



Scholars Research Library

Annals of Biological Research, 2010, 1 (2) : 49-61
(<http://scholarsresearchlibrary.com/archive.html>)



Evaluation of wound healing potential and antimicrobial activity of ethanolic extract of *Evolvulus alsinoides*

U. M. Dhanalekshmi, G. Poovi, Narra Kishore, M. D. Raja and P. Neelakanta Reddy*

Bio - Organic Chemistry Laboratory, Central Leather Research Institute (Council of Scientific and Industrial Research), Adyar, Chennai, India

Abstract

The aim of this study is to explore the wound healing and antimicrobial effects of crude ethanolic extract of the whole plant of *Evolvulus alsinoides* L., (Convolvulaceae). Excision wounds were created in Wistar rats and the area of skin wound in the experimental group was dressed with crude ethanolic extract, while the animal in the control group was dressed with normal saline. The wound area was measured in both groups of animals at the fourth, eighth, twelfth and sixteenth post-operative day and the percentage wound contraction calculated. Sample of granulation tissues and end scar obtained from these wounded animals were used for biochemical and histopathological studies. The result showed significant increase in the percentage wound contraction at day 10 in the experimental group compared with the control. The wound of animals in both groups showed excellent granulation tissue formation. The ethanolic extracts were also tested against nine human pathogenic bacteria and four fungal strains by the agar-well diffusion and slant method. Based on minimum inhibitory concentration (MIC) of the present study, the ethanolic extract showed maximum antibacterial activity and antifungal activity. This study provides a rationale for the topical application of plant extract as a feasible and productive approach to support dermal wound healing with good antimicrobial properties.

Key words: *Evolvulus alsinoides*, ethanolic extract, antimicrobial, wound healing, biochemical.

INTRODUCTION

Medicinal plants are commonly used for the treatment of various ailments in India, as these are considered to have advantages over the conventionally used drugs that are expensive and known

to have harmful side effects [1]. Consumption of medicinal herbs is tremendously increasing over a past decade as an alternative approaches to improve the quality of life and maintain a good health. Medicinal plants have been used for centuries as remedies for human diseases [2, 3].

Wound is defined simply as the disruption of the cellular and anatomic continuity of a tissue [4]. Wound may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue. The process of wound healing consists of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with regain of strength of injured tissue. Clinically, one often encounters non-healing, under-healing or over healing. In traditional medicine, extracts of polysaccharide-containing plants are widely employed for the treatment of skin and epithelium wounds and of mucous membrane irritation [5]. Medical treatment of wound includes administration of drugs either locally (topical) or systemically (oral or parenteral) in an attempt to aid wound repair [6]. The topical agents used include antibiotics and antiseptics, desloughing agents (chemical debridement, e.g. hydrogen peroxide, eusol and collagenase ointment) [7], wound healing promoters and various growth factors [8] necessary for the initiation and promotion of wound healing. Many substances like tissue extracts [9] vitamins & minerals and a number of plant products [10] have been reported by various workers, to possess pro-healing effects. Wound healing herbals encourage blood clotting, fight infection and accelerate the healing of wounds. Plants or chemical entities derived from plants need to be identified and formulated for treatment and management of wounds [11]. Several plant genetic resources were tested for their efficacy in healing wounds, namely *Vernonia scorpioides* [12] and *Argemone mexicana* [13].

Like the alchemist's dream of turning base metal into gold, efforts aimed at achieving a perfect wound healing has pushed many researchers into trying various therapeutic options which were thought to aid or accelerate the wound healing process. The cheaper and more effective the agent, more better for the patient. The aim of this study is to evaluate the wound healing and antimicrobial effect of crude ethanolic extract of the whole plant of *Evolvulus alsinoides* L., (Convolvulaceae).

Evolvulus alsinoides L., (Convolvulaceae) commonly known as 'shankhpuspi' in India, Africa and Philippines. It is an important medicinal plant employed for different ailments in India traditionally and grows in the open and grassy places almost throughout the India and subtropical countries of the world [14, 15]. The oldest reports found of use of *E. alsinoides* are from India and surrounding regions. The herb was used to treat dysentery [16]. Mohammedan physicians used the plant as a general tonic to strengthen the brain and memory and to treat fever [17]. *E. alsinoides* (EA) was used to treat bowel problems and to promote conception [18, 19]. The entire plant was considered astringent and useful for treating hemorrhages and there are a variety of other medical applications, including as an adaptogenic, antiphlogistic, antipyretic, antiseptic, aphrodisiac, febrifuge, stomachic, tonic, vermifuge, against asthma, bronchitis, scrofula, syphilis, or in "controlling night emissions" and to promote wound healing [20- 23]. However, it appears that *E. alsinoides* one of the plant has some phytochemicals that are effective against the maladies for which people use them. The isolation of evolvin, kaempferol-3-O- β -D glucopyranoside, coumarin etc., from *E. alsinoides* was previously reported [24].

The acceptance of traditional medicine as an alternative health care and the development of microbial resistance to the available antibiotics have led researches to investigate the antimicrobial effect of herbal extracts. The potential for developing antimicrobials from higher plants appears rewarding as it may lead to the development of phytomedicine against microbes [25, 26]. The present study describes the wound- healing effect of extract applied on large full-thickness wounds in the rat along with its antimicrobial properties.

MATERIALS AND METHODS

1.1. Collection of plant material and extraction

Fresh whole plants of *E. alsinoides* were collected in Tambaram area of Chennai, Tamil Nadu (INDIA) and identified by Prof Dr. Jayaraman, Plant Anatomy and Research Centre, Tambaram, Chennai, India, where its voucher specimen (PARC/2008/152) was deposited.

The plants materials, was shade dried, powdered (800 g) and subsequently subjected to the extraction process. The solvent was removed at 30°C by using a rotary evaporator. The yield of ethanolic extract was 23 g (2.87 %). This crude ethanolic extract was used for the evaluation of phytochemical, antimicrobial and wound healing activity.

1.2. Animals

Wistar female albino rats (180- 200 g) used for this study were procured from King Institute Guindy, Chennai, India and housed in the Institutional animal house under standard environmental conditions (23± 1°C, 55± 5 % humidity, 12 hours/ 12 hours light/ dark cycle) maintained with free access to standard diet (Hindustan Lever, Bangalore, India) and water *ad libitum*. The 12 animals were divided into two groups, each group containing 6 animals and housed in poly propylene cages. The protocol of animal study was approved by Institutional Animal Ethics Committee (IAEC 03/003/08).

1.3. Wound healing experimentation with animals

For the assessment of wound healing activity excision wound model was used. The animals were divided into two groups each group containing 6 animals.

Group I: Control.

Group II: Ethanolic extract of EA.

1.3.1. Production of full- thickness excision wounds

The fur of the dorsum (below the rib cage) of each animal was removed and a full-thickness skin wound was produced on the dorsum of the animal. The animals were anesthetized using diethyl ether before creation of wound and decontaminated by wiping the whole body with sterile antiseptic. The cleared dorsal surface of skin was marked with a sterile square (2×2 cm) stencil. The skin flap was excised in an aseptic environment using sterile scissors and forceps. A full-thickness wound was created. Each wounded animal was housed in a separate sterile polypropylene cage. Then animals were treated with leaf and flower extract paste. Sterile gauze was reapplied every alternative day. The wound size was calculated and the granulation tissue removed every fourth day.

1.3.2. Measurement of wound contraction

The progression of wound healing can be judged by the periodic assessment of the contraction of excision wounds. Tracing the outline of the wound on tracing sheet and then using graph sheet to calculate the area of the wound size monitored wound contraction. The wound was also pictured. All animals in each group were monitored until complete healing of wounds occurred and the day at which each wound healed was recorded.

1.3.3. Biochemical Analysis

All the assays were performed on the granulation tissue collected. Hydroxyproline was measured using the method of Neuman and Logan [27]. Total protein was assayed using the method of Lowry [28] using BSA as standard. Hexosamine was estimated by Elson and Morgan [29] and Uronic acid by Bitter and Muir [30].

1.3.4. Tensile Strength

Instron Universal testing machine (450 I) was used for testing material under tension or compression. The test specimen was clamped in the jaws and the machine was run at the rate of 100 ± 2 mm/min until the specimen tore apart. The highest load reached was recorded while the sample is subjected to breaking. The distance between the jaws when rupture of the test specimen occurred was noted [31].

1.3.5. Histological Analysis

The granulation tissues were routinely processed by standard procedures and stained with hematoxylin and eosin (H&E). Stained specimens were microscopically evaluated to assess the predominant stages of wound healing.

2.4. Phytochemical analysis

2.4.1. Preliminary phytochemical analysis

Preliminary phytochemical properties of the extract were tested for alkaloids with Mayers and Dragendroffs reagents, saponins glycosides with the ability to produce suds, cardiac glycosides with FeCl_2 and H_2SO_4 , flavonoids with the use of Mg and HCl, anthraquinones with Borntragers reaction, terpenoids with Liebermann- Burchard method and use of H_2SO_4 , tannins with 1% gelatin and 10 % NaCl solutions [32].

2.5. Antimicrobial Studies

The extract was solubilized in dimethyl formamide and tested with solvent control and positive control for antimicrobial activities.

2.5.1. Bacterial and fungal strains used

The test extracts were individually tested against a panel of microorganisms, which includes the gram positive bacteria namely staphylococcus aureus (NCIM 2079), Coagulase negative staphylococcus (clinical strain identified); the gram negative bacterias namely E.coli (NCIM 269), klebsiella (NCIM 2719), Pseudomonas aeruginosa (NCIM 2036), Salmonella typhi (MTCC 735), Salmonella para A (CAS 256), Salmonella para B (CAS 275), Vibrio cholera (NICED 0139) and the fungi Candida albicans (MTCC 227), Aspergillus flavus (MTCC 2206), Aspergillus niger (MTCC 281) and Trichophyton mentagrophytes (CAS 66) were obtained from National collection of Industrial Microorganism (NCIM), Pune, India.

2.5.2. Disc Diffusion Method

Disc diffusion method was used for the determination of antimicrobial activity of the extract and the MIC was calculated by agar dilution method. The drug Ciprofloxacin 5µg/ml was used as a reference drug

Briefly, a suspension of the tested organism was swabbed on Mueller- Hinton agar (MHA) in order to obtain a lawn culture. A filter paper (Whatman no.1) disc of 6mm diameter which contained 10µl of the plant extract was placed on the inoculated plates. The inoculated plates were subsequently inoculated at 37°C for 18 hours. The zone for inhibition was measured in millimeters and compared with standard drug [33-35]. The control consists of filter paper disc covered with dimethyl formamide and evaporated to dryness.

2.5.3. Minimum Inhibitory Concentration Assay

The agar dilution method recommended by National committee for Clinical Laboratory Standards was used [35]. A series of 2 fold dilutions of each extract with dimethyl formamide at a final concentration ranging from 66µg/ml to 333 µg/ml were prepared in MHA at 37°C for antibacterial activity. A final concentration ranging from 83-333µg/ml was prepared in Sabourauds Dextrose Agar (SDA) slant to check antifungal activity.

The plates were spot inoculated with 3µl aliquots of culture contains approximately 10^5 bacteria/ml of each organism. The plates were incubated at 37°C for 18 hours and observed for the presence or absence of growth. 5ml of extract at different concentrations were taken into sterile test tube and mixed with 1 ml of each fungus to be tested. Then 0.5 ml of mixture (culture with extract) was added to 2.5 ml of SDA in the tubes. Afterwards all the tubes were incubated at 30°C for 15 days. The tubes were observed for visible growth of fungi. The highest dilution showing no visible growth was regarded as minimal inhibitory concentration [35-37].

2.6. Statistical Analysis

Data were analyzed and expressed as mean \pm S.E.M

RESULTS

3.1. Rate of wound contraction

A better healing pattern with complete wound closure was observed in treated groups within 16 days while it was about 24 days in control rats (Table 1). There was a significant reduction in wound size from day 4 onwards in treated rats and also in later days the closure rate is much faster when compared with control (Fig 1).

Table 1. Measurement of wound contraction

SAMPLE	DAY 4	DAY 8	DAY 12	DAY 16
	Wound size (l x b)			
Control	1.8 \pm 0.033	1.4 \pm 0.021	0.9 \pm 0.023	0.5 \pm 0.022
Extract of EA	1.5 \pm 0.021	1.1 \pm 0.033	0.7 \pm 0.020	0.3 \pm 0.055

Values are mean \pm S.E.M. (n = 6)

Visual inspection of the wound showed that all the animals had well-formed granulation tissue. Among the experimental groups, animals treated with extract showed more significant wound contraction than control groups.

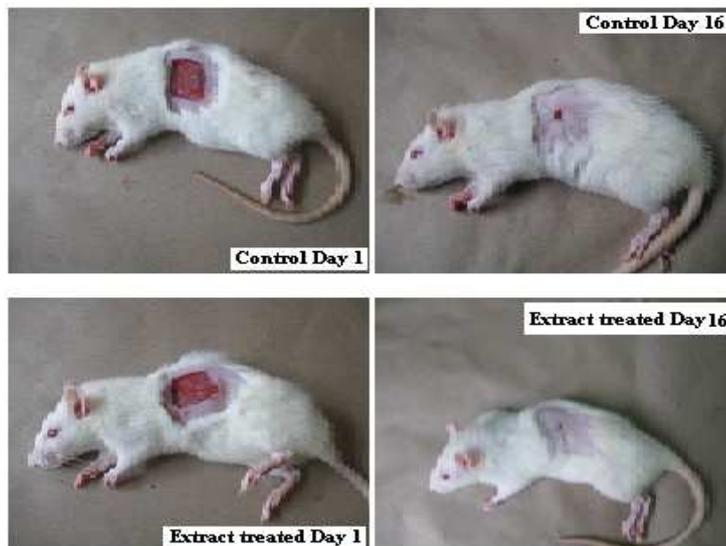


Fig 1. Experimental wound area before and after treatment

3.2. Biochemical Analysis

In the granulation tissue, hydroxyproline and hexosamine level was significantly increased in extract treated group when compared with control group (Fig 2 and 4). A highly significant increase in protein content was also observed in the extract treated group compared to control group (Fig 3). The Uronic acid content of the extract treated group not shown significant difference when compared with control (Fig 5). While on day 12 and 16 granulation tissue collected was not sufficient for doing all the biochemical tests because of better wound contraction

Fig 2. Estimation of hydroxyproline

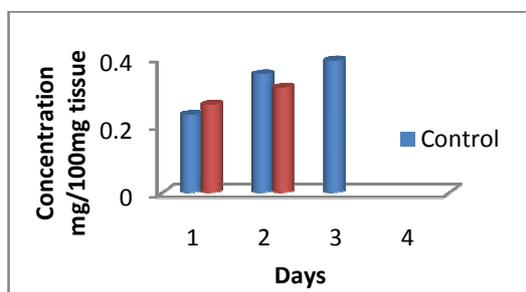
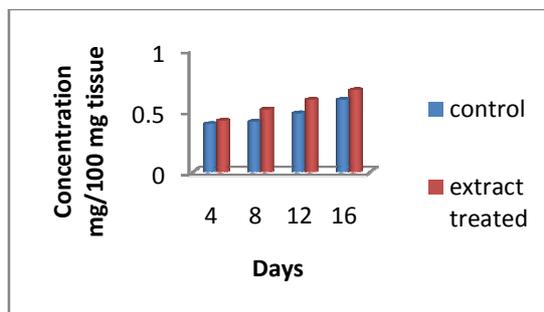
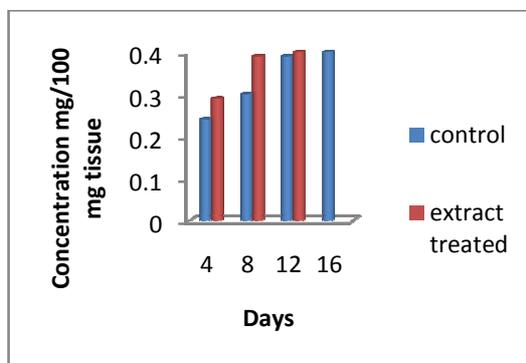
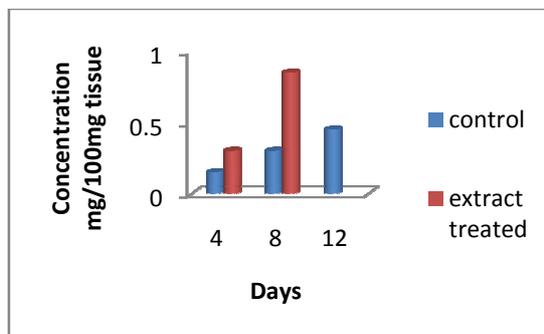


Fig 3. Estimation of protein**Fig 4. Estimation of hexosamine****Fig 5. Estimation of uronic acid**

3.3. Tensile strength

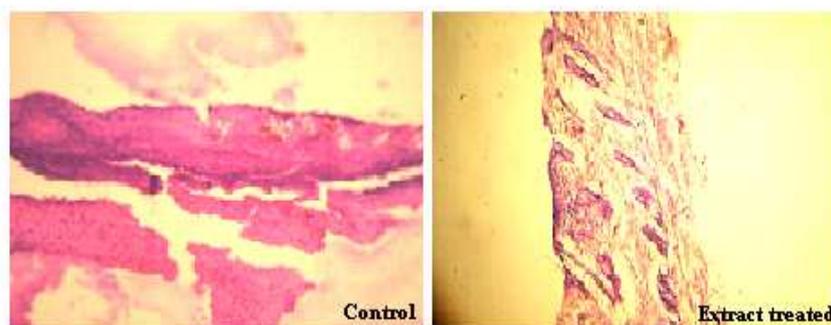
A significant increase in the skin tensile strength of the extract treated group on the post wounding day (Table 2). Tensile strength is the maximum stress a material can withstand under testing. From the data obtained, it was observed that extract treated group has the highest tensile strength when compared with control group. When we consider the maximum percentage strain it clearly reveals that extract treated group has 67 % strain than control which has only 25.4 % strain. Overall the results prove that all healed tissues treated with extract had higher tensile strength than the control group.

Table 2. Tensile Strength in the healed tissue of the wounds treated with Saline and extract of EA

Sample	Max. load (N)	Max. displacement (mm)	Tensile strength (MPa)	Max. Strain (%)
Control	9.879	5.083	2.470	25.418
Extract of EA	3.373	13.470	0.482	67.350

3.4. Histological Examinations

Histological examination reveals that there was a higher expression of macrophages and mast cells in the treated groups than in the control group (Fig 6). The sections of granulation tissue of extract treated animals showed the sign of tissue repair with increased collagen formation and less macrophage. In control animals the wound healing activity was comparatively lesser with moderate collagenation and retention of the macrophages. There was a substantial increase in fibroblast and collagen density in the extract treated group than the control groups. Interestingly, extract treated group showed a clear epidermal layer containing epithelial cells (pinkish layer) interspersed with cells indicating collagen synthesis in the regenerating dermal tissue.

**Fig 6. Histopathological Report**

2.7. Phytochemical studies

Phytochemical studies showed that the applied extract was positive for alkaloids, cardiac glycosides, flavonoids, and negative for saponins and terpenoids.

2.8. Antimicrobial Studies

The ethanolic extract of EA was used in this study to investigate their antimicrobial potential. Both gram negative and gram positive bacteria and fungi were used as test organisms. Ciprofloxacin used as positive control. Regarding antimicrobial activity of the extracts the results are summarized in Table 3 and 4. The ethanolic extract showed activity against all the tested bacteria and maximum activity at higher concentration. The zone of inhibition above 6 mm in diameter was taken as positive result. The disc diffusion method and MIC assay of extract are summarized in table-3.

Table 3. Comparative analysis of disc diffusion method and MIC

Microorganism	Diameter of zone of inhibition in mm		MIC($\mu\text{g/ml}$)
	Ethanollic extract of plant	Ciprofloxacin	Ethanollic extract of plant
<i>S.aureus</i>	30	30	333
<i>CONS</i>	29	30	200
<i>E.coli</i>	15	29	133
<i>Klebsiella spp.</i>	20	27	333
<i>Ps.aeruginosa</i>	21	25	66
<i>Salmonella typhi</i>	11	24	133
<i>Salmonella para A</i>	10	23	66.6
<i>salmonella para B</i>	19	30	133
<i>V.cholera</i>	20	25	133

Table 4. Anti Fungal Activity Ethanollic Extract

Extract concentration	<i>Candida spp</i>	<i>A.flavus</i>	<i>A.niger</i>	<i>T.mentogrophytes</i>
Extract of EA	83	+	+	+
	166	-	-	-
	333	-	-	-

The disc diffusion assay revealed that the ethanollic extract of EA showed broad spectrum of antimicrobial activity. The gram negative organisms were more susceptible to the extracts of EA. The extract showed excellent antimicrobial activity against salmonella species (MIC 66-133 $\mu\text{g/ml}$). Among the tested bacteria, Staphylococcus aureus was found to be more resistant to the extract (MIC 333 $\mu\text{g/ml}$). Other organisms exhibit moderate susceptibility.

EA extract also has got significant antifungal activity and results are tabulated in Table- 4. All the tested organisms namely Candida species, Aspergillus flavus, Aspergillus niger and Trichophyton mentagrophyte responded well to the extract by agar dilution method. The solvents used for solubility purpose not exploited any antimicrobial activity.

DISCUSSION

The screening of plant extracts has been of great interest to scientist for the discovery of new drugs effective in the treatment of several diseases. Indian flora has one of the most extensive floras in the world with more than 9000 plant species. A number of reports concerning the antibacterial, anti-inflammatory and wound healing activity of plant extracts of Indian medicinal plants have appeared in the literature, but the vast majority has yet to be investigated.

Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of area of the wound. It depends upon the reparative abilities of the tissue, type and extent of the damage and general state of the health of the tissue. Granulation, collagen maturation and scar formation are some of the many phases of wound healing, which run concurrently, but independent of each other [38]. The granulation tissue of the wound is primarily composed of fibroblast, collagen, edema, and small new blood vessels. The undifferentiated mesenchymal cells of the wound margin modulate themselves into fibroblast, which start migrating into the wound gap along with the fibrin

strands. The collagen is the major component of extra cellular tissue, which gives support and strength and is composed of amino acid (Hydroxyproline).

Wound contraction is thought to be mediated by specialized fibroblasts found within granulation tissue [39]. The ability of fibroblasts to contract collagen gel is considered to be a specific function, which exists *in vivo*. Increased wound contraction in the treated group may be due to the formation of fibroblasts; increased fibroblast production in turn activates the production of collagen [40, 41].

In the present study, the total protein content of the granulation tissue of the wound treated with extract was found to be higher than control. The fact that the extract treated group had a higher protein content than the control group may be due to either cellular infiltration or increase in collagen synthesis. Higher the protein content implies higher metabolic rate for wound healing. In wound healing, protein metabolism is fundamental for the repair of collagen, a tissue which is dependent on the synthesis of a large quantity of special protein. The hexosamine content of granulation tissue of the wound treated with extract was found to be higher than control group. By correlating hexosamine content it can be judged how fast the wound heals. It is important to note that hexosamine content will increase during wound healing process and decreases when maturation and remodeling phase is attained. The hydroxy proline and total uronic acid content of the granulation tissue of the wound treated with *extract* was found to be higher than control. Hydroxyproline is a major component of the protein collagen. They permit the sharp twisting of the collagen helix and provide stability to the triple-helical structure of collagen by forming hydrogen bonds. For this reason, hydroxyproline content has been used an indicator to determine collagen amount. Wound healing involves interactions of multiple cell types with various cytokines, growth factors, their mediators, and the extracellular protein fibronectin, laminin, tenascin, and collagen [42]. The increased hydroxyproline content agrees with the increase in protein content, which is predominantly due to enhanced collagen synthesis in the treated group. Decrease in uronic acid content was observed in the treated group. The decrease in uronic acid is attributed to an increase in collagen synthesis. This was further supported by Cohen and Haynes, who found that an increase in uronic acid will lead to a decrease in collagen synthesis [43, 44].

Union of the two sides of a wound is believed to be made by the deposition of collagen fibers in a matrix of granulation tissue. Studies of the granulation layer, however, suggested that the changes originated in cells of the surrounding tissue. This concept is supported by several works which showed that new fibroblasts develop in the tissue around wounds [45, 46].

A significant increase in the skin tensile strength of the extract treated group on the post wounding day. Histological examination reveals that there was a high migration of inflammatory cells toward the wound environment in treated groups. High migration of inflammatory cells expresses a wide variety of cytokines and functions to aid in tissue repair in treated groups [47]. An increase in the expression of cytokines activates fibroblasts toward the wound environments. The increase in fibroblast and collagen expression in wound sites by histological examination correlates with the above results, and it is supported by an increase in hydroxyproline content in extract treated groups. Migration of inflammatory cells, a high expression of fibroblasts and collagen, and an increase in wound contraction reveal that extract treated groups follows the normal wound-healing cascade of inflammation, proliferation, and scar formation. Many plant

extracts and medicinal herbs have shown potent antioxidant activity. Tannins the main components of many plant extracts, act as free radical scavengers [48, 49].

As antibiotics provide the main basis for the therapy of bacterial infections the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance. Due to lack of information on screening and evaluation of diverse plants for their antibacterial potential there has been a continuing search for new and more potent antibiotics [50].

In the present study, the ethanolic extract of EA was used to investigate their antimicrobial activity. Human pathogenic bacteria and fungal strain were used to screen possible antimicrobial activity of EA extracts. We found that the extract inhibited the gram negative bacteria better than gram positive. Generally, plant extracts are usually more active against gram positive bacteria than gram negative bacteria [51]. The range of MIC values for all the test micro organism correlated well with the results obtained using the disc diffusion method. Among the tested bacteria, staphylococcus aureus was found to be more resistant to the extract. Other organisms exhibit moderate susceptibility. Several authors had reported that extracts from plants containing antibacterial properties [52]. The detailed nature of the active principles responsible for antimicrobial activity is not known. The preliminary phytochemical screening has however shown that the presence of various constituents, the inhibitory potentials of the extracts might be ascribed to their content of secondary metabolites. The ability of the extract to inhibit the pathogens holds promise for potential application in the pharmaceutical industry [53].

CONCLUSION

This finding thus, justifies its use in folkloric medicine for wound healing. At this stage, it is difficult to say which component(s) of the extracts are responsible for wound healing and antimicrobial activity. However, further phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities.

Acknowledgements

We are grateful to Dr. A. B. Mandal, Director, CLRI, for giving permission to publish this work. We are also thankful to Mr. V. Elango, Department of Bio Organic Chemistry Lab, for helping in the wound healing experiments and careful maintenance of the animals during the experimental period. Author U. M. Dhanalekshmi thanks the Council of Scientific and Industrial Research, India for granting fellowships.

REFERENCES

- [1] CP Khare. In: Encyclopedia of Indian medicinal plant 2nd edition. Rational Western therapy, Ayurvedic and other traditional usage, New Delhi: Springer Verlag. **2004**,153.
- [2] A Nostro; MP Germano; V Dangelo; A Marino; MA Cannatelli. *Lett appl Microbiol.* **2000**, 30, 379-384.
- [3] S Arokiyaraj; R Radha; S Martin; K Perinbam. *Indian Journal of Science and Technology.* **2008**, 1:1- 4.

- [4] RG Bennet. In: Bennett,. Fundamentals of Cutaneous Surgery. St Louis, Mo: Mosby; **1988**,194–239
- [5] MK Bedi; PD Shenefelt. Herbal therapy in dermatology. Arch Dermatol. **2002**, 138, 232–242.
- [6] SS Savanth; RA Shah; D Gore. Text book and atlas of dermatosurgery and cosmetology, ASCAD, Mumbai. **1985**,50-61.
- [7] SS Savanth; RA Shah. In: Savant SS, Shah RA, Gore D. (Eds.). Text book and atlas of Dermatology and Cosmetology, ASCAD, Mumbai. **1992**, 12-17.
- [8] MD Mather; M Sherman; A Frycakowski; JV Jester. *Invest Ophthalmol. Vismal Sci*, **1989**, 30, 2403-2406.
- [9] SL Udupa; HP Shaila ; AL Udupa; KV Ramesh; DR Kulkarni. *Biochem Arch*.**1991**, 7, 207-212.
- [10] SA Dahanukar; RA Kulkarni; NN Rege. *Indian J. Pharmacol.* **2000**, 32, S81-S118.
- [11] Rajinder Raina; Shahid Prawez; Verma; Pankaj. Medicinal Plants and their Role in Wound Healing ISSN 0973-6980, **2008**, vol 3: 1.
- [12] SN Leite; G Palhan; S Almedia; MW Biavatti. *Fitoterapia.* **2002**, 73, 496.
- [13] MB Patil; SS Jalalpure; A Ali. *Indian Drugs*.**2001**, 38, 288–293.
- [14] The Wealth of India - Raw material: Publication and Information Directorate. Volume III. NISCAIR. India. **1952**,223.
- [15] RN Chopra; SL Nayer; IC Chopra. Glossary of Medicinal plants. Publication and Information Directorate. CSIR. India. **1986**,116.
- [16] J Burmanni. Book on flora. Thesaurus zeylanicus: exhibens plantas in insula Zeylana nascentes. Amstelaedami. **1737**.
- [17] G Watt. A Dictionary of the Economic Products of India by Government of India. Reprinted by Cosmo publications. Delhi. **1972**.
- [18] W Ainslie. Materia Medica of Hindoostan and artisan's and agriculturist's nomenclature. Government Press, Madras. **1813**.
- [19] WM Dymock. The Vegetable Materia Medica of Western India. 2nd edition revised and enlarged. Education Society's Press/Trubner & Co. Bombay/London. **1885**.
- [20] FA Daniel. *Journal of Ethnopharmacology.* **2008**, 117, 185–198.
- [21] B Auddy; M Ferreira; F Blasina. *Journal of Ethnopharmacology.* **2003**, 84,131-138.
- [22] V Asolkari; KK Kakkar; OJ Chakre. Second Supplement to Glossary of Indian Medicinal Plant with active Principles, NISCAIR New Delhi, India. **1992**, 1965-1985.
- [23] KR Kritkar, BD Basu. Indian Medicinal Plants. Vol III. International book distributors ,Dehradun, India.**1933**.
- [24] Prasoon Gupta, Akanksha, Kiran Babu. *Chemical and Pharmaceutical Bulletin.* **2007**, 55, 771-775.
- [25] KA Hammer; CF Carson; TV Riley. *J. Appl. Microbiol.* **1999**, 86, 985-990.
- [26] NS Jatap; SS Khadabadi; DS Ghorpade; NB Banarase; SS Naphade. *Research J. Pharm. and tech.* **2009**, 2, 328-330.
- [27] RE Neuman; MA Logan. The determination of hydroxyproline. *J Biol Chem.* **1950**, 184, 299–306.
- [28] OH Lowry; NJ Roseborough; AL Farr; RJ Randall. *J Biol Chem.* **1951**,193, 265–75.
- [29] Elson and Morgan. *The journal of biological chemistry.* **1937**, 21,111-118.
- [30] T Bitter ; HM Muir. *Analytical Biochemistry.* **1962**, 4, 330-334.
- [31] Z Wang; P Ciselli; T Peijs. *Nanotechnology.* **2007**, 18, 455-508.

- [32] GE Trease; WC Evans. Text book of Pharmacognosy. ELBS Baillere Tindall University press, London; **1983**.
- [33] J Smullen; GA Koutsou; HA Foster; A Zumbé; DM Storey. *Caries Res.***2007**, 41, 342-349.
- [34] NCCLS: methods for dilution antimicrobial susceptibility tests of bacteria that grow aerobically .In approved standard M100-S12, Wayne PA, and NCCLS. **2002**.
- [35] U Abbasoglu. *J.pharma Sci.***1996**, 22, 111-118.
- [36] CL Florl; C Speth; G Kofler; MP Dierch. *International Journal antimicrobial Agents.* **2003**, 21,229-233.
- [37] I Rasooli; MR Abyaneh. *Food control.* **2004**, 15, 479-483.
- [38] PV Diwan; LD Tillor; DR Kulkarni. *Indian Journal of Pharmcology.***1979**, 11, 357.
- [39] V Moulin; FA Auger; D Garel; L Germain. *Burns.* **2000**, 26, 3–12.
- [40] CC Chen; FF Mo; LF Lau. *J Biol Chem.* **2001**, 276, 47329–47337.
- [41] H Ochi; H Ogino; Y Kageyama; K Yasuda. *J Biol Chem.* **2003**, 278, 537.
- [42] GS Sidhu; AK Singh; KK Banaudha; JP Gaddipati; Gk Patnaik; RK Maheshwari. *J Invest Dermatol.* **1999**, 113, 773–781.
- [43] IK Cohen; JH Haynes. Clinical aspects of wound healing. In: Altmeyer P, editor. *Wound healing and skin physiology*. Berlin:Springer .**1995**, 27.
- [44] R Ahmed; Gopinath; Gomathi; Sehgal; Jayakumar. *J Biomed Mater Res Part B: Appl Biomater* **2004**, 69 B, 241–248.
- [45] Watts Baddeley ; Wellings. *Lancet.* 1963, 2: 1031.
- [46] HC Grillo. *Annals of Surgery.* **1963**, 157,453.
- [47] ZA Haroon; JM Hettasch; TS Lai; MW Dewhirst; SG Charles. *FASEB.* **1999**, 13, 1787–95.
- [48] M Bekerecioglu; M Tercan;I Ozyazan. *J Scand. Plast Reconstr Hand Surg.* **1998**, 32, 135–139.
- [49] MP Kahkonen; AI Hopia; HJ Vuorela; JP Rauha; K Pihlaja. *Journal of Agriculture and Food Chemistry.* **1999** 47, 3954- 396
- [50] P Heisig. *Planta Med.* **2001**, 67, 3-12.
- [51] D Veeramuthu; A Muniappa; I Savarimuthu. *BMC Complementary and Alternative Medicine.* 2006, 6,35.
- [52] FL Oyetatyoy, VO Oyetatyoy, V Ajewole. *Journal of pharmacology and Toxicology.***2007**, 2, 586-589.
- [53] V Mounisamy; S Darimane; R Gunasegaran. *The Antiseptic.***2002**, 99, 81-82.