



Research Journal of
Phytochemistry

ISSN 1819-3471



Academic
Journals Inc.

www.academicjournals.com

Antimicrobial and Wound Healing Potential of *Canthium coromandelicum* Leaf Extract-A Preliminary Study

¹S. Chandra Mohan, ²K. Sasikala and ³T. Anand

¹Research and Development Center, Bharathiar University, Coimbatore, Tamil Nadu, 641 046, India

²Department of Chemistry, Saranathan College of Engineering, Tiruchirappalli, Tamil Nadu, 620 012, India

³Lab Administration, Thyrocare Technologies Ltd., Turbhe, Navi Mumbai, 400703, India

Corresponding Author: S. Chandra Mohan, Research and Development Center, Bharathiar University, Coimbatore, Tamil Nadu, 641046, India Tel: +917871411720

ABSTRACT

The objective of this study was to evaluate the wound healing potential of the ethanolic extract of *Canthium coromandelicum*. The study was done on male albino rats using excision model. The ethanolic extract, treated wounds were found to epithelize faster and the rate of wound contraction was significantly increased as compared to control and standard wounds ($p < 0.001$ and $p < 0.01$). From the results, the ethanolic extract of *Canthium coromandelicum* had greater wound healing activity than the standard ointment Cipladine. The enhanced wound healing activity of alcoholic extract may be due to free radical scavenging action and the antibacterial property of the phyto constituents (viz., tannins and flavonoids) present in it which either due to their individual or additive effect fastens the process of wound healing. Presence of flavonoids and tannins in alcohol extract was also confirmed by preliminary phytochemical investigation.

Key words: Wound healing activity, phyto constituent, alcohol extract, excision model

INTRODUCTION

Our skin acts as a protective layer from surroundings. The opening or break of skin leads to wounds. Wound healing is complex process that proceeds in three major path viz. inflammation, cellular proliferation and remodeling (Glynn, 1981; Clark, 1996). Healing needs the combined efforts of different tissues and cell lineages (Martin, 1997). Wound healing involves blood clotting, fibrin formation and inflammation. Healing is not complete until the disrupted surfaces are firmly knit by collagen (Buffoni *et al.*, 1993). The basic aim of wound healing is to reduce tissue damage and proper environment to restore the affected wound area (Pierce and Mustoe, 1995). The mechanism of wound repairing means reconstruction of damaged tissues (Phillips *et al.*, 1991). Wound repairing drugs are still limited in drug industry (Udupa *et al.*, 1995). However, the major problem of management of wound healing is the cost effect of therapy and its side effects. (Suh *et al.*, 1998). The Reactive Oxygen Species (ROS) are deleterious to wound healing process due to the harmful effects on cells and tissues. Absorbable synthetic biomaterials are considered to be degraded via ROS (Aliyev *et al.*, 2004). Inflammation results in a coordinated influx of neutrophils at the wound site. Wound related non-phagocytic cells also generate free radicals by involving non-phagocytic NAD(P)H oxidase mechanism (Griendling *et al.*, 2000). Thus, the wound site is rich in free radicals and the presence of free radicals will result in oxidative stress leading to lipid peroxidation, DNA breakage. Evidence for the role of oxidants in the pathogenesis of many diseases

suggests that antioxidants may be of therapeutic use in these conditions. Topical applications of compounds with free-radical-scavenging properties in have shown to improve significantly wound healing and protect tissues from oxidative damage (Thiem and Grosslinka, 2004). Medicinal preparations of plant origin are widely used for the treatment of various diseases of skin. This treatment is especially effective in the case of chronic disorders, since phytotherapy can be carried out over a long period of time without risk of inducing side effects. Most part of the phyto components are non toxic and provide highly effective treatment of many diseases. Many ayurvedic herbal plants have a important role in the process of wound healing. Plants heal the wounds in natural way. The healing process can be monitored by evaluating the rate of contract of the wound. In this present study, *Canthium coromandelicum* leaves were evaluated for wound healing activity.

MATERIALS AND METHODS

Plant materials: The plants were authenticated by Dr. S. Singaravadivel, Senior Scientist, Head of the Department, Department of Microbiology, Indian Institute of Crop Processing Technology, Thanjavur, Tamil Nadu, India. The plant *Canthium coromandelicum* leaves were dried and extracted with ethanol using soxhlet apparatus and prepared as paste.

Phytochemical analysis: Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Mohan *et al.* (2012a).

Microorganisms: *Staphylococcus aureus* (Gram positive), *Escherichia coli* (Gram negative) and *Bacillus subtilis* (Gram positive) and *Candida albicans* were the microorganisms used and they were obtained from the Microbiology Laboratory of the Thanjavur Medical College Hospital, Thanjavur. These microorganisms were identified and confirmed by Microbiologists, Department of Microbiology, Thanjavur Medical College, Thanjavur.

Antimicrobial assay: Antibioqram was done by disc diffusion method (NCCLS, 1993; Awoyinka *et al.*, 2007) using plant extracts. Petri plates were prepared by pouring 30 mL of NA/PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 min. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* were spread on Nutrient agar plates for bacteria and *Candida albicans* was spread on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50, 100 and 150 μ L) were laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24 h for the bacteria and at room temperature (30 \pm 1°C) for 24-48 h for yeasts strains. Each sample was tested in triplicate.

Measurement of zone of inhibition: The antimicrobial potential of test compounds were determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the extracts were measured using a millimeter scale.

Animals: Male albino rats of Wistar strain approximately weighing 180-200 g were used in this study. Male albino rats were purchased from Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature $27\pm 2^{\circ}\text{C}$ and 12 h light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided *ad libitum*. Before one week to the experiment, animals were accustomed to new climate and surroundings. The animal feed composition is crude protein (22.3%), crude oil (4.01%), crude fiber (4.02%), ash (8.02%) and sand silica (1.02%).

Method of ointment preparation: In this preparation 1 g of suitable extract is mixed with 10 g of ointment base (10%). Then it is stirred well until homogenous base is obtained.

Wound healing experiments: The wound site was prepared following the excision wound model (Opara, 1999). The animals were anaesthetized with diethyl ether and the hairs on the skin of the back, shaved with sterilized razor blades. The dorsal fur was shaved and a full thickness of the excision wound of 1.5 cm in width and 0.2 cm depth was done. After complete wounding, wound was washed and cleaned out with normal saline solution and the medicated cream was applied.

Experimental protocol: Animals bearing partial thickness wound were distributed into various groups such as Group I served as control, Group II served as extract and Group III served as standard treated groups. Each group had six animals. Standard drug povidone iodine (Commercially called as CIPLADINE) was selected as standard drug for the comparison of wound healing actions in experimental animals.

Wound treatment: The wounds of the animals were treated topically using the extracts. Group 2 was treated with the plant extract of *Canthium coromandelicum*. Group 3 (positive group) was treated with the standard drug purchased from medical store. It is a broad spectrum antibiotic used in the treatment and prevention of local infections of the teat, hoof and skin diseases in animals. While group I which was serve as the negative control group was left untreated.

Wound measurement: The measurement of the wound areas was taken from the day of the excision of the wound and every two or three days interval until the epithelization of the wound was completed. The area of the wound contraction was measured in different treated and control group on 3rd, 6th ,9th and 12th day. Wound contraction which contributes to wound closure was studied by tracing of the site. A meter ruler was placed over the wound and the estimate taken to produce a scale calculation. The percentage of wound closure was calculated as follows using the initial and final area drawn on glass slides during the experiments (Wall *et al.*, 2002):

$$\% \text{ of wound healing} = \frac{(\text{Wound area on day 0} \times \text{wound area on day n})}{\text{Wound area on day 0}} \times 100$$

Where n is a No. of days (3rd, 6th ,9th and 12th day).

RESULTS AND DISCUSSION

In the present study the phytochemical screening (Table1), antimicrobial activity and the rate of wound contraction by excision wound model was studied. The diameter sizes in mm of the zone of inhibition are shown in the Table 2. The area of wound healing expressed in percentages given

Table 1: Phytochemical screening of *Canthium coromandelicum*

Phytochemicals	Ethanollic extract
Alkaloids	Absent
Flavonoids	Present
Glycosides	Present
Phenols	Present
Tannins	Present

Table 2: Antimicrobial activity of *Canthium coromandelicum* extract

Plant extract (µL)	<i>Staphylococcus aureus</i> (mm)	<i>Bacillus subtilis</i> (mm)	<i>Escherchia coli</i> (mm)	<i>Candida albicans</i> (mm)
50	4±0.02	2±0.03	2±0.03	2±0.02
100	6±0.04	5±0.04	5±0.04	4±0.03
150	9±0.05	8±0.06	8±0.06	6±0.04

Values were expressed as Mean±SD

Table 3: Percentage of wound healing activity of *Canthium coromandelicum*

Groups	Days				
	0	3	6	9	12
Control	-	23.17±1.15	36.66±1.83	56.48±2.82	78.07±3.72
Plant extract	-	35.69±1.78	46.24±2.31	89.14±3.95	98.97±4.86*#
Standard	-	32.58±1.62	45.48±2.17	73.97±3.69	90.46±3.98

Values were expressed as Mean±SD for six rats in each group, *Significantly different from control (p<0.001). # Significantly different from standard (p<0.01)

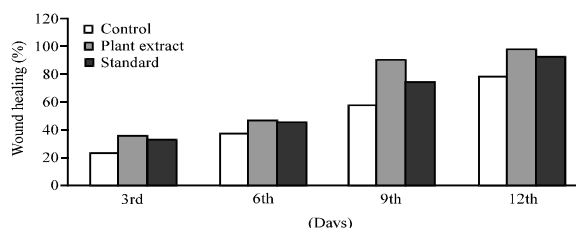


Fig. 1: Percentage of wound healing activity of *Canthium coromandelicum*

in the Table 3. The graphical representation of area of wound healing present in Fig. 1 and the photographic representation in Fig. 2(a-f). The percentage of wound contraction includes by recording the changes in wound area at fixed intervals of time, viz., 3rd, 6th, 9th and 12th day after treated with extract. However, on 12th post wounding day, Group I animal showed 78.07% of healing which may be due to self immunity of animal whereas the standard treated group (Group II) showed 98.97% healing and the extract treated group (Group III) showed 90.46% healing. When obtained result compared with standard and control, the activity of the extract was found to be significant (p<0.01; p<0.001). Wound healing normally involves an initial inflammatory phase followed by fibroblast proliferation, formation of collagen fibers and shrinking, occurring concurrently but independent of one another. Several plants are having wound healing potential. Phytoconstituents such as flavonoids, glycosides and tannins are reported to have wound healing property (Raina *et al.*, 2008; Venkatanarayana *et al.*, 2010). Wound healing effect is also attributed to free radical scavenging activity of flavonoids. Lipid peroxidation refers to the

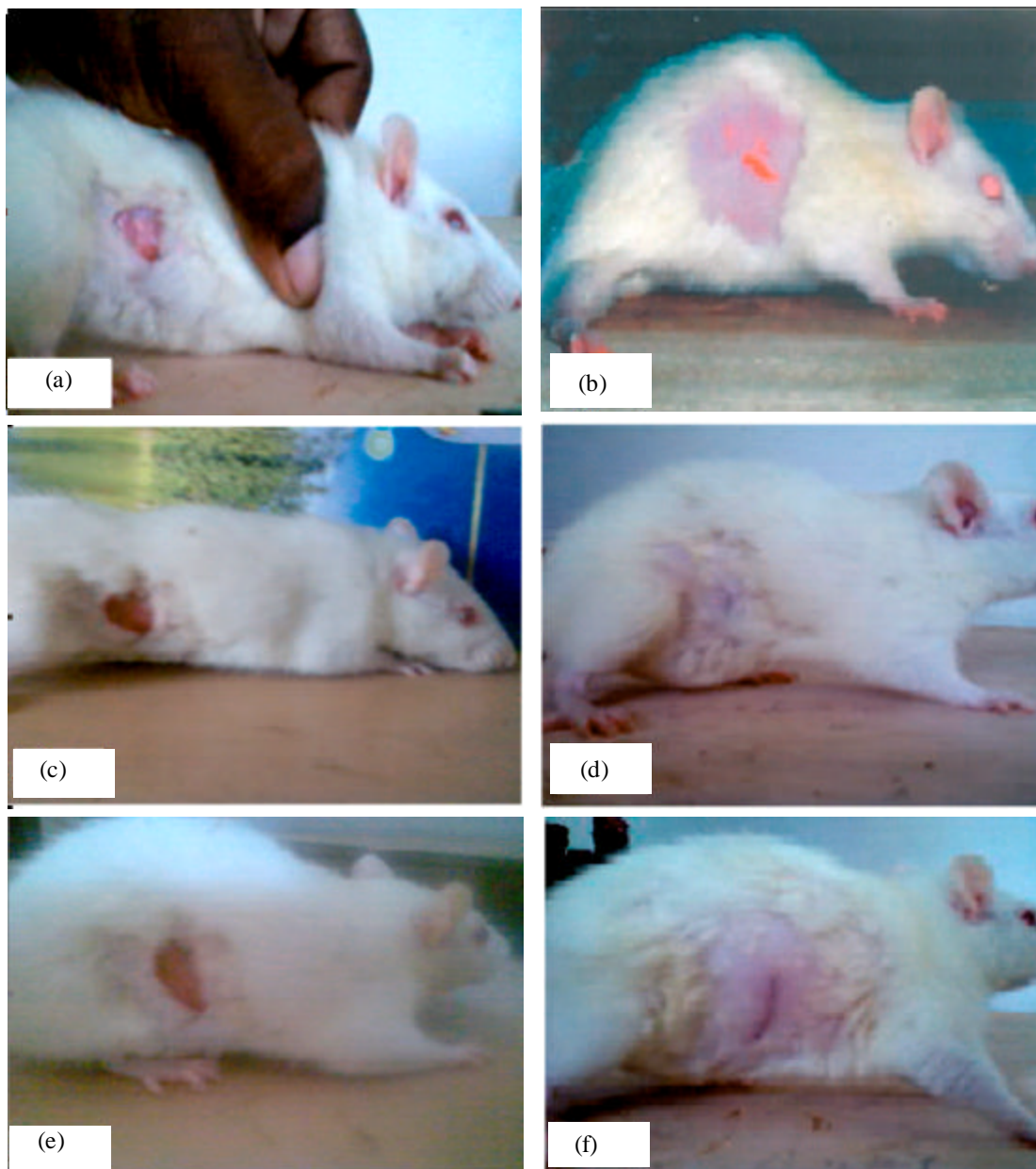


Fig. 2(a-f): Wound healing activity of *Canthium coromandelicum*, (a) Control-0th day (b) Control-12th day, (c) Plant extract-0th day, (d) Plant extract treated rat-12th day, (e) Standard drug 0th day and (f) Standard drug treated rat 12th day

oxidative degradation of lipids, the final products of lipid peroxidation may produce cancer (Mohan *et al.*, 2012b). The process of lipid peroxidation is important in burns, wounds and skin ulcers. It is believed that the increase in strength of collagen fibers by any drug that stops lipid peroxidation. Flavonoids, glycosides and tannins are known to promote wound healing process mainly by their astringent and antimicrobial property (Vinothapooshan and Sundar, 2010; Venkatanarayana *et al.*, 2010).

CONCLUSION

Preliminary phytochemical analysis of the leaves of *Canthium coromandelicum* revealed presence of flavonoids, tannins, phenolic compounds and glycosides. Presence of flavonoids and tannins in extracts of leaves of *Canthium coromandelicum* may be responsible for its wound healing activity. The present study demonstrated that *Canthium coromandelicum* leaves extract was capable of promoting wound healing activity. Enhanced wound contractions suggest that *Canthium coromandelicum* leaves extract has potential in the management of wound healing when compared to standard.

REFERENCES

- Aliyev, E., U. Sakallioğlu, Z. Eren and G. Acikgoz, 2004. The effect of polylactide membranes on the levels of reactive oxygen species in periodontal flaps during wound healing. *Biomaterials*, 25: 4633-4637.
- Awoyinka, A.O., I.O. Balogun and A.A. Ogunnowo, 2007. Phytochemical screening and *in vitro* bioactivity of *Cnidioscolus aconitifolius* (Euphorbiaceae). *J. Med. Plant Res.*, 3: 63-65.
- Buffoni, F., G. Banchelli, S. Cambi, G. Ignesti, R. Pirisino, L. Raimondi and G. Vannelli, 1993. Skin wound healing: Some biochemical parameters in Guinea-pig. *J. Pharm. Pharmacol.*, 45: 784-790.
- Clark, R.A.F., 1996. Wound Repair: Overview and General Considerations. In: *Molecular and Cellular Biology of Wound Repair*, Clark, R.A.F. (Ed.). Plenum Press, New York, pp: 3-50.
- Glynn, L.E., 1981. The Pathology of Scar Tissue Formation. In: *Tissue Repair and Regeneration*, Glynn, L.E. (Ed.). Elsevier/North Holland Biomedical Press, Amsterdam, The Netherlands.
- Griendling, K.K., D. Sorescu and M. Ushio-Fukai, 2000. NAD(P)H oxidase: Role in cardiovascular biology and disease. *Circ. Res.*, 86: 494-501.
- Martin, P., 1997. Wound healing: Aiming for perfect skin regeneration. *Science*, 276: 75-81.
- Mohan, C.S., S. Dinakar, T. Anand, R. Elayaraja and B. Sathiyapriya, 2012a. Phytochemical, GC-MS analysis and antibacterial activity of a medicinal plant *Acalypha indica*. *Int. J. Pharm. Tech. Res.*, 4: 1050-1054.
- Mohan, C.S., V. Balamurugan, R. Elayaraja and A.S. Prabakaran, 2012b. Antioxidant and phytochemical potential of medicinal plant *kalanchoe pinnata*. *Int. J. Pharm. Sci. Res.*, 3: 881-885.
- NCCLS, 1993. Performance Standards for Antimicrobial Disk susceptibility Tests Approved Standard M2-A5. 5th Edn., NCCLS., Villanova, Pa.
- Opara, I.C., 1999. A preliminary investigation into the antiemetic properties of the leaf extract of *Ocimum gratissimum*. D.V.M. Thesis, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria.
- Phillips, G.D., R.A. Whitehead and D.R. Kinghton, 1991. Initiation and pattern of angiogenesis in wound healing in the rat. *Am. J. Anat.*, 192: 257-262.
- Pierce, G.F. and T.A. Muston, 1995. Pharmacologic enhancement of wound healing. *Annu. Rev. Med.*, 46: 467-481.
- Raina, R., S. Prawez, P.K. Verma and N.K. Pankaj, 2008. Medicinal plants and their role in wound healing. *Vet. Scan*, 3: 1-25.
- Suh, D.D., I.P. Schwartz, D.A. Canning, H.M. Snyder, S.A. Zderic and A.J. Kirsch, 1998. Comparison of dermal and epithelial approaches to laser tissue soldering for skin flap closure. *Lasers Surg. Med.*, 22: 268-274.

- Thiem, B. and O. Grosslinka, 2004. Antimicrobial activity of *Rubus chamaemorus* leaves. *Fitoterapia*, 75: 93-95.
- Udupa, A.L., D.R. Kulkarni and S.L. Udupa, 1995. Effect of *Tridax procumbens* extracts on wound healing. *Int. J. Pharmacognosy*, 33: 37-40.
- Venkatanarayana, D., A.S. Kumar and S.M. Lakshmi, 2010. Review on natural wound healing agents. *Int. J. Phytopharm. Res.*, 1: 1-4.
- Vinothapooshan, G. and K. Sundar, 2010. Wound healing effect of various extracts of *Adhatoda vasica*. *Int. J. Pharma Bio Sci.*, 1: 530-536.
- Wall, S.J., D. Bevan, D.W. Thomas, K.G. Harding, D.R. Edwards and G. Murphy, 2002. Differential expression of matrix metalloproteinases during impaired wound healing of the diabetes mouse. *J. Invest. Dermatol.*, 119: 91-98.