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Wound healing potential of *Biophytum sensitivum* (L.) DC.: An ayurvedic drug

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ABSTRACT

Non curative wounds is one of the major problems often encountered in health care system. The resumption of interest to use of Traditional System of Medicine especially plant based medicines is rapidly growing. Herbs are more potent healers and promote the repair mechanisms in a scientific way without causing any side effects. Plants used in Ayurveda are known to play significant role in the management of wound healing. But scientific evaluation of these herbal drugs to assess their safety and efficacy as well their standardization are required for their international recognition and acceptance. Hence in the present study attempts were made to scientifically evaluate the wound healing potentials of an ayurvedic plant, 'LAJJALU' botanically equated as Biophytum sensitivum (L.) DC. (BS). In this study wistar strain albino rats are used as animal model. The wound healing efficacy of the methanolic extracts of source taxon in 1g and 2g dose level was evaluated in excision wound models. The wound treated selected plant showed higher rate of wound contraction, increased level of Hydroxy proline, Hexosamine content, Super Dismutase, Ascorbic acid and decreased Lipid peroxidation. Histopathological studies revealed the presence of newly formed blood vessels amidst the well laid collagen fibres. Significant wound healing activity was observed in both the groups which were administered 1g and 2g of the test drug. But the animals treated with 2g revealed complete healing effect on 15th day.

Key words: Wound healing, Hydroxy Proline, Hexosamine, wound contraction.

INTRODUCTION

Non curative wounds is one of the major problems often encountered in the health care. Wound management needs immediate attention than the other health hazard for quick benefit. Current estimates indicate that worldwide nearly 6 million people suffer from chronic wounds **[1]**. Since last decade, multidisciplinary concepts have been for the treatment of wounds. But they did not give promising results due to their side effects.

The resumption of interest in the use of Traditional System of Medicine is rapidly growing, not only in the developing countries but also in the developed countries. Today, traditional medical practices have been accepted by the World Health Organization (WHO) as a building block of primary healthcare. India has an enormous heritage of traditional medicines such as Ayurveda and Siddha. Ayurveda and Siddha are two systems of medicine that are complementary to each other in contributing towards the health care of the human society. Ayurvedic system of medicine, one of the oldest systems of medicine practiced in India mentions healing of wounds as one of the

important areas of clinical medicines in many Ayurvedic texts under the heading "Vranaropaka". Many of the Ayurvedic herbal plants have a significant role in the management of wound healing. At present people are turning towards the herbal wound healers because they are free from allergy and other complications and also encourage the repair mechanisms in a natural way as compared to the application of synthetic wound healers. But lacuna existing in these systems is lack of proper standardization and scientific validation of the herbs to be used. So there is a need for the scientific evaluation of these herbal drugs so as to assess their safety and efficacy as well as for their international recognition and acceptance.

In the present study attempts were made to evaluate the wound healing potentials of an ayurvedic plant *'LAJJALU'* botanically equated as *Biophytum sensitivum* (L.) DC. (BS) belonging to the family Oxalidaceae employing experimental wistar strain albino rats as animal model.

Source taxon is a slender erect annual herb with a rosette of leaves on top of the stem, leaves abruptly pinnate and sensitive leaflets [2] (Figure 1). Muthuvan, Kerala tribal people use this plant for delivery, cuts and wounds and also against poison [3].

Figure 1: *Biophytum sensitivum* (L.) DC

EXPERIMENTAL SECTION

Collection of Plant material

The aerial parts of *Biophytum sensitivum* DC. selected for the proposed study were collected from in and around Trichy. Care was taken to select healthy plants. The identity of the plant specimen was confirmed using Flora of Presidency of Madras [4]. and authenticated with the Herbarium specimens kept at RAPINAT Herbarium, St. Joseph's College, Trichy, South India.

Preparation of plant extract

Fresh plants of *Biophytum sensitivum* DC. were collected, washed and shade dried. From this 100g of dried plant were coarsely powered and percolated in methanol for 72 hrs at room temperature. After 72 hrs the solvent was filtered and distilled off. The obtained residue was subjected to preliminary phytochemical screening and wound healing activity studies**.**

Preliminary Phytochemical and Standardization Studies

Preliminary phytochemical studies of drug powder as well as extracts were carried out as per the standard methods [5] and important chemical constituents such as alkaloids[6], flavonoids[7], tannins[8], glycosides[9] and lignins[10] were quantitatively estimated.

Wound healing model

Wistar albino rats were used as experimental animals for screening wound healing activities of methanolic extract of *Biophytum sensitivum* DC.

Selection and procurement of animals

Healthy adult wistar strain of albino rats of both sexes, two to three months old and weighing 150g-200g were obtained from Tamilnadu Veterinary and Animal Sciences University, Chennai. The animals were allowed to acclimatize under laboratory conditions for a period of 5 days prior to the experiment. Rats were housed in standard polypropylene cages. All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committees (Approval No: 790/03/ac/CPCSEA at Srimad Andavan College, Trichy).

Experiment design
Wistar strains of albino rats were divided into four groups, each comprising of six rats.

Group I: Excision Wounded- Diseased control rats
Group II: Excision Wounded and treated with 1 g of methanolic BS extract
Group III: Excision Wounded and treated with 2 g of methanolic BS extract
Group IV: Excision Wounded and treated with 2 g of standard drug – Soframycin

Wound creation
Animals were anaesthetized by ketamine (50 mg/kg, i.p) prior to the wound creation. The rats were inflicted with excision wounds. The dorsal fur of the animals was shaved and the anticipated area of the wound to be created was marked. A full thickness excision wound was created by excising areas of skin 200mm^2 in length and 0.2 cm depth from the dorsal region using a sharp surgical blade and pointed scissors [11]. Test drug was applied once daily for 15 days to all experimental animals. Treatment was given as per the treatment schedule till complete epithelization is seen.

Rate of contraction of wounds [12]
The progressive reduction in the wound area is monitored planimetrically by tracing the raw wound boundaries initially on a sterilized transparency paper sheet in mm^2 without causing any damage to the wound area, and then, the wound area recorded is measured using a graph paper, at every 5th day interval starting from the day of operation until the day of complete epithelialization and the degree of wound healing is calculated.

Collection of blood sample and granuloma tissue
After the experimental period, the animals were sacrificed by CO2 inhalation euthanasia, then the blood samples were collected and the regenerated tissues were dissected out from each group. The small portion of regenerated tissue samples was washed in ice-cold saline fixed in 10% buffered formalin for histopathological studies[13]. The remaining regenerated tissues were estimated for hydroxyproline[14] and hexosamine content[15]. The serum was used for estimation of Lipid peroxides[16], Superoxide Dismutase[17] and Ascorbic acid[18].

RESULT AND DISCUSSION

Behaviour of drug powder of BS with various chemical reagent revealed the presences of Saponins, Sterols, Flavonoids, Coumarins and Quinones and the preliminary phytochemical screening of methanolic extracts answered positively for the presence of Steroids, Saponins, Quinones, Coumarins, Flavonoids , Alkaloids and Lignins. The data obtained on the quantitative estimation of the important constituents were presented in Table 1.

Table 1: Quantitative analysis of Important Chemical Constituents

S. No	Phytoconstituents	VALUE (mg/kg)
1.	Total Alkaloids	0.69
2.	Total Flavonoids	0.89
3.	Tannin	0.62
4.	Lignin	0.54
5.	Glycosides	0.10

Table 2: Effect of Methanol extract of BS on the rate of wound contraction

Groups	Parameters	Rate of Contraction in mm^2			
		0th Day	5th Day	10th Day	15th Day
Group I	Wounded Control	2.1 ± 0.06	2.0 ± 0.12	1.5 ± 0.22	1.1 ± 0.23
Group II	Wounded + BS treated (1g/Kg)	2.1 ± 0.07a	1.6 ±0.46a*	1.1 ± 0.38a*	0.3 ± 0.08a*
Group III	Wounded + BS treated (2g/Kg)	2.2 ± 0.04b	1.4 ± 0.32b*	0.5 ± 0.33b*	0 ± 0.07b*
Group IV	Wounded + Soframycin (2g/Kg)	2.0 ± 0.03c	1.2 ± 0.09c*	0.4 ± 0.27c*	0 ± 0.03c*

Values are expressed as mean ±S.E (n = 6) Statistical Comparison
**P < 0.05 significant when compared with control a: Group I and II, b: Group I and III c:Group I and IV.*

Figure 2: Effect of Methanol extract of BS on the rate of wound contraction

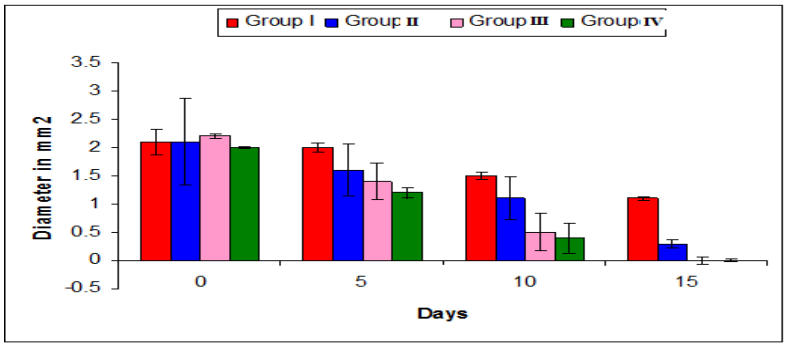
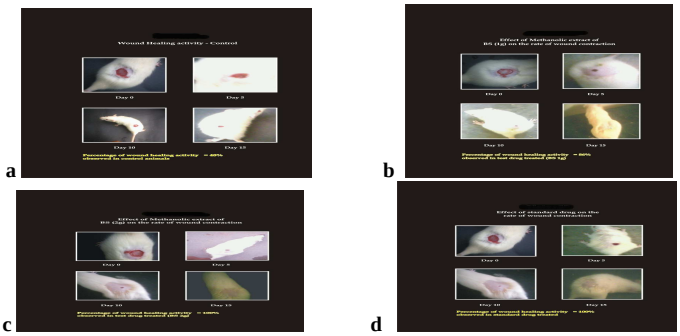


Figure 3: Rate of Wound Contraction in Experimental animals



a. Rate of wound Contraction in Control rats.
b. Effect of methanolic extract of BS (1g) on the rate of wound contraction.
c. Effect of methanolic extract of BS (2g) on the rate of wound contraction.
d. Effect of standard drug on the rate of wound contraction

The effect of methanolic extract of BS on the rate of contraction of wound area was presented in Table 2. Figure 2 & 3. The wound treated with plant extracts showed a higher rate of contraction during the treatment period. Group II and Group III received the test drug at the dose levels of 1g/kg bw and 2g/kg bw respectively. Significant wound healing activity was observed in both the groups. The group III animals which received the dose of 2g/kg bw showed a higher rate of contraction as compared to Group II animals which received 1g/kg bw. and also the group III animals showed complete healing effect on 15th day. Group III showed rapid tissue repair as compared to control animals.

The data of the results of the present study revealed that the test drug demonstrated highly efficient wound healing activity throughout the experiment displaying a significant reduction in wound area as compared to the normal control. Hence, it is inferred that the test drug not only actively promotes faster wound contraction, but also acts as a potent agent in aiding the process of tissue granulation and in the remodeling of the connective tissue during the healing process.

Table 3: Effect of Methanol extract of BS in the Biochemical markers of wounded animals

Groups	Treatment	Hydroxy Proline	Hexosamine	LPO	SOD	Ascorbic acid
Group I	Wounded Control	14.2 ± 3.56	9.0 ± 2.56	6.2 ± 2.45	2.1 ± 0.75	5.82 ± 0.97
Group II	Wounded +BS treated (1g/Kg)	29.8 ± 4.04a*	24.82 ± 3.09a*	3.66 ± 2.18a*	3.0 ± 0.95a*	9.09 ± 1.12a*
Group III	Wounded +BS treated (2g/Kg)	34.5 ± 3.94b*	29.6 ± 3.47b*	2.92 ± 1.84b*	3.5 ± 0.43b*	9.78 ± 1.56b*
Group IV	Wounded + Soframycin (2g/Kg)	35.2 ± 3.82c*	31.3 ± 2.98c*	2.86 ± 1.45c*	3.6 ± 0.62c*	9.87 ± 0.98c*

Values are expressed as mean ±S.E (n = 6) Statistical Comparison
**P < 0.05 significant when compared with control a: Group I and II, b: Group I and III c:Group I and IV*

Table 3 and Figure 4 depicts hydroxyproline concentration in the tissue of animals. The group III animals which received the dose of 2g/kg bw showed increased hydroxy proline content (34.5 ± 3.94) as compared to Group II animals (29.8 ± 4.04) which received 1g/kg bw. Both the doses of drug showed significant increase of hydroxy

proline content compared to the control rats. The results were comparable to that of standard drug used in the study. Significant increase in the hydroxyproline content of the granulation tissue indicated increased collagen turnover. Breakdown of collagen liberates free hydroxyproline and its peptides. Measurement of this hydroxyproline has been used as an index of collagen turnover. Collagen is the major component which strengthens and supports extra cellular tissue which is composed of the amino acid hydroxyproline, and has been used as a biochemical marker for tissue collagen [19] Increase in the hydroxy proline was found among the treated rats in this present study which suggest a good turn over content of collagen.

Figure 4: Level of Hydroxy Proline in experimental animals

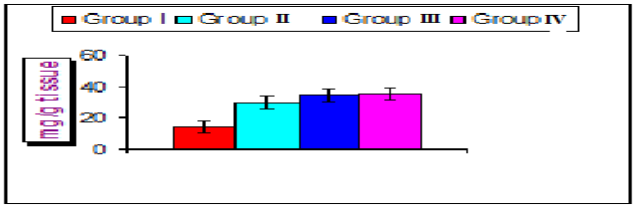
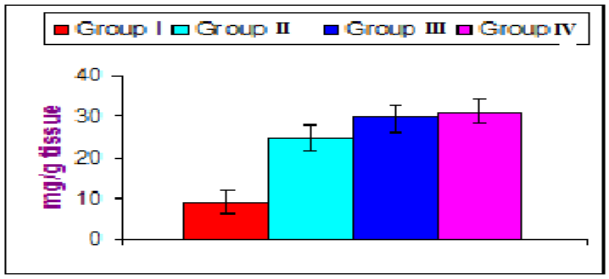


Figure 5. shows the effect of methanolic extract of BS on Hexosamine content in the treated and untreated rats. Hexosamine content increases in the early stages of wound healing and is an indicative that the fibroblasts are actively synthesized. This is the ground substances (mucopolysaccharides) on which the collagen can be laid on [20]. BS extract treated animals showed to increase the hexosamine level which was evident from the obtain data (Table: 3) A significant increase observed in the Hexosamine levels in group II (24.82 ± 3.09) and group III (29.6 ± 3.47).

Figure 5: Level of Hexosamine in experimental animals



The levels of LPO in the experimental animals were given in Figure 6. The cytokine cascade activated after an injury with stimulation of phagocytic cells will lead to the formation of oxygen free radicals and lipid peroxidation. The control group showed an elevation in the lipid peroxidation levels which indicated the decreased scavenging capacity of the wounded tissues. Lipid peroxidation is oxidative deterioration of PUFA [21]. It leads to cell injury and further to the generation of peroxides and increased lipid peroxides. In the present study, treatment with BS extract at a dose of 2g/kgbw (group III) and 1g/kg bw (group II) showed a significant decrease in the levels of LPO. This data revealed the hastening of the wound healing process by the test extracts.

Figure 6: Level of LPO levels in experimental animals

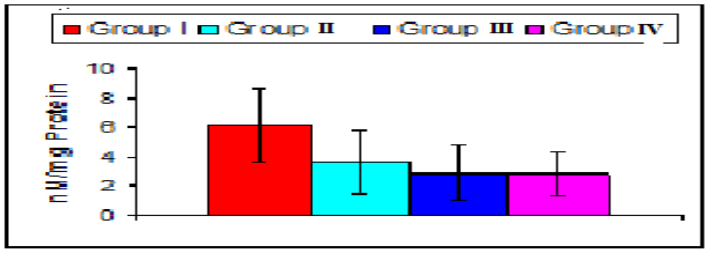


Figure 7. shows the effect of BS extract on SOD levels. Studies on the estimation of antioxidant enzyme revealed that the BS extract significantly increased the levels of superoxide dismutase. This enzyme was known to quench the superoxide radical and thus prevent the damage of cells caused by free radicals [22]. In the present work, experimental animals (Group III & Group II) which were given the test drugs at the doses of 1g/kg bw & 2g/kg bw were found to have increased (3.5 ± 0.43 & 3.0 ± 0.95) levels of SOD whereas disease control (GroupI) had very low level (2.1 ± 0.75) of SOD.

Figure 7: Level of SOD levels in experimental animals

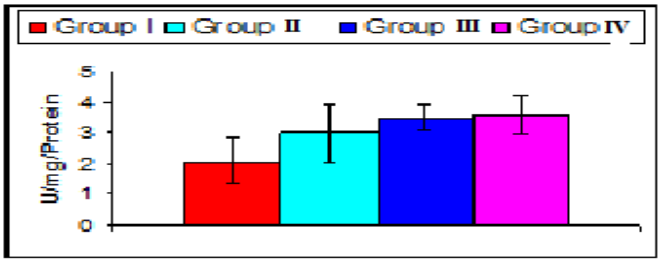


Figure 8. showed the level of of Ascorbic acid in experimental animals. Ascorbic acid is an essential cofactor for the synthesis of collagen and other organic components of the intracellular matrix of tissues such as bones, skin, capillary walls, and other connective tissues. In addition to collagen production, ascorbic acid enhances neutrophils function, [23] increases angiogenesis [24] and functions as a powerful antioxidant [25]. Ascorbic acid has a role in both the formation and maintenance of collagen in healing wounds. In the present study wounded control animals showed the decreased level of ascorbic acid as compared to drug treated animals. Group III and Group II animals were found to have profound increase of ascorbic acid content. Animals which received 2g/kg bw showed remarkable increase in the Ascorbic acid levels.

Figure 8: Level of Ascorbic acid content in experimental animals

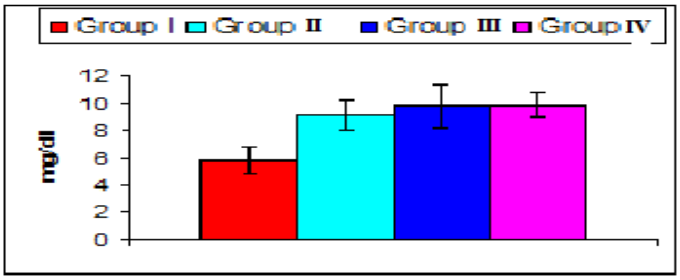
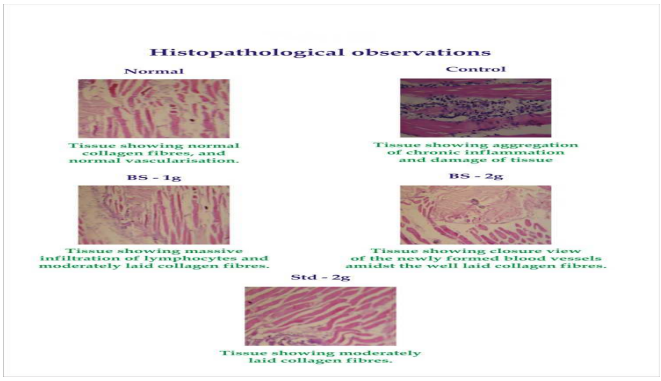


Figure 9: Histopathological Observations



Histopathological observations [26]
Histopathological observations were given in figure 9. Histopathological studies of the tissue obtained from BS treated group showed closure view of the newly formed blood vessels amidst the well laid collagen fibres compared to the normal, control and standard drug treated animals.

The wound treated with plant extract showed the higher rate of wound contraction compared to control rats which revealed that the drug is actively promoting wound contraction and also acting as a potent agent in aiding the process of rapid tissue repair, tissue granulation and remodelling of the connective tissue. The increased hydroxyproline content indicates faster collagen turnover leading to rapid healing process with concurrent increase in the breaking strength of the drug treated wounds. Hexosamine concentration which is one of the components of ground substance on which the collagen can be laid on, significantly increased in plant extract treated animals as compared with control indicating better stabilization of collagen fibers in plant extract treated animals. The production of free radicals is decreased by decreasing the lipid peroxidase level in drug treated animals. elevated level of ascorbic acid has a role in both formation and maintenance of collagen and also lead to improved antioxidant status that contributed towards faster wound healing process. The drug treated animals showed an increased levels of superoxide dismutase leading to quenching of the superoxide radical and thus preventing the damage of cells caused by free radicals. Histopathological observations also provide supportive proof for the wound healing properties of the test drug (BS). BS treated animals showed normal collagen fibres and normal vascularization with newly formed blood vessels admist the well laid collagen fibres. The flavonoids [27] and Alkaloids [28] present in the *Biophytum sensitivum* DC. might have contributed in quickening the wound healing process and were also responsible for wound contraction. Amentoflavone [29] present in the BS might have caused better wound healing process. Thus the biochemical parameters and histopathological studies carried on wounded animal model depicts re-epithelialization potential, improved antioxidant enzyme activity, and higher collagen cross-linking properties of the test drug BS.

CONCLUSION

Significant wound healing activity was observed in both the groups which were administered 1g and 2g methanolic extract of *Biophytum sensitivum* DC. But the animals treated with 2g of the test drug revealed complete healing effect on 15^{th} day in par with the standard drug. Scientific evidences that emerged through present work proved the wound healing effect of the test drug. Further indepth studies can be taken up on their wound healing extract for the better health care and beauty care of the skin as it is possible that present test drug could be scar free human friendly wound healer.

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