraction, hydroxyproline content and decreased epithelization period in excision wound model as compared to ethanol extract. The enhanced wound healing activity of aqueous extract may be due to free radical scavenging action and antibacterial property of the phytoconstituents(viz; tannins and flavonoids) present in it.

key words: *Jasminum sambac*, leaves extract, wound healing, hydroxyproline

INTRODUCTION

A wound is a disruption of tissue integrity that results in damage and is typically associated with loss of function. Wound healing can be defined as a complex dynamic process that results in the restoration of anatomic continuity and function. It involves regeneration of specialized cells by proliferation of surviving cells. Wound healing is divided into four sequential, yet overlapping 4 phases: hemostasis, inflammatory phase, proliferative phase, remodelling phase.Wounds are generally classified as, wounds without tissue loss (e.g. in surgery), and wounds with tissue loss, such as burn wounds, abrasions or as secondary events in chronic ailments e.g.venous stasis, diabetic ulcers or pressure sores and iatrogenic wounds such as skin graft donor sites and derma abrasions[(1)]. Proper healing of wound is essential for the restoration of disrupted anatomical continuity and disrupted functional status of the skin[(2)]. Many Ayurvedic herbal plants have a very important role in the process of wound healing. Plants are more potent healersbecause they promote the repair mechanisms in the natural way. The healing process can be physically monitored by assessing the rate of contraction of the wound. *Jasminum sambac* Linn. (Family-*Oleaceae*) commonly known as Motia or lily jasmine is a scandent or sub-erect shrub with young pubescent branches, broadly ovate or elliptic, opposite leaves, white, very fragrant flowers cultivated nearly throughout the tropical and sub-tropical parts of the world [(3)]. Traditionallyleaves are used infever or cough, indolent ulcer, abdominal distension, diarrhoea, lowering the blood glucose level,regulating menstrual flow, to clean kidney waste, inflamed and blood shot eyes [(3,4)]. The plant is reported to have to have antidiabetic[(5)], antitumor[(6)], antimicrobial[(7)], antioxidant[(8)], anti-acne[(9)],suppression of puerperal lactation[(10)], A.N.S stimulating effect[(11)]. To validate the ethnotherapeutic claim of the plant in skin diseases, wound healing activity was studied. In this communication we report the preliminary phytochemical investigations of the various extracts, the acute toxicity studies, and wound healing activity of the plant.

MATERIALS AND METHODS

The plant material *Jasminum sambac* was collected from the Herbal Garden, Ambala Cantt, The plant was authenticated by Dr. H.B Singh, Scientist F and Head, Raw Materials Herbarium and Museum, NISCAIR, New Delhi under the voucher specimen no: NISCAIR/RHMD/Consult/-2010-11/1696/294 and a specimen was submitted to the Department of Pharmacognosy and Phytochemistry, Hindu College of Pharmacy, Sonipat, Haryana (India)

Preparation of Extracts: The collected sample was washed thoroughly shade dried, powdered and successively extracted with different solvents like petroleum ether, chloroform, ethanol and water so as to get the respective extracts. All the extracts were filtered individually, evaporated to dryness using the rotatory evaporator. The traces of the solvents were removed by keeping the dried extracts into a desiccator.

Preliminary phytochemical studies: The individual extracts were subjected to qualitative chemical investigation for the identification of the phytoconstituents; alkaloids, carbohydrates, flavonoids, tannins, proteins, glycosides, saponins, sterols.

Preparation of Simple Ointment (BPC): Weighed quantity

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Table 1: Results of phytochemical screening of extracts of *Jasminum sambac*

Tests for constituents	Ethanol extract	Aqueous Extract
Alkaloids	-ve	-ve
Carbohydrates	+ve	+ve
Flavanoids	+ve	-ve
Tannins and Phenolic compounds	+ve	+ve
Proteins and Amino-acids	-ve	+ve
Mucilages	-ve	-ve
Steroids	+ve	-ve
Glycosides	+ve	-ve
Saponins	+ve	+ve
Fats and fixed oils	-ve	-ve

+ve Present, -veAbsent

of Macrogol 4000 (solid form) was melted on hot plate. Macrogol 600 (liquid form) was warmed to the same temperature, and then added to the melted macrogol 400. It was stirred until cool.

Preparation of Test Sample

Formulation I**:** Aqueous extract ointment containing 200mg/kg Body weight of extract

Formulation II: Aqueous extract ointment containing 400mg/kg Body weight of extract

Formulation III: Ethanol extract ointment containing 200mg/kg Body weight of extract

Formulation III: Ethanol extract ointment containing 400mg/kg Body weight of extract

Experimental Pharmacological Activity: Animals:

Albino mice of either sex weighing (25±2g) were procured from Institutional animal house, Hindu college of pharmacy, Sonipat. Throughout the experimental period, the animals were housed in cages. The animals were provided with (pellet diet) and water ad libitum. Animals were maintained at temperature range of 22-25°C. Study was conducted after obtaining clearance from the institutional Animal Ethical Committee of the Hindu college of pharmacy, Sonipat.

Acute toxicity study: Swiss albino mice of either sex weighing (25±2g) were used for acute dermal toxicity study. The study was carried out as per the guidelines set by OECD 434. The animals were divided into 3 groups (n= 5) and were applied dermally with dose (2000mg/kg B.W.) of the aqueous and ethanol extract). Group I was considered as control and applied simple ointment base. Group II and III were applied with aqueous and ethanol extracts of leaves. The animals were continuously observed for mortality and behavioural responses immediately after dosing during first 30 min, periodically during the next 24 hrs and daily thereafter for 14 days[12].

Excision wound model: Swiss albino mice of either sex weighing (25±2g) were divided in to 6 groups(n=6) used for wound healing study.All the animals in each group were anaesthetized under ether, before wound creation hair on the back was removed using depilatory cream (Veet). Before applying depilatory cream skin sensitivity test was also performed by applying cream on small area of mice (on back), and was observed for 24 hrs. No allergic reaction was noticed. An impression was made on the dorsal thoracic region 1cm away from vertebral column and using a round seal of 10 mm diameter on the pre-shaved, area. The skin of impressed area was excised to the full thickness to obtain a wound area of 78 mm^2.

Homeostasis was achieved by blotting the wound with cotton swab soaked in normal saline. 5mg of ointment was applied daily and different parameter was noted. Wound contraction, which contribute for wound closure was noted on 1, 4, 8, 12, 16 day by retracing the wound on a millimetre scale graph paper[13]. The degree of wound healing was calculated as shown in table 2.

$$\% \text{ wound contraction} = \frac{\text{initial wound area - final wound area}}{\text{Initial wound area}} \times 100$$

Grouping of Animals

GROUP I: (control group): Received application of simple ointment Base.

GROUP II:(Standard Group): Received application of standard drug of povidone iodine.

GROUP III: (Aqueous Extract of leaves 200mg/kg b.wt.). Received application of formulation I

GROUP IV: (Aqueous extract of leaves 400mg/kg b.wt.). Received application of formulation II

GROUP V: (Ethanol extract of leaves 200mg/kg b.wt.). Received application of formulation III

GROUP VI: (Ethanol extract of leaves 400mg/kg b.wt.). Received application of formulation IV

Epithelialization Peroid:–Epithelialization period is the number of days required for falling of the scab without any residual raw wound behind[14]**.**The results were noted and shown in table 3.

Hydroxyproline estimation: Hydroxyproline, an amino acid which is found almost exclusively in collagen and which provides a direct measure of collagen content.Regenerated tissues from the healed lesion of wound were collected for the estimation of hydroxyproline. Free hydroxyproline is released from the tissues sample by carrying out acid hydrolysis, the acid is then neutralized which is then further oxidized to pyrrole with chloramines T at pH 6. This intermediate then gives pink colour with 4-dimethylaminobenzaldehyde[15].The

Photos depicting reduction in wound area are shown in Fig. 1.

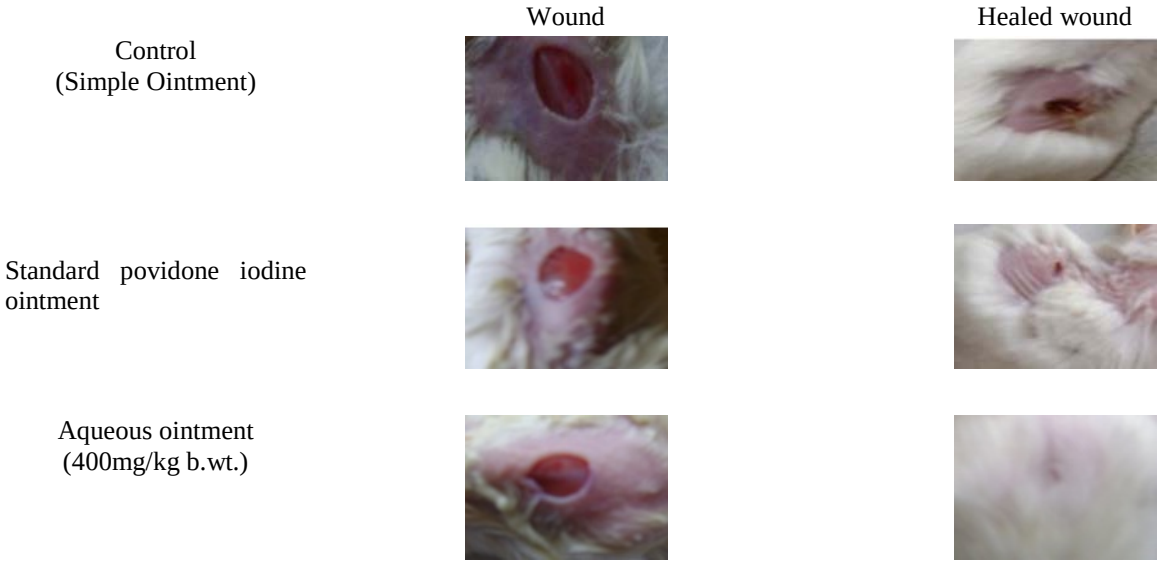


Table 2: Percentage wound contraction in various extracts

Extract	Day 4	Day 8	Day 12Day 16	
Control (Simple Ointment)	15.8±.68	49.6±2.7	66.4±.88	87.0±.38
Standard (Povidone iodine)	24.2±.59***	65.2±2.4***	79.2±.1.4***	96.3±.31***
Aqueous extract ointment (200mg/kg B.W)	20.9±2.3	56.3±1.8	73.5±1.1**	93.0±.77***
Aqueous extract ointment (400mg/kg B.W)	27.6±1.0***	70.0±1.8***	82.3±1.5***	98.0±.32***
Ethanol extract ointment (200mg/kg B.W)	19.6±1.4	60.0±2.0**	72.1±1.4	91.5±1.3**
Ethanol extract ointment (400mg/kg B.W)	22.8±1.1**	63.1±2.0***	77.1±1.0***	95.2±.47***

results for hydroxyproline content was calculated and are shown in table 3

Histopathology: A section of granuloma tissue was subjected to histopathological examination so as to determine the pattern of lay-down for collagen.

STATISTICAL ANALYSIS

All the results were analyzed by One-way Analysis of Variance (ANOVA) followed by Dunnett's test. The level of significance was set at $P<0.05$.

RESULTS

Acute dermal toxicity study: The safe dose came out to be 2000mg/ kg b.wt acute toxicity testing. Then $1/10^{th}$ and $1/5^{th}$ value of the safe dose was selected as two dose level for the purpose of wound healing investigation.

Preliminary Phytochemical Evaluation: The phytochemical tests revealed that the leaves of the plant containscarbohydrates, flavonoids, tannins, phenolic compounds and glycosides in ethanol and aqueous extracts. The results of phytochemical screening are given in table 1

DISCUSSION

To evaluate the wound healing property of the leaves of *Jasminum sambac*, wound contraction, epithelisation period, hydroxyproline content parameters were estimated. Wound healing effect is attributed to free radical scavenging activity of flavonoids. Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing onset of cell necrosis, but also by improving vascularity. Lipid peroxidation is an important process in several types of injuries like burns, infected wounds and skin ulcers. Hence any drug that inhibits lipid peroxidation is believed to increase strength of collagen fibres, by increasing circulation or by preventing cell damage or by promoting DNA synthesis. Flavonoids, glycosides and tannins are known to promote wound healing process mainly by their astringent and antimicrobial property[16,17].

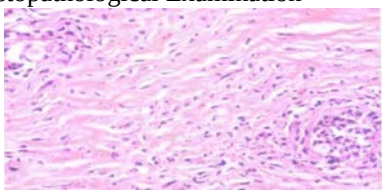
Wound contraction is a factor, which indicates rates of reduction of unhealed area during the course of treatment. This centripetal movement of wound margin is believed to be due to the activity of myofibroblast[18]. Greater the reduction better is the efficacy of medication. In other

Table 3: Epithelization period & hydroxyproline content of extracts of *Jasminum sambac*

Extract	Epithelization peroid	Hydroxyproline(mg/g of wet tissue)
Control (Simple Ointment)	19.2 + .33	1.8±.06
Standard (Povidone iodine)	15.0+ .25***	3.5±.15***
Aqueous extract ointment (200mg/kg Body weight)	16.5 + .22***	2.5±.10**
Aqueous extract ointment (400mg/kg Body weight)	15.0 + .25***	3.9±.13***
Ethanol extract ointment (200mg/kg Body weight)	17.1 + .30***	2.3±.08*
Ethanol extract ointment (400mg/kg Body weight)	16.0 + .25***	2.8±.19***

*Values expressed as Mean ± SEM), * p< 0.05, ** p< 0.01, *** p< 0.001*

Fig. 2: Histopathological Examination



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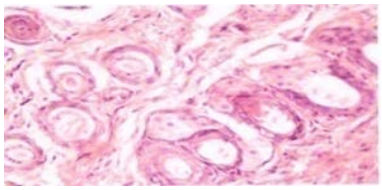
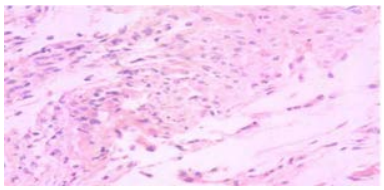
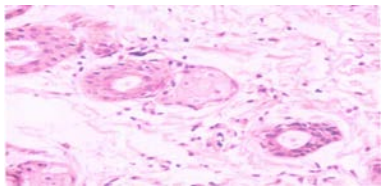


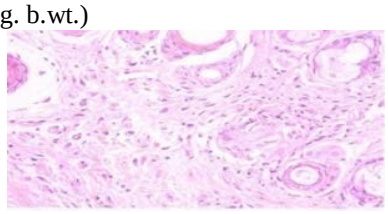
Standard



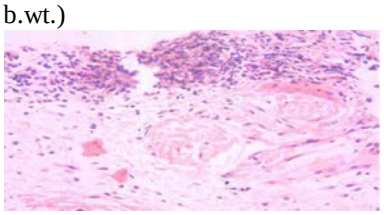
Aqueous extract (200mg/kg. b.wt.)



Aqueous extract (400mg/kg b.wt.)



Ethanol extract (200mg/kg b.wt.)



Ethanol extract (400mg/kg b.wt.)

words the wound will close at fast rate if the medication is more efficient. The increased wound contraction in the treated group may be due to the enhanced activity of fibroblasts and macrophages. The slow rate of wound closure in the control group might be attributed to the presence of microorganisms and their metabolites, which inhibit wound contraction and delay the wound healing. Table 2 records the reduction of wound area of the different groups over the period of 16 days. Depicting photographs of wound healing has been shown in photograph. Photograph clearly indicate the significant wound healing activity of aqueous extract(400mg/kg B.W).

It may be seen that the fastest healing of wound took place in case of animals treated with the aqueous (400mg/kg B.W) extract of *Jasminum sambac* Ait. leaves as compared to standard followed by other extracts.

The days required for falling off of eschar leaving no raw wound was taken as the period of epithelisation[14]. Epithelisation period was found to be lesser in groups treated with aqueous extract(400mg/kg) as compared to other groups.

Hydroxyproline is an indicator of improved conditions of wound healing in animals. Hydroxyproline is an amino acid present in the collagen fibres of granular tissue. Its measurement quantitatively is direct related to formation of collagen and its estimation helps to understand progress rate at which healing process is going on in the connective tissue of the wound. Collagen occupies the central role in healing of wound[19].Hydroxyproline content in granulation tissue of the animals treated with aqueous extract(400mg/kg) was significantly increased when compared to control group indicating increased collagen content.

The Histopathological examination of mice skin treated with aqueous extract (400mg/kg b.wt) showed increased number of fibroblasts, well-formed hair follicles with no macrophages and inflammatory cells which are comparable to the standard. Histopathological examination of control group showed large no of macrophages and inflammatory cells with lesser no of fibroblasts and destroyed hair follicles.

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