ORIGINAL ARTICLE

Antimicrobial and wound healing activities of leaves of *Alternanthera sessilis* Linn

Sunil S. Jalalpure, Nitin Agrawal, M.B. Patil[1], R. Chimkode[2], Ashish Tripathi[3]
Departments of Pharmacognosy and Phytochemistry, KLES's College of Pharmacy, Belgaum, Karnataka, [1]KLES's College of Pharmacy, Ankola, Karnataka, [2]N. R. Vekaria Institute of Pharmacy and Research Centre, Junagadh, Gujrat, [3]College of Pharmacy, Agra, India

The leaves of *Alternanthera sessilis* (Linn.) R.Br.ex DC (Family Amaranthaceae) were exhaustively extracted by soxhlet apparatus with different solvents like, petroleum ether (40-60°C), chloroform, acetone, methanol and distilled water in ascending order of the polarity. All the five extracts were subjected to antimicrobial screening by using the cup plate and turbidimetric methods. The chloroform extract among all five extracts showed maximum zone of inhibition and significant MIC values in above two methods respectively. Hence chloroform extract was taken for the screening of wound healing activity and models adopted for wound healing activity were, incision, excision and granuloma studies. The chloroform extract of leaves of *Alternanthera sessilis* at a dose of 200 µg/mL (orally) in all models showed significant results. Contraction of wound area ($P < 0.005$) at 16th day, time for complete epithelization in 16 days ($P < 0.0001$), and scar area after complete epithelization was found to be 33.2 ± 0.0730 ($P < 0.0008$) in excision wound model, wound breaking strength 388 ± 5.85 grams ($P < 0.0001$) in incision wound model, granuloma dry weight 47.7 ± 2.29, granuloma breaking strength 247 ± 10.2 ($P < 0.022$) in granuloma studies. All the results were significant for different parameters in wound healing activity when compared with control group. Presence of sterols in chloroform extract was also confirmed by preliminary phytochemical investigation, TLC and HPTLC methods.

Key words: *Alternanthera sessilis,* antimicrobial, chloroform extract, wound healing

INTRODUCTION

Alternanthera sessilis is an annual or perennial prostate weed, found throughout the hotter part of India, ascending to an altitude of 1200 m.[1] The plant has been scientifically proven to consist of chemical constituents like α-and β- spinasterols,[2] lupeal isolated from roots.[3] Apart from the above, plant also contains β- sitosterol, stigmasterol etc.[4] The herb has been reported as galactagogue, chologague, abortificient, and febrifuge and is also said to be used in indigestion.[5] The leaves are used in eye diseases; in cuts and wounds; antidote to snake bite and scorpion sting; in skin diseases.[3]

A wound is the result of physical disruption of the skin, one of the major obstacles to the establishment of infections by bacterial pathogens in internal tissues. When bacteria breach this barrier, infection can result.[6] Wounds are generally classified as, wounds without tissue loss (e.g. in surgery), and wounds with tissue loss, such as burn wounds, wounds caused as a result of trauma, abrasions or as secondary events in chronic ailments eg: venous stasis, diabetic ulcers or pressure sores and iatrogenic wounds such as skin graft donor sites and derma abrasions.[7] Wound healing involves complex series of interactions between different cell types, Cytokine mediators and the extracellular matrix. The phases of normal wound healing include hemostasis, inflammation, proliferation, and remodeling.[8]

To validate the ethnotherapeutic claim of the plant in skin diseases, wound healing activity was studied.

MATERIALS AND METHODS

Plant Material and Preparation of Extracts

Fresh leaves of *Alternanthera sessilis* were collected in the month of June 2007 from the Belgaum and same were authenticated by Dr. P. S. N. Rao, Botanical Survey of India, Koregaon Road, PUNE (voucher specimen No-ASNAI), shade dried and powdered then passed from 40# mesh size.

Preparation of Various Extracts of *Alternanthera sessilis* Linn

Around 1 kg fresh shade dried leaves were powdered and around 800 gms were extracted by hot percolation method by soxhlet apparatus[9] with five liters of each pet ether (40-60°C), chloroform, acetone, and methanol successively and around 200 gms of powdered drug by cold maceration method for seven days with chloroform water I.P.[10] All the extracts finally reduced to dryness at 40° C by Rotovapour (Rotavapour Buchii, Switzerland). The quantity of each extract after extraction was 12.73 gms (Pet ether 40-60°C), 12.95 gms (Chloroform), 15.21 gms (Acetone), 31.01 gms (Methanol), 26.87 gms (Aqueous).

For correspondence: Dr. Sunil S. Jalalpure, Department of Pharmacognosy, K.L.E.S's College of Pharmacy, Belgaum, Karnataka, 590010, India.
E-mail: jalalpuresunil@rediffmail.com
Received: 28-02-08; **Accepted:** 05-05-2008

Microorganisms

The test microorganisms used for the antimicrobial activity screening were 4 bacteria (2 Gram positive) - *Enterococcus faecalis, Staphylococcus aereus*, (2 Gram negative) - *Klebsiallea pneumoniae, Escherichia coli*, and 2 fungai- *Candida albicans* and *Aspergillus fumigatus*.

These organisms were identified and procured from National Chemical Laboratory (NCL), Pune, India.

Antimicrobial Activity

The agar diffusion method[11] was used to evaluate the antimicrobial activity. Bacteria were cultured overnight at 37°C in Mueller Hinton 10 µl Broth (MHB, Oxoid) and fungi at 28°C for 72h in Potato Dextrose Broth (PDB, Oxide) and used as inoculums. A final inoculums, using 100 µl of suspension containing 10^8 CFV/ml of bacteria 10^4 spore/ml of fungi spread on Mueller Hinton Agar (MHA) and Potato Dextrose Agar (PDA) medium respectively.

The disc (6 mm in diameter) was impregnated with 10 µl of 75 µl/ml, 50 µl/ml, 25 µl/ml, 10 µl/ml and 5 µl/ml of each extracts and for each organism placed on seeded agar. Ciprofloxacin and Fluconazole (75 µl/ml, 50 µl/ml, 25 µl/ml, 10 µl/ml and 5 µl/ml) were used as positive control bacteria and fungi respectively. The test plates were incubated at 37°C for 24h for bacteria and at 28°C for 72h for fungi depending on the incubation time required for a visible growth.

MIC values were also studied for microorganisms by turbidimetric method, which were determined as sensitive to the extracts in cup plate method. MIC was defined as the lowest concentration of extract that inhibit visible growth.

Pharmacological Activity

Experimental Animals

Albino rats of either sex (weighing 150 to 200 gms) were employed for wound healing activity. The above animals were purchased from National Toxicology Centre, Pune. They were housed under standard experimental conditions in polypropylene cages with standard pellet diet (Amrut laboratory animal feed, Sangli–Maharashtra) and water *ad libitum*. Rats were divided into six groups, each group having six animals. This project was cleared by the ethical committee of our institution (CPCSEA 221).

Acute Toxicity Activity

Albino rats of either sex weighing between 150-200 gm were used for acute toxicity study. The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD). The therapeutic dose was selected based upon the maximum cut off value i.e. 2000 mg/kg b.w.[12]

Wound Healing Activity

In the experiment, the rats were divided into six groups (n = 6): group 1 and 2 was inflicted with excision wound; group 1 was the control group which received normal saline water and group 2 received chloroform extract, group 2 and 3 was inflicted with incision wound model; group 3 received normal saline water and group 4 received chloroform extract and similarly group 5 and 6 were inflicted with dead space wound model; group 5 received normal saline water and group 6 received chloroform extract.

Excision Wound Model

An impression was made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear using a round seal of 2.5 cm diameter on the anaesthetized rat. The skin of impressed area was excised to the full thickness to obtain a wound area of about 500 mm^2 diameters. Hemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. Contractions, which contribute for wound closure in the first two weeks, were studied by tracing the wound on a transparency paper initially then an impression was taken on a millimeter scale graph paper, scar area after complete epithelization and time for complete epithelization in days was evaluated to calculate the degree of wound healing.[13]

Incision Wound Model

In the incision model,[14] the rats were anesthetized by anesthetic ether and two longitudinal paravertebral incisions of 6 cm length were made through the skin and cutaneous muscle at a distance of about 1.5 cm from the midline on each side of the depilated back. After the incision, the parted skin was sutured 1 cm apart using a surgical thread (No. 000) and curved needle (No. 11). The wounds were left undressed. The drugs were given by oral route once a day, till complete healing. The sutures were removed on eighth post-wound day. The skin-breaking strength of the 10-day-old wounds was measured by the method of Lee.[15]

Dead Space Wound Model

In this model the physical and mechanical changes in the granuloma tissue were studied. Under light ether anesthesia the hairs in the axilla and groin were clipped out and a subcutaneous dead space wound were inflicted in the same region, by making a pouch through a small nick in the skin.

Granuloma formation was induced by implanting both sterile cotton pellets and grass piths.

Two sterile cotton pellets weighing 10 g (sterilized by autoclaving) were used to grow granuloma by the technique as described by, but the granulomas were removed on 10^{th} day.

As described above two cylindrical grass piths measuring (25 x 3 mm) were also introduced in the subcutaneous pouch in each animal.

The sutured wounds were mopped with an alcoholic swab and animals were placed into their individual cages. The granuloma was excised from the surrounding tissue on 10th post-wounding day under light ether anesthesia. Cotton pellet granuloma excised from dead space wounds were dried overnight 60°C so as to obtain constant dry weight. Their weights were expressed as mg/100 gms body weight as suggested by Dispaquale and Meli.[16]

Granuloma surrounding grass piths were excised and slit opened by a longitudinal incision in one planeso as to obtain rectangular strips. The breaking strength of a strip of granuloma measuring about 15 mm in length and 8 mm in width (obtained by trimming the rectangular strip of granuloma tissue) was measured employing the method described under incision wounds.

Statistical Analysis

Data obtained for each set of wound healing models were expressed as mean ± SE and analyzed by unpaired Student's t test. Level of significance was set at $P < 0.05$.[17]

RESULTS AND DISCUSSION

The LD_{50} was found to be more then 2000 mg/ kg BW p.o. in acute toxicity testing. The therapeutic dose 200mg/ kg BW p.o. was calculated as 1/10th of the lethal dose for the purpose of wound healing investigation.

Antimicrobial activity was done for all the five, pet ether, chloroform, acetone, methanol and aqueous extracts. During antimicrobial study chloroform and acetone extracts showed maximum zone of inhibition against almost all organisms in cup plate method and again chloroform extract showed significant MIC values in turbidimetric method. So the chloroform extract was taken for wound healing activity [Tables 1-3].

In an excision wound model, chloroform extract at a dose 200mg/kg BW p.o. of *Alternanthera sessilis* showed significant wound healing activity (% wound contraction on 16th day 97.4 ± 0.940 $P < 0.0053$) compared to control (90.2 ± 1.79). It also showed complete epithelization (14.5 ± 0.428 days $P < 0.0001$) when compared to control (19.8 ± 0.601). The chloroform extract showed a scar area of 33.2 ± 0.703 mm^2 as compared to control 41.0 ± 1.51 mm^2 [Table 5].

In incision and grass pith granuloma studies, the chloroform extract showed significant ($P < 0.0001$) breaking strength when compared to control and also showed significant increase in the weight of cotton pellet (47.7 ± 2.29, $P < 0.0001$), compared to control (32.3 ± 1.01) in cotton pellet model [Table 4].

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissues as closely as possible to its normal state. Wound contraction is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. In the maturation phase, the final phase of wound healing the wound undergoes contraction resulting in a smaller amount of apparent scar tissue.

Granulation tissue formed in the final part of the proliferative phase is primarily composed of fibroblasts, collagen, edema

Table 1: Zone of inhibition (mm) of chloroform extract at various concentrations on some microorganism (Bacterial strain)

	Staphylococcus aureus					*Enterococcus faecalis*					*Klebsiella pneumoniae*					*Escherichi coli*				
Concentration in µgms	75	50	25	10	5	75	50	25	10	5	75	50	25	10	5	75	50	25	10	5
Standard drug (Ciprofloxacin)	17.6	15.8	13.9	11.7	9.5	18.9	15.8	13.8	11.7	9.5	21.9	19.5	16.5	14.4	-	21.2	18.6	16.9	15.8	13.5
Pet ether	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	28	24	16	-	-
Chloroform	14	12	12	-	-	12	12	-	-	-	-	-	-	-	-	26	20	-	-	-
Aqueous	14	-	-	-	-	16	-	-	-	-	20	16	14	-	-	40	32	36	26	20
Methanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	28	22	16	-	-
Acetone	14	12	-	-	-	-	-	-	-	-	-	-	-	-	-	28	24	-	-	-

Table 2: Zone of inhibition (mm) of chloroform extract at various concentrations on some microorganisms (Fungi)

	Candida albicans					*Aspergillus fumigatus*				
Concentration in µgms	75	50	25	10	5	75	50	25	10	5
Standard drug (Fluconazole)	18.5	15.9	14.7	-	-	19.9	17.6	15.6	13.5	-
Pet ether	-	-	-	-	-	-	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-	-	-
Aqueous	-	-	-	-	-	16	-	-	-	-
Methanol	-	-	-	-	-	12	12	12	-	-
Acetone	-	-	-	-	-	-	-	-	-	-

Table 3: Minimum inhibitory concentration (MIC) of chloroform and acetone extracts on various microorganisms

	Chloroform extract	Acetone extract	Standard drug ciprofloxacin
Staphylococcus aureus	<50.00	6.25	1.0
Enterococcus faecalis	<50.00	<12.5	<2.0
Escherichi coli	<6.25	<0.25	<0.1
Klebsiella pneumoniae	3.15	0.4	<0.2

Table 4: Effect of chloroform extract of *Alternanthera sessilis* on wound healing in incision and dead space wound models

	Re-sutured incision breaking strength	Dead space	
		Breaking strength (gms)	Granuloma weight (mg %)
Control group	288 ± 7.37	203 ± 12.3	32.3 ± 1.01
Chloroform extract	388 ± 5.85***	247 ± 10.2*	47.7 ± 2.29***

(Values are mean ± SE from 6 animals in each group) Data analyzed by unpaired Student's t- test, *P* values: <0.0001***, <0.0219*

Table-5: Effect of chloroform extract *Alternanthera sessilis* on excision wound model

Wound model		Control group	Chloroform extract
Wound closure (days)		19.8 ± 0.601	14.5 ± 0.428***
Mean scar area (mm^2)		41.0 ± 1.51	33.2 ± 0.703***
% Wound contraction by day	4th	20.1 ± 1.76	35.2 ± 1.08***
	8th	40.2 ± 1.81	55.2 ± 1.08***
	12th	70.2 ± 1.81	95.3 ± 1.06***
	16th	90.2 ± 1.79	97.4 ± 0.940**

[Values are mean ± SE from 6 animals in each group], Data analyzed by unpaired Student's t- test, *P* values: <0.0001, ***<0.0053**

and new small blood vessels. The increased dry granulation tissue weight in the test treated animals suggests higher protein content.

The wound healing property of *Alternanthera sessilis* may be attributed to the phytoconstituents present in the plant, and the quicker process of wound healing could be a function of either the individual or the additive effects of the phytoconstituents.[18] Sterols were the major constituents of chloroform extract during phytochemical investigation and there are reports that sterols are responsible for wound healing activity.[19] So sterols may increase collagen content and degree of collagen cross-linkage within the wound they may also promotes cell division and the growth of bone, cartilage and other connective tissues.[8]

CONCLUSION

All the results were significant for different parameters in wound healing activity when compared with control group. Sterols were the major constituents of chloroform extract during phytochemical investigation and there are reports that sterols are responsible for wound healing activity.

ACKNOWLEDGEMENTS

We thank Dr. F. V. Manvi, Principal, K.L.E.S's College of Pharmacy, Belgaum, Karnataka, India for providing all the facilities to conduct this work.

REFERENCES

1. The Wealth of India. Raw materials. Vol 1(Revised), New Delhi: CSIR; 1985. p. 318-9.
2. Rastogi RP. Compendium of Indian medicinal plant. 2nd ed. Lucknow: CDRI; 1993. p. 1970-9.
3. Gupta AK, Indian medicinal plants. New Delhi: ICMR; 2004. p. 151-7.
4. Sinha P, Arora VK, Wahi SP. Chemical investigation on *Alternanthera sessilis*. Indian Drug 1984;1:139-40.
5. Anandkumar BH, Sachidanand YN. Treatment of acne vulgaris with new polyherbal formulation clarina cream. Indian J Dermatol 2001;46:1-3.
6. Giacometti A, Cirioni O, Schimizzi AM, Prete DM, Barchiesi F, Errico DM, *et al*. Epidemiology and microbiology of surgical wound infections. J Clin Microbiol 2000;38:918-22.
7. Paul W, Sharma CP. Chitosan and alginate wound dressings: A short review. Trends Biomater Artificial Organs 2004;18:18-23.
8. Douglas M, Alan LM, Support for wound healing. Alter Med Rev 2003;8:359-60.
9. Indian Herbal Pharmacopoeia. RRR Jammu Tawai and IDMA Mumbai: 1999. p. 47-9.
10. Pharmacopoeia of India, 4th ed, Vol. 1, New Delhi: Government of India, Ministry of Health and Family Welfare; 1985. Appendix 3:3:10, p. 69.
11. Murray PR, Baron EJ, Pfallar MA, Tenover FC, Yolke RH. Manual of clinical microbiology. 6th ed. Washington, DC: ASM; 1995.
12. Organization for Economic Co-operation and Development, revised draft guidelines 423, "OECD Guideline for the testing of chemicals" Revised document – October 2000.
13. Werner S, Breededen M, Hubner G, Greenhalgh DG, Longaker MT. Introduction of keratinocyte growth factor expression is reduced and delayed during wound healing in the genetically diabetic mouse. J Investig Dermatol 1994;103:469-73.
14. Ehrlich HP, Hunt TK. The effect of cortisone and anabolic steroids on the tensile strength of healing wounds. Ann Surg 1968;167:324.
15. Lee KH. Studies on the mechanism of action of salicylate retardation of wound healing by aspirin. J Pharma Sci 1968;57:1042-3.
16. Dispasquale G, Meli A. Effect of body weight changes on the formation of cotton pellet induced granuloma. J Pharm Pharmacol 1965;17:379-82.
17. Rathi BS, Bodhankar SL, Baheti AM. Evaluation of aqueous extract of *Moringa oleifera* for wound healing in albino rats. Indian J Exp Biol 2006;44:898-901.
18. Nayak BS, Anderson M, Pereria Pinto LM. Evaluation of wound healing potential of *Catharanthus roseus* leaf extract in rats. Fitoterapia 2007;78:540-4.
19. Patil MB, Jalalpure SS, Ali A. Preliminary phytochemical investigation and wound healing activity of leaves of *Argemone maxicana* Linn. (Papaveraceae). Indian Drug 2001;38:288-93.

Source of Support: Nil, **Conflict of Interest:** None declared.