International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 3, 2011

Research Article

# EVALUATION OF THE WOUND HEALING EFFECT OF HERBAL OINTMENT FORMULATED WITH *SALVIA SPLENDENS* (SCARLET SAGE)

**SAILESH NARAYAN[1*], D. SASMAL[2], PAPIYA MITRA MAZUMDER[2]**

**Sagar Institute of Pharmacy and Technology , Gandhi Nagar, Near Airport, Bhopal.(M.P.), Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi.(JH.) Email: saileshcology@yahoo.co.in**

*Received: 02 April 2011, Revised and Accepted: 03 May 2011*

**ABSTRACT**

The evaluation of wound healing effect of herbal ointment formulated with *Salvia splendens* (Scarlet Sage) embedded in the ointment bases having strength of 5%, 10% ,and 15% which have been evaluated in vivo using the excision wound healing model, incision wound healing model and various biochemical parameters like Collagen, Mucopolysaccarides,DNA and Proteins. The evaluation of wound healing is compared with that of Nitrofurazone ointment(0.2% w/w). *Salvia splendens* was extracted using methanol and the extract formulated as herbal ointments. The herbal ointments were used to treat wounds inflicted on experimental Albino Mice. The wound healing effect of the formulations were compared to that of a standard antibiotic Nitrofurazone. In all cases, there as a progressive decrease in wound area with time, indicating the efficacy of the formulations in healing the induced wounds. By the 18th day, the ointment containing 15% w/w of *Salvia splendens* in ointment base showed 100% healing. The wound areas in the animals treated with the standard Antibiotics, Nitrofurazone showed a 100% healing by the 18th day, indicating that the plant extract, at that given concentration, had a better wound healing property than the standard antibiotic. The granulation tissue weight and hydroxyproline content in the dead space wounds were also increased significantly, in the herbal ointment formulated with *Salvia splendens* in the treated animals as compared with the standard antibiotic Nitrofurazone treated animals.

**Keywords:** Wounds, Healing, Herbal ointment, *Salvia splendens*, Nitrofurazone (0.2% w/w)

## INTRODUCTION

Various plant species have served as a source of medicine for people all over the world, for year's plant is one of the most intense areas of natural product research yet the field is far from being exhausted .Plants and their extracts have immense potential for the management and treatment of wound. The phytomedicine for wound healing are not only cheap and affordable but are also purportedly safe as hypersensitive reactions are rarely encountered with the use of these agents. These natural agents induce healing and regeneration of the tissue by multiple mechanisms, however, there is need for scientific validation, standardization and safety evaluation of plants of traditional medicine before they could be recommended for healing of wounds.[1]

Wound healing, or wound repair, is an intricate process in which the skin (or another organ-tissue) repairs itself after injury.[1] In normal skin, the epidermis (outermost layer) and dermis (inner or deeper layer) exists in a steady-state equilibrium, forming a protective barrier against the external environment. Wounds are unavoidable events of life; wounds may arise due to any agent that induces stress & injury and their healing has been one of the well-known problems. Healing is a survival mechanism and represents an attempt to maintain normal anatomical structure and function. Treatment is therefore aimed at minimizing the undesired consequences. Management of under healing of wounds is a complicated and expensive program and research on drugs that increase wound healing is a developing area in modern biomedical sciences. Several drugs obtained from plant sources are known to increase the healing of different types of wounds. Some of these drugs have been screened scientifically for evaluation of their wound healing activity in different pharmacological models, but the potential of many of the traditionally used herbal agents remains unexplored. In few cases, active chemical constituents were identified.[2] Hence, there is dearth of rational pro-healing agents for the wound management programme, which can hasten the healing process.

*Salvia Splendens (Scarlet Sage or Red Salvia) family Lamiaceae,* is one of the Ornamental plant, having medicinal value which has not been fully studied scientifically. The plant is found in Brazil and in India. Traditionally, the leaves are used for the treatment of wounds,ulcer and also applied for itchy skin. The roots are used as cold and cough. The seeds of the plant is useful in emetic, dysentry, Haemorroids and colic disorders. It has been reported that the leaves contain essential oil are Salviorin A, α-pinene, β-pinene, linalyl, thujone, camphor, borneol and bornyl acetete. A large number of mono- and sesquiterpenoids and small amount of triterpenoids and steroids.

Even though, traditionally, leaves of *Salvia splendens (Scarlet Sage)* were extensively used for the treatment of variety of wounds; however, no scientific data in its support is available. The present study was undertaken to ascertain the effect of hydroalcoholic extract of *Salvia splendens (Scarlet Sage)* leaves on experimentally induced wounds in rats.[3,4,5,6,7,8,9,10,12,13,14,15,16,17]

## MATERIALS AND METHODS

### Collection, Authentication and Preparation of plant sample

The leaves of *Salvia splendens* ( Lamiaceae) was collected from Meccon Nursery, Lac Research Center, Namkum, Hazaribag Nursery of Hazaribagh and Ranchi Districts.The plant were authenticated by ***National Herbarium, Botanical Survey of India***, Shibpur, Botanical Garden, Kolkata. The voucher specimen was submitted in the Department of Pharmaceutical Sciences, B.I.T., Mesra.(Herbarium no. CNH/I-I(44)/2006/Tech.II)

The leaves of *Salvia splendens* were dried in shade for about a week followed by drying at $30^0$C – $40^0$C in oven for a day. The leaves were then grinded to coarse powder in an iron Motar and Pestle. The powdered materials was passed from sieve no. 20 . Finally, the powder is used for extraction.

### Preparation of extract

Dried leaves of *Salvia splendens* ( about 60 grams) will be extracted with 300 ml Methanol (80%) in a soxhlet apparatus for 72 hrs. After extraction, the solvent will be filtered and then evaporated under reduced pressure.

Total yield =0.341g

### Ointment preparation for topical application

An alcohol free extract of *Salvia splendens* leaf extract was used for the preparation of the ointment for topical application.[18] A 10% (w/w) and 15% (w/w) of extract ointment was formulated using soft white paraffin base.

**Chemicals**

All chemicals and reagents used were of analytical grade.

**Experimental animals**

Albino rats (*Rattus novergicus*) of 140±20g body weight and were used in study. Animals were procured from Laboratory Animal house (Reg no. 621/02/ac/CPCSEA) of Birla Institute of Technology, Mesra. All animals were kept in polyacrylic cages and maintained under standard housing conditions (room temperature 24-27$^0$C and humidity 60-65% with 12:12 light: dark cycles. Food was provided in the form of dry pellets and water ad libitum. All experiments involving animals complies with the ethical standards of animal handling and approved by Institutional Animal Ethics Committee.

**Model design**

The design of wound healing activity was performed by two models-

1) Excision Wound Model
2) Incision Wound Model

**1) Excision wound model[19-22]**

The back of each rat was shaved under Pentobarbitone (4mg/kg) anesthesia and prepared for operation. Thereafter open circular wound of 500mm$^2$ area was produced in each rat by excising the skin. For this purpose a marker was used to mark the area to be excised. The wounded animals were kept separately. Rats wound were left undressed to the open environment, this model was used to monitor wound contraction and epithelisation time. The standard drug (0.2% w/w nitrofurazone ointment), simple ointment; methanolic extract ointment 10%w/w and 15%w/w of leaves of *Salvia splendens* were applied everyday till the wound was completely healed.(Table no.1, Fig. 2)

**Measurement of wound area:[23]**

The progressive changes in wound area were measured planimetrically by tracing the wound margin on a graph paper every alternate day. The changes in healing of wound i.e the measurement of wound on graph paper was expressed as unit (mm$^2$).Wound contraction was expressed as percentage reduction of original wound size.

$$\% \text{ Wound Contraction} = \frac{\text{Healed Area}}{\text{Total Area}} \times 100$$

**Method**

30 albino rats were used for excision wound model and the ointment is applied topically and animal were divided into following groups:

Group- I : No ointment was applied and served as control.

Group- II : Ointment base was applied and served as vehicle control.

Group- III : 10%w/w ointment was applied once daily.

Group- IV : 15%w/w ointment was applied once daily.

Group- V : Nitrofurazone ointment (0.2%w/w) was applied once daily

Six animals were taken in each groups. All the above mentioned treatments were started from the day of operation and continued till the 20$^{th}$ day of healing. On 2$^{nd}$, 4$^{th}$, 8$^{th}$ , 10$^{th}$ , 12$^{th}$ , 14$^{th}$ , 16$^{th}$ , 18$^{th}$ and 20$^{th}$ days the wound area of each rat was traced on a graph paper and measured with the help of planimeter.[23]

**Biochemical analysis of wound tissue[24,25,26]**

The animals were anaesthetize on 4$^{th}$, 8$^{th}$ and 12$^{th}$ day after treatment. Then, the granulation tissue were removed from each wound and tissue was divided into two parts for following analysis:

♦Estimation of mucoplysacharides (Table 2) and collagen (Table 3)

♦Estimation of DNA (Table 4)

**2) Incision wound model[27,28]**

A 6 cm long abdominal incision was made in shaved area of anaesthetized rat and closed with interrupted sutures at a distance of 1cm. Thereafter they were kept individually in different cages.

**Method**

30 albino rats were used and an incision wound was made. These animals were divided into following groups:

Group- I : No ointment was applied locally and served as controls.

Group- II : Ointment base was applied once daily vehicle control.

Group- III : 10%w/w ointment was applied once daily.

Group- IV : 15%w/w ointment was applied once daily.

Group- V : Nitrofurazone (0.2%w/w) ointment was applied once daily

Six animals were taken in each group. On the 10$^{th}$ day the animals were sacrificed and there tensile strength was measured as follows: After sacrificing the animals after anaesthesia, sutures were gently pulled out. Both wound areas from each animal were removed carefully. Wound stripes of equal size (width) were then cut using a knife in which two blades were fixed at a fixed distance. Both ends of each strip were fixed with the help of a pair of steel clips. One clip allowed hanging on a stand and a polyethylene bottle was then allowed to fill with water gradually till the wound strip was broken at the site of wound. The amount of water required to break the wound was noted and expressed as tensile strength of wound in gms. (Table 5, Fig. 1)

**Fig. 1: View of incision wound model**

**Table 1: Evaluation of *Salvia splendens* leaves extract and Nitrofurazone ointment on**

| Post wounding days | Wound area (mm²) (mean±SE) and percentage of wound contraction | | | |
|---|---|---|---|---|
| | Simple ointment | Nitrofurazone ointment | *Salvia* extract 10%w/w ointment | *Salvia* extract 15%w/w ointment |
| 0 | 526±3.1 | 512±2.7 | 531±2.9 | 522±4.2 |
| 2 | 438±2.2 (16.7%) | 414±14.1 (19.1%) | 426±1.7 (19.80%) | 407±2.2 (22.00%) |
| 4 | 392±3.4 (25.5%) | 306±2.6** (40.20%) | 352±1.6 (33.70%) | 331±5.1* (36.60%) |
| 6 | 314±4.2 (35.2%) | 233±2.8 ** (54.50%) | 293±3.3* (44.80%) | 246±2.8* (52.9%) |
| 8 | 306±3.9 (41.8%) | 189±1.6** (63.10%) | 228±4.3* (57.1%) | 176±5.2** (66.3%) |
| 10 | 289±0.8 (45.1%) | 108±2.2 ** (78.90%) | 165±1.7** (68.9%) | 117±3.6** (77.60%) |
| 12 | 268±2.7 (49.0%) | 64±1.8 ** (87.50%) | 128±3.4** (75.9%) | 73±1.8** (86.00%) |
| 14 | 242±1.6 (54.0%) | 30±2.2 ** (94.10%) | 82±1.4** (84.50%) | 34±2.1** (93.50%) |
| 16 | 218±0.8 (58.5%) | 8±0.2 ** (98.40%) | 36±1.1** (93.20%) | 11±0.1** (97.90%) |
| 18 | 196±2.4 (62.7%) | 00±00** (100%) | 12±0.8** (97.7%) | 00±00** (100%) |
| 20 | 187±3.6 (64.4%) | 00±00* (100%) | 00±00** (100%) | 00±00** (100%) |

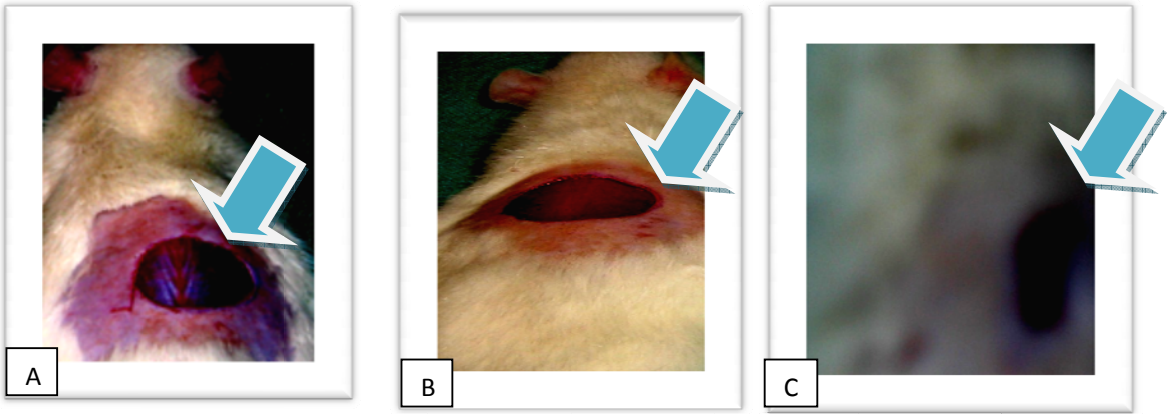



**Fig. 2: Excision wound model--- A. Day 0 (Control); B.Day 0 (Treated); C.Methnolic extract (after 18 days)**

**Table 2: Effect of *Salvia* extract (10% and 15%w/w) ointment and Nitrofurazone (0.2%w/w) on mucopolysacride content of granulation tissue**

| Treatment | Mucopolysaccride content in granulation tissue on post wounding day (Mean+SEM) | | |
|---|---|---|---|
| | 4th | 8th | 12th |
| Vehicle(simple ointment) | 0.92±0.11 | 0.73±0.06 | 0.5±0.18 |
| Nitrofurazone(0.2%w/w ointment) | 1.81±0.02[e] | 0.97±0.03[d] | 0.81±0.005[a] |
| Salvia extract (10% ointment) | 1.21±0.02[c] | 0.61±0.02[b] | 0.53±0.02 |
| Salvia extract (15% ointment) | 1.43±0.01[e] | 0.68±0.01[a] | 0.67±0.01[a] |

Result were statistically significant compared with the corresponding control values; (simple ointment) and P-values were calculated by student's t-test (n=6); [a]P<0.5, [b]P<0.1, [c]P<0.05, [d]P<0.01, [e]P<0.001

**Table 3: Effect of *Salvia* extract (10% and 15%w/w) ointment and Nitrofurazone (0.2%w/w) ointment on collagen content of granulation tissue**

| Treatment | Collagen content in granulation tissue on post wounding day (Mean+SEM) | | |
|---|---|---|---|
| | 4th | 8th | 12th |
| Vehicle(ointment base) | 2.1±0.24 | 3.36±0.13 | 3.93±0.13 |
| Nitrofurazone (0.2%w/w ointment) | 3.57±0.11**** | 6.49±0.07**** | 8.0±0.07**** |
| Salvia extract (10%w/w ointment) | 2.23±0.18 | 5.53±0.03** | 4.53±0.03**** |
| Salvia extract (15%w/w ointment) | 3.0±0.05*** | 5.92±0.06**** | 5.1±0.06**** |

Result were statistically significant compared with the corresponding control values (simple ointment) and P-values were calculated by student's t-test (n=6) *P<0.5, **P<0.02, ***P<0.01,****P<0.001

**Table 4: Effect of *Salvia* extract (10% and 15%w/w) and Nitrofurazone (0.2%w/w) ointment on DNA content of granulation tissue**

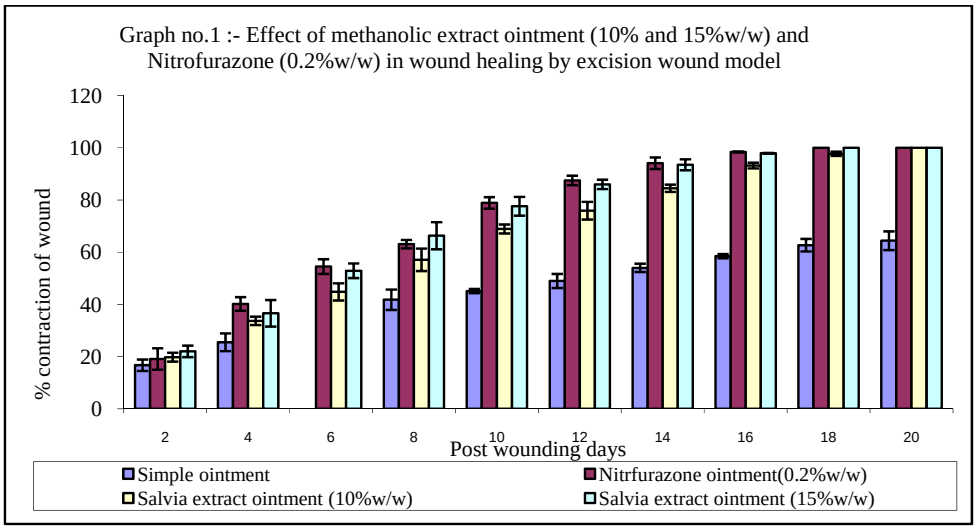
| Treatment | DNA content in granulation tissue on post wounding day (Mean+SEM) | | |
|---|---|---|---|
| | 4th | 8th | 12th |
| Vehicle (ointment base) | 5.15±0.13 | 4.69±0.17 | 2.02±0.05 |
| Nitrofurazone (0.2%w/w ointment) | 9.83±0.12** | 6.76±0.07** | 3.33±0.10** |
| Salvia extract (10%w/w ointment) | 6.25±0.02** | 5.08±0.04* | 2.53±0.07** |
| Salvia extract (15%w/w ointment) | 7.18±0.04** | 6.16±0.02** | 3.26±0.10** |

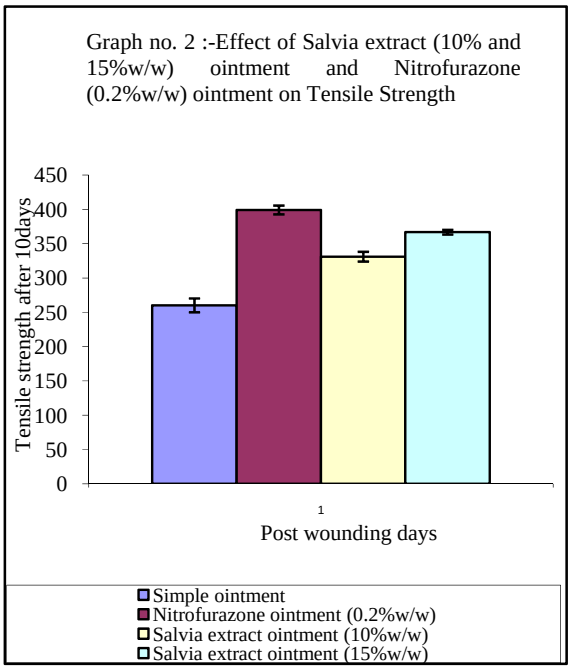
Result were statistically significant compared compared with the corresponding control (simple ointment) and P-values were calculated by student's t-test (n=6) *P<0.05, **P<0.001,

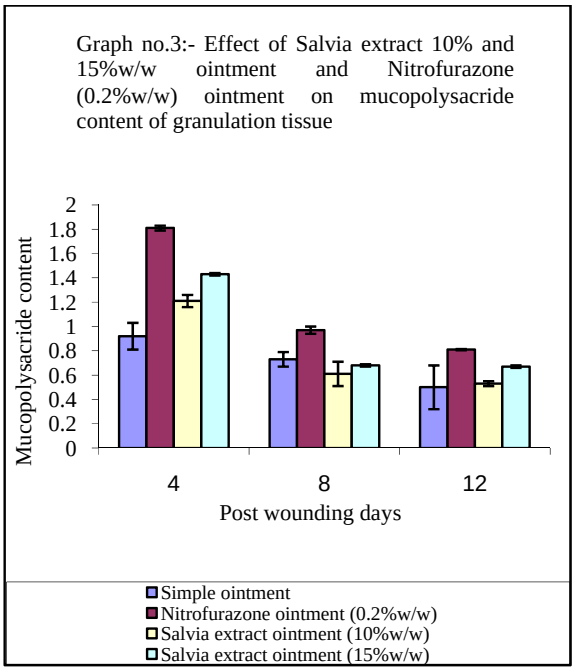
**Table 5:Effect of *Salvia* extract(10% and 15%w/w)ointment and Nitrofurazone (0.2%w/w) ointment on Tensile Strength**

| Treatment | Tensile strength after 10 days of incision wound (Mean+SEM) |
|---|---|
| Control (simple ointment base ) | 260.16±10.11 |
| Nitrofurazone (0.2%w/w) ointment | 399.16±6.45* |
| Salvia extract (10%w/w) ointment | 331.16±7.14* |
| Salvia extract (15%w/w) ointment | 366.83±3.41* |

Result were statistically significant compared with the corresponding control (simple ointment) and P-values were calculated by student's t-test (n=6) *P<0.001




## RESULTS

### Wound healing by excision wound method in rats

Result were statistically significant compared with the corresponding control values (simple ointment) and P-values were calculated by student's t-test (n=6) *P<0.01, **P<0.001

### Effect of topical application of leaves of *Salvia* extract (10% and 15%w/w) on Excision and Incision wound model

For excision wound model the effect of topical treatment of extract at 10% and 15% w/w ointment showed, the significant (P<0.01) increase in the contraction rate of animals treated as compared with control on all days of the treatment.(Table 1, Graph 1). where as the tensile strength of incised wound on 10th day of wounding, were

treated with extract (10% and 15% w/w) was significant at (P<0.001) as compared with control (Table 5, Graph 2).

**Biochemical studies**

**Effect of topical application of extract (10% and 15% w/w) on Mucopolysaccharide content of granulation tissue.**

The topical treatment group of 10% and 15% w/w was significant at P<0.05 and P<0.001, the amount of hexosamine content of granulation tissue on 4th day increases as compared to control.

**Effect of topical application of extract (10% and 15% w/w) on Collagen content of granulation tissue.**

The effect of topical application of extract 10% and 15% w/w was highly significant at P<0.001, the content of collagen increases on 12th day as compared to control (Table no.3, Graph 3)

**Effect of topical application of extract (10% and 15% w/w) on DNA content of granulation tissue.**

Effect of topical treatment of extract 10% and 15% w/w on DNA content of the granulation tissue was highly significant (P<0.001) on the 4th day other than 8th and 12th day and as compared with control (Table no. 4, Graph 3)

**DISCUSSION**

**Excision wound model**

The progress of the wound healing induced by *Salvia splendens* leaves extract ointment (10% and 15% w/w) treated groups, simple ointment (control) treated group and Nitrofurazone (standard drug, 0.2%w/w) treated group of animals are shown in table no.1. It is observed that the wound contraction ability of the ointment containing Salvia extract in different concentrations were significantly greater than that of the control (i.e., simple ointment treated group). The 15%w/w extract containing ointment treated groups showed significant wound healing from the fourth day onwards, which was comparable to that of the Nitrofurazone treated group of animals. The wound closure time was lesser, as well as the percentage of wound contraction was much more with the 15%w/w extract ointment treated group (18th days for 100% contraction which was almost similar to that of the Nitrofurazone treated group). 10%w/w extract ointment treated group of animals showed significant wound contraction from the 18th day onwards and achieved 100% with the wound closure time of 20th days.

**Biochemical analysis**

Mucopolysaccharide content was increases initially when collagen(hyrodroxyproline content was low which was confirmed from the healing process. It was seen that the decrease in Mucopolysaccharide content was associated with a concomitant increase in collagen. The mucopolysaccharides are made up of repeating disaccharides containing uronic acid and hexosamine. They are the first components of the extra cellular matrix to be synthesized during wound healing and form the template for collagen and elastin deposition. Thus the increase in mucopolysaccharide content of the treated wound might have contributed in matrix synthesis, formation of new tissues will ultimately leading to the acceleration of wound healing. The extract 10% and 15%w/w showed increase in the collagen content as compared to control.

**Incision wound model**

Collagen is known to play important role in the process of wound contraction and gain in tensile strength. Tensile strength depends upon the increase in the collagen content. The tensile strength of 10% and 15%w/w extract ointment is highly significant as compared to control.

**ACKNOWLEDGEMENT**

The authors are very thankful to Dr. D.Sasmal (Professor & Head, Birla Institute of Technology, Mesra, Ranchi) and Dr.Papyia Mitra Mazumdar ( Professor, Birla Institute of Technology, Mesra, Ranchi) for their encouragement and providing facilities to carry out this work.

**REFERENCES**

1. Shanker D, Unnikrishnan PN. An overview: introduction to herbal plants used in medicine. Amrut: 2001. 5(4); 9-16.
2. Nguyen DT, Orgill DP, Murphy GF. The Pathophysiologic Basis for Wound Healing and Cutaneous Regeneration: Biomaterials For Treating Skin Loss. Woodhead Publishing (UK/Europe) & CRC Press (US), Cambridge/Boca Raton: 2009. Chapter 4: 25-57.
3. Bobbi A. Splendid salvias-14 Tender Perennials for summer and fall Bloom. Brooklyn Botanic Garden. New York: 2003. 18(3). (www.plantdelidgts.com)
4. Nadkarni KM. The Indian Metria Medica: 1991. 2: 1093-1096.
5. The Wealth of India. A Dictionary of Indian Raw Material and Industrial Products. Council of Scientific and Industrial Research. New Delhi: 1998. 9; 195-198.
6. Kaplan DR. Salvia Splendens of the Lamiaceae family. Plant Biology: 2001. 107.
7. Erv E. Salvia splendens. Consumer horticulturist: NC State University Florida. 2000-2003. (www.Salviasplendens.com)
8. info@fleuroselect.com
9. www.floridata.com
10. Lowell CE. Salvia splendens-sacarlet sage. Flower seed Trials: Michigan state university 1997. (www.Salvia splendens%20sage.htm)
11. Edward FG, Teresa H. Salvia splendens; Institute of food and Agriculture sciences: university of Florida Florida: 1999. 528.
12. Salvia splendens-scarlet sage. (www.Salvia plant/calphotos.htm)
13. Fowers of Red sage. (www.Salvia plant/calphotos.htm)
14. KitchenGardensseed-Salvia. (www.coustomerservice@kitchengardenseeds.com)
15. Bentley R, Triman H. Introduction to Medicinal plants. Medicinal Plants: 1880. 3; 206.
16. Evans WC. Trease and Evans – Pharmacognosy. 15th edition Saunders W.B. Edirburgh: 2002. 259.
17. Salvia splendens. (www.pfaf.org)
18. Simple ointment. British Pharmacopoiea: 1996. 1360.
19. Mukherjee PK, Verpoorte R, Suresh B. Evaluation of in-vivo wound healing activity of *Hypericum patulum* (Family:Hypericaceae) leaf extract on different wound model in rats. Journal of Ethnopharmacology: 2000. 70: 315-321.
20. Charde MS. Fulzele SV. Satturwar PM. Joshi SB. Kasture AV. Dorle AK. Study on wound healing activity of Mulathiaadi Ghrita. Journal of Pharmaceutical Research: 2005. 4(1): 08-12.
21. Majumdar MR, Kamath JV. Herbal concept on wound healing. Journal of Pharmaceutical Research: 2005. 4(1): 01-07.
22. Mortan JJK. Evaluation of Vulnerary by an open procedure in rats. Arcch. Int. Pharmacodyn: 1972. 196-117.
23. Mustafa MR, Mahmood AA, Sidik K, Noor SM. Evaluation of wound healing potential of Ageratum conyzoides leaf extract in combination with honey in rats as animal model. Interational Journal of Molecular Medicine and Advance Science: 2005. 1(4): 406-410.
24. The phases of cutaneous wound healing, Expert Review of Molecular Medicine. Cambridge university press: 2003.(www.expertreview.org).
25. Robert FD, Melissa CE. Wound healing: An overview of Acute, Fibrotic and Delayed healing. Frontiers in Bioscience: 2004. 9: 283-289.
26. Gillian SA, Teresa G, Michael AH, Sharon MW, Mark WJF. Topical estrogen Accelerates cutaneous wound healing in Aged humans Associated with an Altered inflammatory Response. American Journal of Pathology: 1999. 155(4): 1137-1146.
27. Park EH, Chun MJ. Wound healing activity of opuntia ficus-indica. Fitoterapia: 2001. 72: 165-167.
28. Vogel H Gerhard. Pharmacology Model in Dermatology. In Drug Discovery and Evalution: Pharmacology Assay. 2nd edition. Springer Germany: 2002. 360-1362.