Arun et al., Afr J Tradit Complement Altern Med. (2015) 12(3):135-142
http://dx.doi.org/10.4314/ajtcam.v12i3.17

EXPERIMENTAL WOUND HEALING ASPECTS OF *JASMINUM GRANDIFLORUM* LINN: A PRECLINICAL STUDY

Mittal Arun[1]*, Sardana Satish[1], Pandey Anima[2]

[1]Department of Pharmacognosy and Phytochemistry Hindu College of Pharmacy Sonipat, Haryana-131001, India.
[2]Birla Institute of Technology Mesra, Jharkhand-835215, India.
*Address for Correspondence Arun Mittal Hindu College of Pharmacy, Sonipat, Haryana-131001, India.
E mail: mittalarun07@rediffmail.com. 91-9354806431.

Abstract

Background: *Jasminum grandiflorum* is an important shrub of family Oleaceae and ancient literature mentions its various medicinal uses especially its efficacy in wound healing. To substantiate its traditional claim the present study was conceived.
Materials and Methods: Preliminary wound healing activity of successive extracts of leaves was assessed by measuring wound contraction using excision wound model and the detailed study of most potent extract was evaluated by measuring wound contraction, epithelialization period, hydroxyproline content, tissue breaking strength and histopathology parameters by excision and incision wound models.
The antioxidant activity was determined by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. The agar well-diffusion method and minimum inhibitory concentration (MIC) was used to established antimicrobial efficacy.
Results: The results show that successive ethanolic extract of the leaves exhibited promising wound healing, antioxidant and antimicrobial properties.
Conclusion: From results; it is concluded that its traditional use for the treatment of wound and in microbial infection is justified.

Key words: *Jasminum grandiflorum;* Wound healing; Hydroxyproline content; Antioxidant; Antimicrobial activity.

Introduction

Nature has showered rich sources of medicinal agents on humanity and as such, a remarkable number of modern drugs have been isolated from natural sources, based on their traditional uses as medicine. Over the years, plants like *Aloe, Cinchona bark* and *Digitalis* have enjoyed immense importance in indigenous medicinal systems (Trease and Evans, 1991).

Recently, advancement in science and technology, developing trends in orthodox medicine began and traditional medicine was side lined. However, in some rural areas, due to lack of appropriate and adequate health facilities, age-long medicinal systems are still extensively used to prevent and cure diseases.

Owing to the increasing unwanted effects of orthodox medicines, the inclination toward indigenous system is growing rapidly. Thus, significant rise in interest of scientific community towards exploration of hidden aspects of herbal medicines and the folklore claims of herbal drugs has been witnessed.

Based on ethno-botanical surveys, several species of plants and herbs with promising healing boon for wounds in addition to antioxidant and antimicrobial potential; are found to be widely distributed in India and extensively used by traditional healers. However, only a few of them have been investigated to substantiate their potential healing effect while others await the same. Therefore it is necessary to introduce a scientific validation for the medicinal effect of plants used in traditional medicine. From the literature survey, the *Jasminum grandiflorum* has been selected for evaluation in order to prove its claim in folklore medicine. Extracts from the dried or wet leaves and flowers of these plants were applied as paste on wounds in some rural communities (Warrier et al., 2004). The fresh juice from the leaves is employed in treating skin problems, dermatitis, eczema and cancer (Kirtikar and Basu, 2003).

Hence the present study was designed with special emphasis to investigate the wound healing activity of different extracts of *J. grandiflorum* leaves and also to find out the most bioactive extract, which was then used for detailed wound healing, antioxidant and antimicrobial activity.

The acute dermal toxicity study of the extracts of leaves of this plant was carried out to know the safety profile and for the selection of dose.

Materials and methods

Plant collection and authentication

Leaves were collected from the medicinal garden of Hindu College of Pharmacy, Sonepat and authenticated by Dr. H.B. Singh, Scientist F & Head, Raw Materials Herbarium & Museum, National Institute of Science Communication and Information Resources, New Delhi. A voucher specimen has been deposited at the NISCAIR Herbarium (NISCAIR/RHMD/Consult-2010-11/1627/225).

Preparation of extracts and phytochemical screening

Leaves were air dried in the shade and coarsely powdered. The extracts were prepared by successive solvent extraction method using petroleum ether (60-80^{o}C), chloroform, ethanol and purified water.

http://dx.doi.org/10.4314/ajtcam.v12i3.17

The extracts thus obtained were concentrated using rota-evaporator and the solvents recovered. The concentrated extracts were then evaporated to dryness, in vacuum oven at temperature not more than 50^{o}C. The dried extracts were stored at 2-8^{o}C in refrigerator. These extracts were then used during study.

All the extracts were tested qualitatively for various plant constituents by carrying out standard tests (Wagner et al., 1984; Brain and Turner, 1975).

Animals and microorganism

The healthy albino rats of either sex, weighing 150-200gms were housed under standard environmental conditions of temperature and humidity (25±0.5^{0}C) and 12 hr light/dark cycle. The animals were fed with standard pellet diet and water *ad libitum*. The animal studies were performed in the institute with due permission from Institutional Animal Ethics Committee (Reg No. 585/02/c/ CPCSEA India). Bacterial culture (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aureginosae, Micrococcus luteus* and *Escherichia coli*) and fungal culture (*Candida albicans and Aspergillus niger*) were procured from Microbial Type Culture Collection, Chandigarh, India.

Preparation of ointment

The ointment was prepared by using water soluble base i.e. PEG 4000 and PEG 400. PEG 4000 was melted on hot plate at 60^{o}C. PEG 400 was also warmed to the same temperature and then added to the melted PEG 4000. Stirred continuously till cooled to room temperature. Extracts were incorporated in the prepared ointment base (Yaduvanshi et al., 2011).

Acute dermal toxicity

The study was carried out to calculate the therapeutic dose. The limit test for acute dermal toxicity study was carried out in adult female albino rats by fixed dose method of OECD guidelines No. 434. The ointment was applied topically to the shaved area, at dose level 2000 mg/kg body weight (OECD guidelines, 2004).

Selection of dose

For the assessment of cutaneous wound healing activity, dose level was selected in such a way that the dose was approximately one tenth of the maximum tested dose during acute toxicity study (Nalwaya et al., 2009).

Experimental Protocol
Preliminary wound healing screening of extracts

By measuring the wound contraction using excision wound model; the most bioactive extract from various extracts was selected (Morton and Malone, 1972). For study the animals were divided into five groups of six animals each.

Group I: served as control treated with ointment base.

Group II, III, IV, V: received the application of successive petroleum ether, chloroform, ethanolic and aqueous extract ointments.

All the animals in each group were anaesthetized by open mask method using anesthetic ether. The rats were depilated at dorsal thoracic region and full thickness of skin (500mm^{2}) was cut off from a pre-determined area on the dorsal back of rats 1cm away from vertebral column and 5cm away from the ear. The wound area was measured immediately by placing a transparent paper upon the wound and tracing it out, area of this impression was calculated using 1mm graph sheet (Muthusamy et al., 2008).

The same protocol was followed, for measurement of area; every 3rd day till complete healing. The percentage wound contraction area was calculated. The area of wound at the time of wounding was considered as 100% and the wounding day was considered as day zero. The whole experimental protocol was approved by IAEC (Reg No. 585/02/c/CPCSEA India.

Wound healing activity

The detailed wound healing activity of most bioactive extract was evaluated by monitoring various parameters of excision and incision wound models.

The animals in both models were divided into three groups of six animals each.

Group I: Control group, received ointment base.

Group II: Standard group, treated with 0.2%w/w Nitrofurazone ointment.

Group III: Test group, treated with successive ethanolic extract (most bioactive extract) ointment.

The treatment was given topically once a day, starting from the wound induction till complete healing.

Excision wound model

The excision wound model was performed according to the method as described above. The various parameters such as percentage wound contraction, epithelialization period, scar area, hydroxyproline content and histopathology of granular tissues; were evaluated (Neuman and Logan, 1950; Nayak et al., 2007).

http://dx.doi.org/10.4314/ajtcam.v12i3.17

Incision wound model

The animals were grouped and treated as described in excision model. The animals were anesthetized under ether anesthesia. One full thickness paravertebral incision of 6 cm length was made through the skin, on either side of the vertebral column with the help of sterile scalpel (Ehrlich and Hunt, 1969; Udupa et al., 1994 b). After complete haemostasis, the parted skin was kept together and stitched with black silk surgical thread (no. 000) and a curved needle (no. 11) at 0.5cm intervals. The continuous threads on both wound edges were tightened for better closure of the wounds. The wounds of animals in different groups were treated with topical application of the ointment as described above for a period of 10 days. The wounding day was considered as day zero.

After thorough healing of wounds, the sutures were removed on the 8th post wounding day and the wound breaking strength was determined on the 10th day by continuous constant water flow technique (Lee, 1968). The hydroxyproline content of the healed tissue was also determined (Neuman and Logan, 1950).

Histopathology

For histopathology examination of the samples of healed skin tissue were taken from control, standard and treated groups of excision a

nd incision wound models (Shenoy et al., 2011).

Antioxidant activity

Total phenolic content estimation

Total phenolic content was determined by the Folin-Ciocalteu method (Djeridane et al., 2006). The extract (0.2 ml) was pipetted into a 10.0 ml volumetric flask containing 4.0 ml water. To this, 0.5 ml Folin-Ciocalteu's reagent and after 1 minute 2.0 ml of 20% aqueous solution of sodium carbonate was added. The volume was made up to 10.0 ml with distilled water. After 30 min, absorbance was measured at 760 nm against the reference solution. The results were expressed as mg gallic acid equivalents per gram of dry mass of extract (mg GAE/g).

DPPH free radical scavenging activity

The free radical-scavenging activity of successive ethanolic extract of *J. grandiflorum* was measured in terms of hydrogen donating or radical-scavenging ability using stable radical DPPH (Anna et al., 2012). A solution of DPPH (2, 2-diphenyl-1-picrylhydrazyl) was prepared in methanol and 4.0 ml of this solution was added to 1.0 ml of extract solution in methanol at different concentration (10-200µg/ml). Thirty minutes later, the absorbance was measured at 517nm. Lower absorbance of the reaction mixture indicates higher free radical- scavenging activity. Ascorbic acid was used as a standard antioxidant. The results were expressed as IC_{50} (inhibitory concentration 50) value i.e., concentration of samples exhibited 50% inhibition of DPPH radicals.

Antimicrobial activity

Sensitivity Test (Agar well diffusion method)

The successive ethanolic extract was dissolved in dimethyl sulphoxide and used at 50 mg/ml concentration. Molten nutrient agar medium (for bacteria) and Sabouraud dextrose agar medium (for fungus) were taken in sterile petridishes and allowed to solidify. Broth cultures of the test isolates (0.1 ml) containing 1.0×10^5 c.f.u./ml of organism were spread on the solidified media using sterile spreader. Each petridish was divided into three sectors and wells were bored in the sectors using a standard sterile cork borer of 8 mm diameter and equal volumes of the plant extract samples, standard agents and control solvent (0.1 ml) were poured into the wells using micropipette. The plates were kept for 1 hr for pre-diffusion and then incubated as per respective microorganisms. At the end of incubation period, zone of inhibition was measured in all the plates. Ciprofloxacin and fluconazole (100 µg/ml) were used as standard antibacterial and antifungal agents, respectively (Cappucino et al., 1996; Pandey et al., 2012; Vats et al., 2011).

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the successive ethanolic extracts was determined for each of the test microorganisms by serial dilution method. To 1 ml of varying concentrations of the extracts (25.0, 12.5, 6.25, 3.125, 1.562, 0.781 and 0.39 mg/ml), 1 ml of nutrient broth was added and then 0.1 ml of broth culture of test organism was introduced to the tubes.

The procedure was repeated on the test organisms using the standard antibiotics (ciprofloxacin for bacteria and fluconazole for fungal isolates). A tube containing nutrient broth alone was seeded with the test organisms as described above to serve as control.

Tubes were then incubated as per respective microorganisms. After incubation the assay tubes were removed, observed for any deposits, shaken to aerate the solution and to suspend microbes which may have settled at the bottom of the assay tubes. The lowest concentration of the extracts and the standard drug, which caused apparently a complete inhibition of the growth of micro-organism was taken as the minimum inhibitory concentration of that particular extract (Cappucino et al., 1996; Vats et al., 2011).

Statistical Analysis

The mean value ± SEM was calculated for each parameters. Results were statistically analyzed by one-way-analysis of variance (ANOVA) followed by Dunnet's t-test. $P< 0.05$ was considered as significant.

Results and Discussion

Repairing of injured tissues occurs as a sequence of events which include coagulation, inflammation, collagenation, wound contraction and epithelialization. While the phase between coagulation to collagenation is intimately inter-linked, the phase of wound contraction and epithelialization are independent to each other and run concurrently (Bairy and Rao, 2001). No single wound model thus, be sufficient to assess the influence of drugs on wound healing.

Therefore, in the present study two wound models viz., Excision wounds (for wound contraction and epithelialization phase) and Incision wound (for collagenation phases) were employed. The effect of extract on various parameters of wounds is discussed below:

Acute dermal toxicity study revealed that extracts of leaves were safe upto 2gm/kg body weight.

The preliminary wound healing screening of all the extracts showed that the successive ethanolic extract owed best prospective for wound contraction compared to other extracts (Graph. no.1). So, for the further detailed wound healing activity, this extract was selected.

Wound contraction is the major parameter used to assess wound healing. It has been suggested that wound contraction is fibroblast-dependant and involves the deposition and maturation of collagen (Gabbiani et al., 1971). Collagen not only confers strength and integrity to the wound matrix but also plays important role in haemostasis and in epithelialization at the later stage of healing (Raghow, 1994; Chithra et al., 1998b).

While measuring the wound contraction, it was observed that there was better healing pattern with complete wound closure on the 18th day in successive ethanolic extract treated group as compared to control group (Table 1).

Epithelialization is the process of restoring an intact epidermis after cutaneous injury (Clark, 1996). Falling of scab leaving no raw wound behind was taken as end point of complete epithelialization and the days required for this was taken as period of epithelialization (Shirwaikar et al., 2003). Shorter epithelialization period indicates more collagen content and hence better healing (Table 2). Hence successive ethanolic extract of *J. grandiflorum* promotes epithelialization either by facilitating the proliferation of epithelial cells or by increasing the viability of epithelial cells.

Table 1: Effect of Successive Ethanolic Extract of *J. grandiflorum* on excision wound model in rats

Treatment	Percentage Wound Contraction in Days							
	3	6	9	12	15	18	21	24
Control	1.90±0.19	10.42±0.38	21.94±0.73	37.32±0.55	57.53±0.87	74.96±0.81	90.59±0.94	100.00±0.00*
Standard	15.04±0.83*	36.69±0.85*	57.03±1.14*	72.93±1.50*	94.48±1.61*	100.00±0.00*	100.00±0.00*	100.00±0.00*
S.E.Extract	10.5±0.65*	31.97±0.81*	52.32±1.06*	68.22±1.45*	88.28±1.30*	100.00±0.00*	100.00±0.00*	100.00±0.00*

Values are represented as mean± SEM (n=6). Data was analysed by one-way Anova followed by Dunnett's test *p<0.05.

Table 2: Effects of Successive Ethanolic Extract on different parameters of excision and incision wound models

Treatment	Excision Wound Model			Incision Wound Model	
	Epithelialization Period(days)	Scar Area (mm^2)	Hydroxyproline Content(µg/mg)	Hydroxyproline Content(µg/mg)	Wound Breaking Strength
Control	22.83±0.40	23.83±1.27	79.48±0.95	61.37±0.74	144.7±0.25
Standard	16.33±0.21*	19.50±0.76*	145.4±1.02*	138.30±1.07*	195.0±1.46*
S.E.Extract	17.33±0.33*	20.17±1.22*	143.2±0.89*	136.6±0.69*	185.8±2.00*

Values are represented as mean± SEM (n=6). Data was analysed by one-way Anova followed by Dunnett's test *p<0.05

Table 3: Study of various histological parameters

WoundModel	Treatment	Parameters of Histology			
		Keratinization	Epithelialization	Collagenization	Neovascularisation
Excision	Control	Present Mild	Present Complete	-	-
	Standard	Present Mild	Present Complete	Present Marked	Present Mild
	S.E.Extract	Present	Present Complete	Present Marked	Present Mild
Incision	Control	Present Moderate	Present Complete	Present Mild	-
	Standard	Present Moderate	Present Complete	Present Marked	Present Marked
	S.E.Extract	Present Mild	Present Complete	Present Marked	Present Moderate

http://dx.doi.org/10.4314/ajtcam.v12i3.17

Table 4: Zone inhibition and minimum inhibitory concentration effects of successive ethanolic extracts of *J. grandiflorum* on microbes

Micro-organism	Zone of Inhibition in mm		Minimum inhibitory concentration	
	Test Drug (mg/ml)	Standard Drug(µg/ml)	Test Drug (mg/ml)	Standard Drug(µg/ml)
Bacillus subtilis	14.87±0.9	16.96±1.8	1.56	2.5
Staphyloccocus aureus	13.62±1.1	15.67±2.22	3.12	2.5
Pseudomonas aeruginosa	18.60±2.1	24.33±3.01	6.25	1.25
Micrococcus luteus	15.77±1.8	17.21±2.49	0.78	2.5
Escherichia coli	18.33±1.6	19.67±2.11	0.78	2.5
Candida albicans	----	16.77±1.11	-----	2.5
Aspergillius niger	----	17.43±1.19	-----	2.5

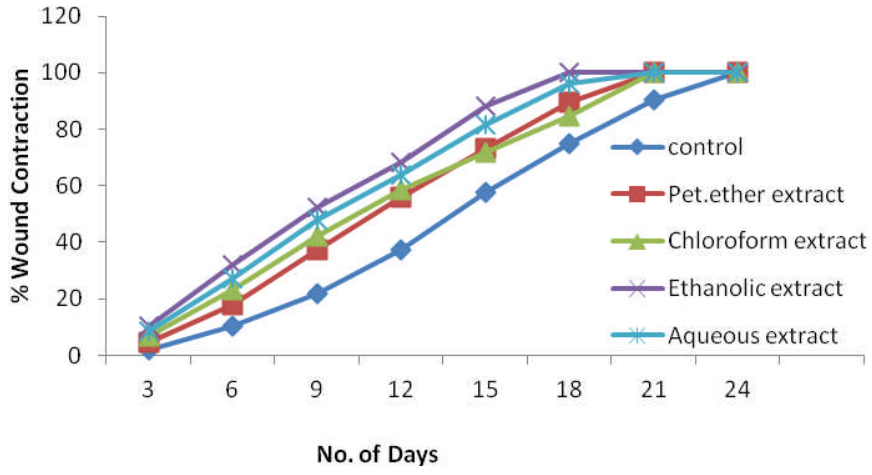
All values are expressed as mean± SEM (n=3). (Ciprofloxacin and Fluconazole are the standards for bacteria and fungus, respectively).

Table 5: *In-vitro* antioxidant activity of successive ethanolic extract of *J. grandiflorum*

Sample	Total Phenolic content(mg GAE/gm)	DPPH radical scavenging activity(IC_{50} value µg/ml)
Successive ethanolic extract of *J.grandiflorum*	21.94±0.74	33.62±0.52
Standard(Ascorbic acid)	-------	35.41±0.66

All values are expressed as mean± SEM, n=3, IC_{50}= Inhibitory concentration50, DPPH= 2,2-diphenyl-1-picrylhydrazyl

Graph 1: Effect of different extracts of *J. grandiflorum* on excision wound expressed as percentage of wound contraction
Values are represented as mean± SEM (n=6). Data was analysed by one-way Anova followed by Dunnett's test *p<0.05.



Scar area is the measurement of the entire healed wound area after falling of eschar leaving no residual wound behind. Scar area will be less if there is faster wound contraction due to increased collagen content (Krishnan et al., 2011). Scar area was found to be lesser in the group treated with successive ethanolic extract compared to control (Table 2).

Hydroxyproline is an amino acid, one of the major constituents of collagen and found in collagen fibers of granular tissue. Breakdown of collagen liberates free hydroxyproline and its peptides. So the estimation of hydroxyproline is an accepted method of biochemically evaluating the total collagen content of a sample and also used as a marker of collagen synthesis (Lin et al., 2003; Rasik et al., 1992).

A biochemical analysis of wound tissue in both the models demonstrated a significant increase in hydroxyproline content in successive ethanolic extract treated wounds compared to controlled wounds (Table 2). Therefore higher concentration of hydroxyproline in *J.grandiflorum* successive ethanolic extract treated group indicates that there is replacement of granulation tissue in the wound area by collagen.

Tensile strength is the amount of force required to open the healed skin and it represents the degree of wound healing. It has commonly been associated with the organization, content and physical properties of collagen fibril network.

By carrying out the determination, it was found that successive ethanolic extract treated incised wound exhibited an increased skin breaking strength compared to control (Table 2). It indicates increased collagen concentration and stabilization of fibers (Udupa et al., 1995).

The above results are further supported by histo-pathological evidences i.e. enhanced wound contraction in extract treated wounds compared to control (as shown in Table 3 and Figure 1-6). Thus the Successive ethanolic extract of leaves promotes wound healing by increased cell proliferation and collagen deposition.

As per literature, it was found that wound healing takes place in different stages, but some bacteria like *S. aureus, E. coli. P. auregenosa* and *Bacillus spp*. will delay the healing by showing their effects at inflammatory phase (Subramoniam et al., 2001). This delay will also causes generation of reactive oxygen species. Over production of reactive oxygen species result in oxidative stress thereby causing cyto-toxicity and delaying wound healing (Dissemond et al., 2002). Hence estimation of both antioxidant and antimicrobial property of extract is relevant because these agents have synergetic effects on wound healing by destroying the free radicals and preventing the delaying of angiogenesis. From the result it was clear that successive ethanolic extract of leaves possess good antimicrobial and antioxidant effects (Tables 4, 5).

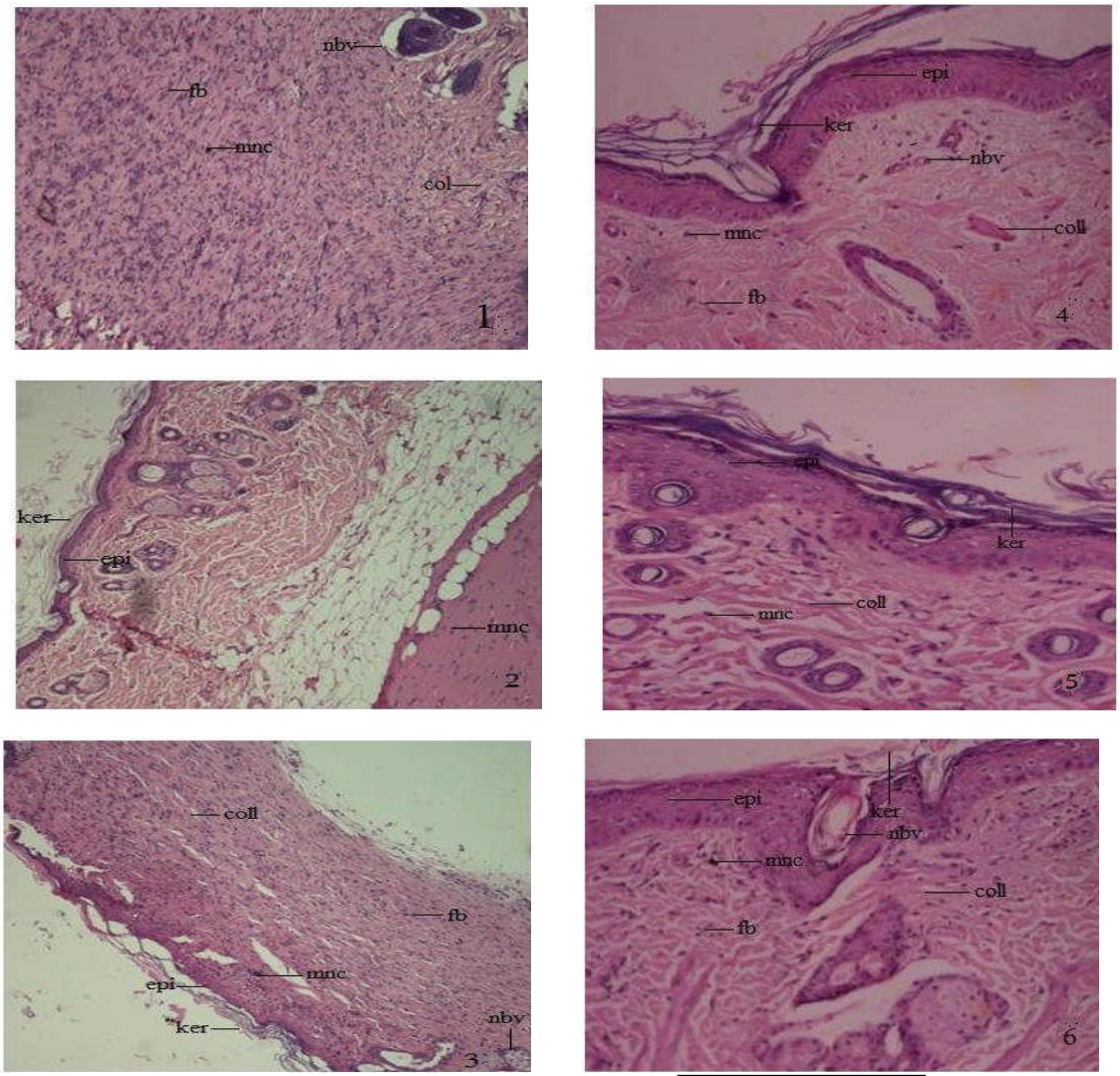


Figure 1: Standard drug; Figure 2: Control drug , Figure 3: Successive ethanolic extract of *J. grandiflorum* ; Figure 4: Standard drug
Figure 5: Control drug ; Figure 6: Successive ethanolic extract of *J. grandiflorum*
Figure 1,2,3 are of excision wound and Figure 4,5,6 are of incision wound.
where as fb: Fibroblast cell; mnc: Mononucleated cell; epi: Epithelilization; ker: Keratinization; nbv: Newblood vessel; coll: Collagen.

Active principles of the plant like flavonoids, triterpenoids, alkaloids, steroids, tannins and other bio-molecules promotes the process of wound healing . These agents usually influence one or more phase of healing process (Udupa et al., 1995). Our preliminary phytochemical analysis revealed the presence of flavonoids, triterpenoids, steroids, alkaloids, saponins, tannins and phenolic compounds in successive ethanolic extract. Flavonoid, Triterpenoids and Tannins are known to promote the wound-healing process mainly due to their astringent, anti-microbial and free radical

scavenging, which seems to be responsible for wound contraction and increased rate of epithelialization (Tsuchiya et al., 1996; Scortichini et al., 1991; Chaudhari et al 2006; Mittal et al., 2013).

Whereas sterols are also responsible for wound healing by increasing collagen content and degree of collagen-cross linkage within the wound, thereby promotes cell division, growth of bone, cartilage and other connective tissues (Megha et al., 2012).

These results demonstrate that successive ethanolic extract *of J.grandiflorum* shows wound healing property partly by increasing the collagen synthesis, probably due to the presence of useful mixture of phytoconstituents in the leaves and also due to antioxidant and antimicrobial activity.

Conclusion

It was concluded that all successive extracts of *J. grandiflorum* have wound healing activity irrespective of how early wound contraction, lesser epithelialization period, increased tensile strength, increased collagenation, histopathology, antioxidant, antimicrobial and phytochemical analysis support that successive ethanolic extract shows remarkable effect. Further study with purified constituents of *Jasminum grndiflorum* needs to be performed to understand the complete mechanism of wound healing potential and constituents responsible for same.

References

1. Anna, G.G., Marlena, D.M. and Irena, M. (2012). DPPH radical scavenging activity and Phenolic compound content in different leaf extracts from selected Blackberry species. Acta Biologica Cracoviensia Series Botanica 54(2):32–38.
2. Bairy, K.L. and Rao, C.M. (2001). Wound healing profiles of *Ginkgo biloba*. J. Nat. Remedies 1: 25.
3. Brain, K.R. and Turner, T.D.(1975) The Practical Evaluation of Phytopharmaceuticals. Bristol, Wright- Scientechnica, p.4
4. Cappucino, J.G. and Sherman, N. (1996). Microbiology: A Laboratory Manual, fourth ed. Addison-Wiley Longman, England, p. 263.
5. Chaudhari, M. and Mengi, S. (2006). Evaluation of phytoconstituents of *Terminalia arjuna* for wound healing activity in rats. Phytother. Res.. 20: 799.
6. Chithra, P., Sajithlal, G.B. and Chandrakasan, G. (1998b). Influence of *Aloe vera* on the glycosamino-glycans in the matrix of healing dermal wounds in rats. J Ethnopharmacol. 59: 179.
7. Clark, R.A.F. (1996). Wound repair: Overview and general consideration in the molecular and cellular biology of wound repair. edited by Clark, R.A. and Henson P.M. Plenum Press, New York. p.3.
8. Dissemond, J., Goos, M. and Wagner, S.N. (2002). The role of oxidative stress in the pathogenesis and therapy of chronic wounds. Hautarzt. 53:718.
9. Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P. and Vidal N. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compound. Food Chemistry. 97: 654–660.
10. Ehrlich, H.P. and Hunt, T.K. (1969). Effect of anabolic steroids on tensile strength of a healing wound. Ann. Surg. 170: 203.
11. Gabbiani, G., Ryan, G.B. and Majno, G. (1971). Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. Experientia. 27: 549.
12. Jha, M., Sharma, V. and Ganesh, N. (2012). Antioxidant and wound healing potential of *Pistia stratiotes* L. Asian Pacific J. of Tropical disease. S579-S584.
13. Kirtikar, K.R. and Basu, B. D. (2003). Indian Medicinal Plants, second ed. Popular Prakashan, Bombay, Vol.7, p. 2093.
14. Krishnan, S.N., Kumar, P.S., Usha, V. and Mohideen, S. (2011). Evaluation of wound healing activity of *Sida spinosa* in rats. Pharmacologyonline.3: 1017.
15. Lee, K.H. (1968). Studies on the mechanism of action of salicylates II. Effects of vitamin A on wound healing retardation action of aspirin. J. Pharm. Sci. 57: 1238.
16. Lin, Z.Q., Kondo, T., Ishida, Y., Takayasu, T. and Mukaida, N. (2003). Essential involvement of IL-6 in the skin wound healing process as evidenced by delayed wound healing. J. Leukocyte Bio. 73: 713.
17. Mittal, A., Sardana, S. and Pandey, A. (2013). Herbal boon for wounds. Int. J. Pharmacy and Pharma. Sci. 5: 1.
18. Morton, J.J.P. and Malone, M.H. (1972). Evaluation of vulnerary activity by an open wound procedure in rats. Arch. Int. Pharmacodyn. Therap.196: 117.
19. Muthusamy, S.K., Kirubanandan, S., Sripriya. and Sehgal, P.K. (2008). Triphala promotes healing of infected full-thickness dermal wound. J. Surg. Res.144: 94.
20. Nalwaya, N., Pokharna, G., Deb, L. and Jain, N.K. (2009). Wound healing activity of latex of *Calotropis gigantea*. Int. J. Pharmacy and Pharm. Sci.1: 176.
21. Nayak, B.S., Anderson, M. and Pereire, P. (2007) Evaluation of wound healing potential of *Catharanthus roseus* leaf extract in rats. Fitoterpia.78: 540.
22. Neuman, R.E. and Logan, M.A. (1950). The determination of collagen and elastin in tissues. J. Biochem. 186: 549.
23. OECD guidelines for testing of chemicals. (2004). Acute Dermal Toxicity- 434, 1.
24. Pandey, A., Mittal, A., Gupta, A.K. and Sardana, S. (2012). Antimicrobial activity of leaves of *Cassia occidentalis.* Linn. Int. R. J. Pharm. Sci. 3(1): 27-28.
25. Raghow, R. (1994). The role of extracellular matrix on post-inflammatory wound healing and fibrosis. FASEB J. 8: 823.
26. Rasik, A.M., Raghubir, R., Gupta, A., Shukla, A., Dubey, M.P., Srivastava, S. and Jain, H.K. (1992). Healing potential of *Calotropis procera* on dermal wounds in guinea pigs. J. Ethnopharmacol. 68: 261.
27. Scortichini, M. and Pia, R.M. (1991). Preliminary *in-vitro* evaluation of the antimicrobial activity of terpenes and terpenoids towards *Erwinia amylovora* (Burrill). J. Applied Bacteriol.71: 109.

http://dx.doi.org/10.4314/ajtcam.v12i3.17
28. Shenoy, R.R., Sudheendra, A.T., Nayak, P.G. and Paul, P. (2011) Normal and delayed wound healing is improved by sesamol, an active constituent of *Sesamum indicum* Linn. in albino rats. J. Ethnopharmacol.133: 608.
29. Shirwaikar, A., Somashekar, A.P., Udupa, A.L., Udupa, S.L. and Somashekar, S. (2003). Wound healing studies of *Aristolochia bracteolate* Lam. with supportive action of antioxidants enzymes. Phytomedicine.10: 558.
30. Subramoniam, A., Evans, D.A., Rajasekharan, S. and Nair, G.S.(2001). Effects of *Hemigraphis colorata* (Blume) H.G. Hallier leaf on wound healing and inflammation in mice. Indian J. Pharmacol.33: 283-285.
31. Trease, G.E. and Evans, W. C. (1991). A Text book of Pharmacognosy. ELBS Baillere Tindal, London, p.1-10.
32. Tsuchiya, H., Sato, M., Miyazaki, T., Fujiwara, S., Tanigaki, S. and Ohyama, M. (1996). Comparative study on the antibacterial activity of phyto-chemical flavones against methicillin-resistant *S. aureus.* J. Ethnopharmacol.50: 27.
33. Udupa, A.L., Kulkarni, D.R. and Udupa, S.L. (1995). Effect of *Tridax procumbens* extracts on wound healing. Int. J. Pharmacol. 33: 37.
34. Udupa, S.L., Udupa, A.L. and Kulkarni, D.R. (1994b). Studies on the anti-inflammatory and wound healing properties of *Moringa oleifera* and *Aegle marmelos.* Fitoterapia.65: 119.
35. Vats, M., Singh, H. and Sardana, S. (2011). Phytochemical screening and antimicrobial activity of roots of *Murraya koenigii* (Linn.) Spreng. (Rutaceae). Brazilian J. Microbiol.42: 1569-1573.
36. Warrier, P.K., Nambiar, V.P.K and Ramankutty. (2004). Indian Medicinal Plants- A Compendium of 500 Species, Orient Longman Pvt. Ltd, Chennai. Vol. 3, 249.
37. Yaduvanshi, B., Mathur, R., Mathur, S.R. and Velpandian, T. (2011). Evaluation of wound healing potential of topical formulation of leaf juice of *Tridax procmbens* L. in mice. Indian J. Pharm. Sci. 73(3): 303-306.