ISSN: 2320 - 4230

Journal of Drug Discovery and Therapeutics
Available Online at www.jddt.in
CODEN: - JDDTBP (Source: - American Chemical Society)
Volume 3, Issue 31, August, 2015, 01-06

Research Article

Wound healing activity of the ethanolic extract of *Solanum spirale* leaves indigenous to north east India

Vinod Kumar Verma[1]*, Manas Pratim Boruah[2], Manju Bais[3], Bankim Chandra Nandy[4]

*[1]Department of Pharmaceutical Science, Dibrugarh University, Dibrugarh, Assam, India

[2] Department of Chemistry, Silapathar Science College, Silapathar, Dhemaji, Assam, India

[3]Department of Anthropology, Pt. Ravi Shankar Shukla University, Raipur, (C.G.) India

[4]Department of Pharmaceutical Science, Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India

Received 08 May 2015; Accepted 25 July 2015

ABSTRACT

As a part of present work objective are to investigate indigenous plant used in wound healing in northeast India, we hereby reported our findings related to wound healing activities of plant *Solanum spirale* (Solanaceae) leaves from *in vitro* and *in vivo* model in rats following topical application studies. The ethanolic extracts of the leaves of *Solanum spirale* was tested for phytochemical constituents and wound healing activity against rats. The important secondary metabolites alkaloid, glycosides, saponins, flavonoids, carbohydrates, tannins, polyphenolic compounds, protein, and fats were identified in extracts. The animals were divided into three groups with six rats in each group. Topically applied 10% w/v of plant *Solanum spirale* leaves extracts in saline taking 0.2% w/w nitrofurazone, ointment as standard. The results showed that ethanolic extract of *Solanum spirale* leaves on topical application was reduced the epithelization period from 26.12±0.73to 20.47±0.63 days control and ethanol extract respectively along with a marked decrease in the scar area from 49.42±0.83 to 35.12±0.84 mm^2 control and ethanol extract respectively. Significant increase in tensile strength and hydroxyproline content of plant extract were also observed and compared to the control and silver sulphadiazine. The above result revealed that the plant has remarkable wound healing potency and appear to justify the traditional use of *Solanum spirale* in wound healing in India and offer a scientific support to the treatment of traditional healers.

Keywords: Excision; Incision; *Solanum spirale*; Wound healing activity

1. Introduction

In developing countries due to the poor hygienic conditions, wound infection is one of the most common diseases[1]. Traditionally villagers are use the poultice prepared from different plant crude extracts to treat a variety of skin ailments including wounds. Wound healing process involves systematic steps, which involves coagulation, inflammation, formation of granulation tissue, matrix formation, remodeling of connective tissue, collagenization and aquisation of wound strength. During the formation of new tissue, endothelial cells proliferate and form new blood vessels. The wound healing process can be broadly categorized into three stages; inflammatory phase, proliferate phase, and finally the remodeling phase which ultimately determines the strength and appearance of the healed tissue [2, 3, 4].

The traditional use of plants for wound healing has recently more attention by the scientific community and research on wound healing agents through the plants is one of the developing areas in modern biomedical sciences[5].

There are many reports stating that the various traditional plants and their extracts used for wound healing properties [4-18].

Plant *Solanum spirale* (locally known as Bari-hundari) belong to the family Solanacea. In Assam these leaves are traditionally used wound healing and to kill intestinal worms and roots are found to be narcotics and diuretic. In Arunachal Pradesh, the Adi tribes use this plant as vegetable to cure urinary and stomach disorders, high blood pressure. In some places, this plant is used for the treatment of various liver diseases. The use of these plant as a wound healing activity evaluated at first time therefore, the present study, explore the wound healing activity of alcoholic extracts of *Solanum spirale* leaves were studied and justify the traditional use.

*Corresponding author: Vinod Kumar Verma |E-mail: vinodmod@yahoo.com

2. Materials and Methods

2.1. Plant materials and Preparation of Extracts

The fresh tender leaves of *Solanum spirale* were collected from their natural habitats from nearby areas of Dibrugarh University, Assam. The plants were identified by Prof. P. J. Handique, Department of Biotechnology, Guwahati University, Guwahati, India. Voucher specimens (*Solanum spirale:* DCH-27) were deposited at the Herbarium of the Department of Chemistry, Dibrugarh University, Dibrugarh, India.

The collected plant materials were dried in shade and made course powder for the preparation of extracts. The powdered plant materials were subjected to ethanolic extracts by keeping 500g of material in 1L of ethanol for 36 hours separately. The ethanol extract were lyophilized in to powder and prepare 33.3% w/w *Solanum spirale* ethanolic extract powder in simple ointment BP used for the study.

2.2 Phytochemical Analysis

The plant ethanolic leaves extract was phytochemically screened for presence of alkaloid, glycosides, saponins, flavonoids, carbohydrates, tannins, polyphenolic compounds, protein, and fats using the procedures outlined by Wall et al.,1952 and Harbon 1973[19,20] .

2.3. Animals

Adult healthy Wistar albino rats either (170-200 g) were purchased from M/S Chakroborty Enterprises, 3/1 D, Grish Vidyaratan Lane Kolkata (WB) India were used in the experiment, which were performed in Pinnacle Biomedical Research Institute (PBRI), Bhopal. These rats aged between 2 and 2.5 months and were housed in PBRI in well ventilated stainless-steel cages at room temperature (24±2ºC) in hygienic condition under natural light and dark schedule with not more than four animals per cage. They were allowed free access to standard laboratory diet (Golden Feed, New Delhi) and water regularly. Food and water were given *ad libitum*. All the experimental studies were performed in accordance with the National Institute of Health's guideline for Survival Rodent Surgery (1985) after approval from institutional animal ethical committee (IAEC) of PBRI, Bhopal. (Reg. No.-1283/c/09/CPCSEA)

2.4. Animal groping

In present experiment, the rats were divided into three groups consisting six animals in each group. Group-I the control group animal which received simple ointment BP base. Group-II animals were locally applied with the standard drug (0.2% w/w nitrofurazone, a standard antimicrobial agent in topical wound dressing). Group-III treated group animal received *Solanum spirale* ointment (33.3% w/w *Solanum spirale* ethanolic extract in simple ointment BP) topically on rat wounds that were created on the dorsal back of rats daily until the wounds completely healed. The extracts ointment was given to compare the same amount and concentration as given in standard nitrofurazone ointment.

2.5. Wound healing activity

The excision wound model and incision wound model mainly these two models were used for study of the potential wound healing activity.

2.5.1 *Excision wound model*

Some earlier reported version of excision wound healing model was used with little modification[13-15]. In short animals were anesthetized with 1 ml of intravenous ketamine hydrochloride (10 mg/kg body wt) prior to and during creation **of** the **wounds**. An full impression was created on the dorsal thoracic region 1 cm away from the vertebral column and 5 cm away from the ear using a biopsy punch of uniform 2 cm diameter. The skin of the created impression area was excised to its full thickness to get a wound area of about 500 mm^2. The wound was left undressed to the open environment and no local or systemic anti-microbial agents were used. Hemostasis was done by blotting the wound with normal saline swab soaked cotton. The rats were randomly distributed in groups and each rat was placed in a separate cage. Contributions of contractions, for wound closure in first 2 week were studied by tracing the raw wound. Wound area was measured by retracing the wound on a millimeter scale graph paper. The degree of wound healing was calculated using formula: 1− (wound area on corresponding day/wound area on zero days) ×100. The number of days for complete epithelization was noted [13]. Hydroxiproline constituent of collagen was measured according to Shukla[8] *et. al.,* 1999, using spectrophotometer measured at 557 nm.

2.5.2 *Incision wound model*

The incision wound model used according to Udupa *et al.,* 1995; Govindarajan *et al.,* 2004; Perez Gutierrez *et al.,* 2006; Shivhare *et al.,* 2010 and Stephen *et al.,* 2010 with some modification. The animals were divided into three groups of six rats each and kept in separate cages. Rats were anesthetized and two paravertebral long incisions made through the skin and cutaneous muscles at particular distance of about 1.5 cm from the midline on each side of their depilated back. The animals not provide any systemic antimicrobial as well as any antiseptic throughout the experiment. Each of the three groups of animals was treated in the same manner as for the

excision wound model. The parted skin was kept together by stitching with a black silk surgical thread (No.000) and curved needle (No.11) and continuous threads on both wound edges were tightened for good wound closure. Group I animals (control) were treated topically with simple ointment base, group II treated group animal received *Solanum spirale* ointment (33.3% w/w *Solanum spirale* ethanolic extract in simple ointment BP) topically twice a day for 9 days. The tensile strength of a wound represented the degree of wound healing, so wound healing agents usually provide a gain in tensile strength. The tensile strength measured on 10th day after removing sutures. The animals were anesthetized and strips of healing tissue (8 mm width and 20 mm long) along with normal skin at two ends were excised. The tensile strength was measured by loading a strip between the upper and lower holder of the Tensile Testing Machine TKG-20 in such a way that the effective load size 8*8 mm with the wound remaining in the centre. The total breaking load was measured in Newtons and the tensile strength was calculated from the following equation:

$$\text{Tensile strength} = \frac{\text{total breaking load}}{\text{cross - sectional area}}$$

The mean tensile strength of the two-paravertebral incisions on both sides of the animals was taken as the measures of the tensile strength of the wound for an individual animal. The tensile strength of *Solanum spirale* ointment treated wound was compared with the control and nitrofurozone ointment (as standard) treated wounds tensile strength. Further, after tensile strength determination, also measured the epithelization period and scar area daily for 20 days.

2.6 Statistical data analysis

Results were expressed as mean ± SEM. Statistical comparison were made by using Student's t-test analysis and difference were considered statistically significant when P-value were <0.05.

3. Result and Discussion

The preliminary phytochemical investigation of the plant extracts showed positive results for important secondary metabolites alkaloid, glycosides, saponins, carbohydrates, tannins, polyphenols compounds, and flavonoids [Table-1].

The wound healing is an extreme complex phenomenon in which involving a number of well-arranged processes, including regeneration of parenchymal cells, migration and proliferation of both parenchymal and connective tissue cells, formation of extracellular matrix protein, remodeling of connective tissue parenchymal components, collagenization and acquisition of wound strength[2]. Phytochemical work of *S. spirale* leaves reveal that plant contains high amount of tannins, presence of polyhenolic compound and flavones, implied that tannin and flavonoids is one of the active compounds which may be responsible for the antioxidant activity. Therefore, in this study scavenging effect might be one of the most important components of wound healing which may be responsible to support wound healing property. Thus, the enhanced wound healing may be due to the free radical scavenging action of the plant as well as enhanced antioxidant enzyme level in granuloma tissues.

The complete wound healing of ethanol extract of *Solanum spirale* leaves were studied by counting the number of days and results are presented in Tables 2 and 3. (Figure 1& 2)

The animals treated with ethanolic extract of plant shows wound healing potency treatment was continued upto 20th days, no raw wound left after 16th days. The present experiment showed ointment of plant ethanolic extract possesses a good wound healing activity, there was a reduction in the epithelization time from 26.12±0.73to 20.47±0.63 days and the scar area reduced on complete epithelization from 49.42±0.83 to 35.12±0.84 mm^2. The hydroxyproline content and tensile strength of control and ethanolic extract based ointment of plant treated group increased from 152.67±7.21 to 272.34±5.33 µg/100mg and from 270.45±7.34 to 348.35±9.62 g respectively, results were compared to control and standard group on the 16th day of post healing are significant. The single model of wound healing is inadequate to confirm the wound healing activity of the drug because the wound healing process involved various phases and no *in vitro* experiment exists that collectively represent the various components of wound healing. There are *in vivo* assay are highly recommended to evaluate and confirm the *in vitro* observation. Some of the *in vivo* assays include the determination of hydroxyproline content and tensile strength significantly, which is an indication of quality of the healing[12]. The major protein in the extracellular matrix component is collagen that is ultimately contributes to wound strength. On breakdown of collagen liberates free hydroxyproline and measurement of hydroxyproline could be used as an index for the collagen turnover.

In present study, a significant elevation in the hydroxyproline content of the granulation tissue of the animal treated with *S. spirale* ethanolic extract was recorded and compared with control group, thus indicating positive effect of the *S. spirale* ethanolic extract

on collage synthesis hence it indicates wound healing activity. Similar result also found on the previous work done by Verma *et al.,* 2011 on Solanum *xanthocarpum* Schrad and Wendl (Solanaceae) in *in-vitro* and *in-vivo* model, which supported that the some plant of solanaceae family having wound healing properties.[4] The increase in tensile strength of the granulation tissue indicated enhanced collagen maturation by increase cross-linking. The increase in epithelization, as well as the tensile strength could be attributed to the increased hydroxyproline content in the wound tissue[10]. Thus the ethanolic extract of *Solanum spirale* leaves possesses good wound healing activity.

Table 1: Results of preliminary screening of plant extracts

Test for the presence of Phytoconstituents	*Solanum spirale*
Polyphenols	+
Flavonoids	+
Alkaloid	+
Glycosides	+
Saponins	+
Tannins	+
Carbohydrates	+

Table 2: Effect of topically applied *Solanum spirale* leaves extracts ointment on excision wound model in rats. [a]

Animal Treatment Groups [b]	Contraction of excision wound area (mm^2) after days [a]				
	4th Day	8th Day	12th Day	16th Day	20th Day
Group-I Control	6.75±0.36	7.475±0.45	9.36±0.35	11.23±0.23	12.98±0.45
Group-II Standard Drug (0.2% w/w nitrofurazone)	9.94±0.56	12.67±0.42	15.12±0.32	17.45±0.62	19.42±0.43
Group-III Treated (33.3% w/w *S. spirale* ointment)	7.34±0.72	9.23±0.58	14.28±0.22	16.92±0.54	18.84±0.35

Values are mean ±SEM (n=6) [a] statically significant difference in comparison with control group: *P< 0.05. [b] Once a day, for 20 day; control, no treatment.

Table 3: Effect of topically applied *Solanum spirale* leaves extracts ointment on incision wound model in rats.

Animal Treatment Group	Epithelization Period (days) [c]	Scar area (mm^2)	Hydroxyproline content (µg/100mg) [d]	Tensile strength (g)
Group-I Control	26.12±0.73	49.42±0.83	152.67±7.21	270.45±7.34
Group-II Standard Drug (0.2% w/w nitrofurazone)	22.43±0.38	38.87±0.74	248.74±5.43	352.22±8.42
Group-III Treated (33.3% w/w *S. spirale* ointment)	20.47±0.63	35.12±0.84	272.34±5.33	348.35±9.62

Values are mean ±SEM (n=6) statically significant difference in comparison with control group: *P< 0.05. [c] On complete epithelization. [d] On day 16th post wound healing.

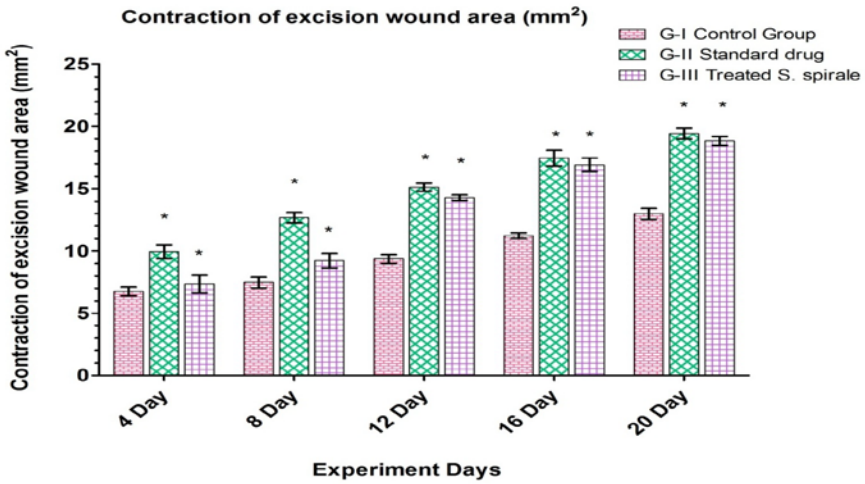

Figure 1: Effect of topically applied *S. spirale* leaves extracts ointment on contraction of excision wound area

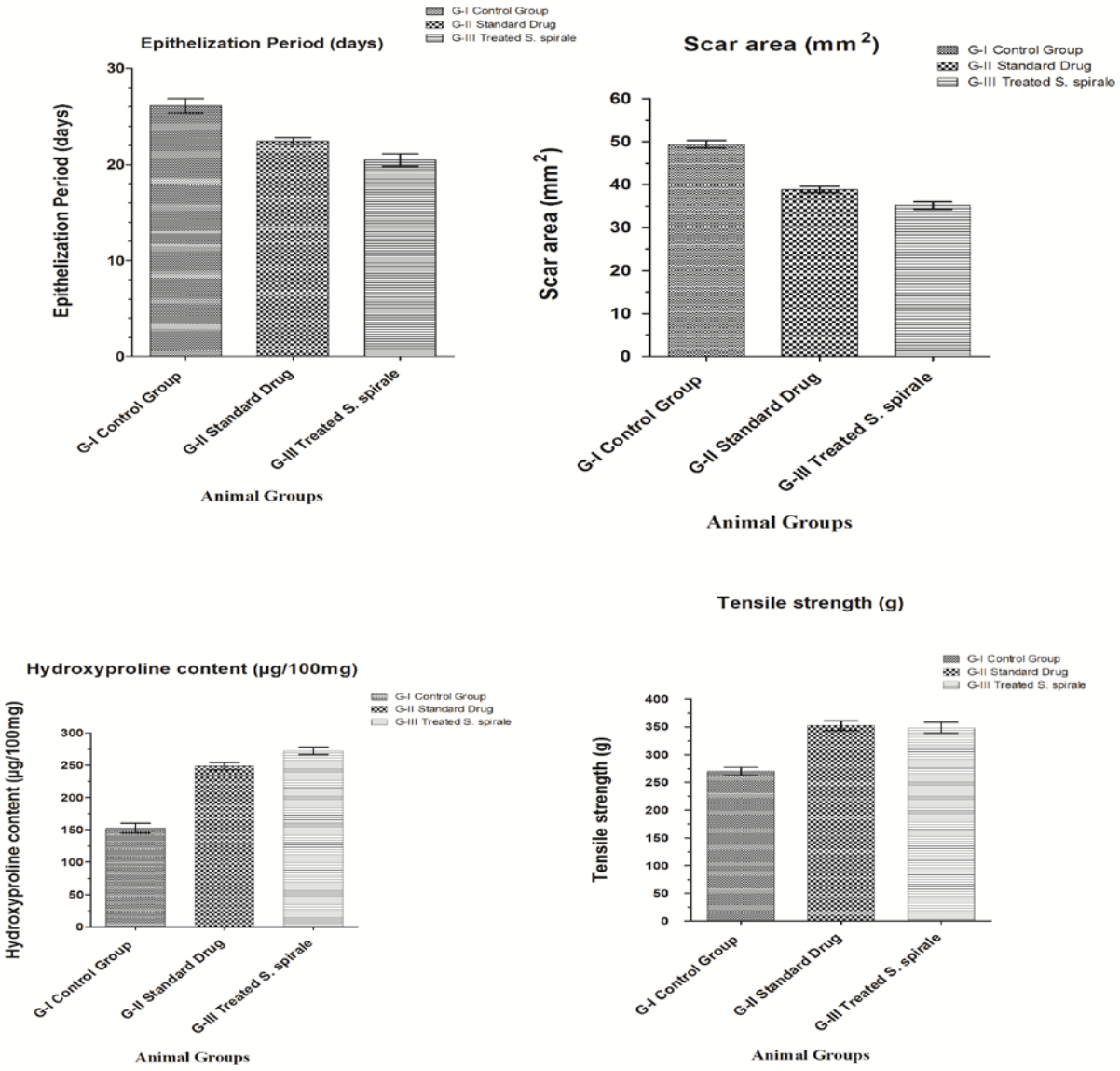

Figure 2: Effect of topically applied *S. spirale* leaves extracts ointment on different parameters of incision wound model

4. Conclusion

Finding of the present study suggested, that the ethanolic extract of plant *Solanum spirale* leaves has potential wound healing. The wound healing activity of the ethanolic extract may be due to the individual or combined effect of the phytoconstituents present in plant extract. *Comprehensive evaluation on the plants with wound healing activity based on traditional medicine may possibly give new compounds that could be used as prominent drugs in wound healing therapy.* Further investigations are needed for isolation and identification of active principles responsible for the wound healing activity. The present investigation offers a scientific support to the traditional healer account in use of the plant *Solanum spirale* leaves for treatment of cuts and wounds.

5. References

1. Senthil Kumar M, Sripriya R, Vijaya Raghavan H and Sehgal P (2006). Wound Healing Potential of *Cassia fistula* on Infected Albino Rat Model. *J. Surg. Res.,* 131: 283-289.
2. Reddy JS, Rajeswara R and Reddy MS (2002). Wound healing effects of *Heliotropium* indicum, *Plumbago zeylanicum* and *Acalypha indica* in rats. *J. Ethnopharmacol.,* 79: 249-251.
3. Sumitra M, Manikandana P and Suguna L. (2005). Efficacy of *Butea monosperma* on dermal wound healing in rats. *Int. J. Biochem. Cell Biol.,* 37: 566-573.
4. Verma VK, Dewangan H, Bais M, and Jaiswal V, 2011: "Potential wound healing activity of the ethanolic extract of *Solanum xanthocarpum* schrad and wendl leaves." *Pakistan Journal of Pharmaceutical Sciences,* 25:1; 189-194.
5. *Houghton PJ, Hylands PJ, Mensah AY, Hensel A, Deters AM. 2005. In vivo test and ethanopharmacological investigations: Wound healing as an example. J ethanopharmacol 100:100-7.*
6. Diwan, PV, Tillo LD and Kulkarni DR (1982). Influence of *Tridax procumbens* on wound healing. *Indian J. Med. Res.*, 75: 460-464.
7. Sunil Kumar, Parameshwara S and Shivakumar HG (1998). Evaluation of topical formulations of aqueous extract of *Centella asiatica* on open wounds in rats. *Indian J. Exp. Biol.,* 36: 569-572.
8. Shukla A, Rasik AM, Jain GK, Shankar R, Kulshrestha DK and Dhawan BN (1999). *In vitro* and *in vivo* wound healing activity of asiaticoside isolated from *Centella asiatica., J. Ethnopharmacol.,* 65: 1–11.
9. Suguna L, Sivakumar P and Chandrakasan G (1996). Effects of *Centella asoatica* extract on dermal wound healing in rats. *Indian J. Exp. Biol.*, 34: 1208-1211.
10. Stephen Y Gbedema, Kisseih Emelia, Adu Francis, Annan Kofi and Woode Eric (2010). Wound healing properties and kill kinetics of *Clerodendron splendens* G. Don, A Ghanaian wound healing plant. *Pharmacog. Res.,* 2(2): 63-68.
11. Udupa AL, Rao SG and Kulkarni DR (1989). Hound healing profile of septilin. *Indian J. Physiol. Pharmacol.*, 33: 39-42.
12. Perez Gutierrez RM and Vargas SR (2006). Evaluation of the wound healing properties of *Acalypha langiana* in diabetic rats. *Fitoterapia,* 77: 286-289.
13. Nagappa AN and Cheriyan Binu (2001). Wound healing activity of the aqueous extract of *Thespesia populnea* fruit. *Fitotherapia,* 72: 503-506.
14. Govindarajan R, Vijayakumar M, Rao CV, Shirwaikar A, Mehrotra S and Puspangadan P (2004). Healing potential of *Anogeissus latifolia* for dermal wound in rats. *Acta Pharm.,* 54: 331-338.
15. Saha K, Mukherjee PK, Das J, Pal M and Saha BP (1997). Wound healing activity of *Leucas la_ endulaefolia* Rees. *J. Ethnopharmacol.,* 56: 139-144.
16. Park EH and Chun MJ (2001). Wound healing activity of *Opuntia ficus–indica. Fitoterapia,* 72: 165-167.
17. Rasik AM, Raghubir R, Gupta A, Shukla A, Dubey MP, Srivastava S, Jain HK and Kulshrestha DK (1999). Healing potential of *Calotropis procera* on dermal wounds in guinea pigs. *J. Ethnopharmacol.,* 68: 261-266.
18. Mukherjee PK and Suresh B (2000). The evaluation of wound-healing potential of *Hypericum hookerianum* leaf and stem extracts. *J. Alter. Compl. Med.,* 6: 61-69.
19. Wall ME, Eddy CR, Mc Clenna ML, Klump ME,. 1952. Detection and estimation of steroids and sapogenins in plant tissue. Anal Chem 24:1337-42.
20. Harbone JB, Phytochemical methods: A guide to modern techniques of plant analysis London: Chapman and Hall; 1973.p.279.