RESEARCH ARTICLE

COMPARATIVE WOUND HEALING ACTIVITY OF PLANT *ICHNOCARPUS FRUTESCENS* (L.) W. T. AITON

***Meher Ashutosh[1], Padhan Amiya Ranjan, Mohapatra Tapas. Kumar, Agrahari Anuj kumar, Nayak Reena Rani, Nanda Bikash Kumar, Prusty Debasish**

The Pharmaceutical College, Samaleshwari vihar, Tingipali, Barpali - 768029, Bargarh, Odisha, India

***For Correspondence:** Ashutosh Meher, Asst. Prof., The Pharmaceutical College, Department of Pharmacognosy, Samaleswari vihar, Tingipali, Barpali - 768 029, Dist. Bargarh, Odisha, India, E-Mail - mashumeher@gmail.com, mashu_meher33@yahoo.co.in, Contact : 09861007801*

Received 15 Nov 2012; Review Completed 30 Nov 2012; Accepted 28 Dec 2012, Available online 15 Jan 2013

ABSTRACT

The present work objective are to investigate indigenous plants used in wound healing in India, we hereby reported our findings related to wound healing activities of plants *Ichnocarpus frutescens* (L.) W. T. Aiton *in vivo* model studies. The plant hydro-alcoholic (Ethanol 70:Water 30) extracts of the leaves, stems and roots of *Ichnocarpus frutescens* was identified and tested wound healing activity. The important secondary metabolites like Ursolic acid, Kaempferol, Trifolin, Mannitol, Flavon, Phenolic acid, Triterpene glycoside(1,4-β-D-glucopyranosyl-(1,3) α-amyrin, β –Sitosterol, alkaloid and Flavonoid identified in extracts. *Ichnocarpus frutescens* roots hydro-alcoholic extract showed wound healing activity significantly in the excision wound model in rats on topical application. The animals were divided into five groups with six rats in each group. Topically applied *Ichnocarpus frutescens* leaves, stems and roots extracts ointment. Taking povidone iodine ointment as standard. The results showed that hydro-alcoholic extract of *Ichnocarpus frutescens* stems on topical application was reduced the scar area from 2.5±1.5 to 0.0±0.0 cm^2, hydro-alcoholic extract of *Ichnocarpus frutescens* leaves on topical application was reduced the scar area from 2.5±1.5 to 0.2±0.0 cm^2 , hydro-alcoholic extract of *Ichnocarpus frutescens* roots on topical application was reduced the scar area from 2.5±1.5 to 0.7±0.4 cm^2, control on topical application was reduced the scar area from 2.5±1.5 to 1.2±1.0 cm^2 and standard povidon iodine ointment on topical application was reduced the scar area from 2.5±1.5 to 0.5±0.02cm^2 respectively. Significant increase in tensile strength were also observed and compared to the control and povidon iodine. The above result revealed that the hydro-alcoholic stems extract has remarkable wound healing potency and appear to justify the traditional use of *Ichnocarpus frutescens* in wound healing in India and offer a scientific support to the treatment of traditional healers.

Keywords: *Ichnocarpus frutescens*, Hydro-alcoholic, Wound healing, Wistar rat, povidone iodine

INTRODUCTION

Wound infection is one of the most common diseases in developing countries because of poor hygienic conditions[1]. Research on wound healing agents is one of the developing areas in modern biomedical sciences and many traditional practitioners across the world particularly in countries like India and China have valuable information of many lesser known hitherto unknown wild plants for treating wounds and burns[2]. Many medicinal plants are claimed to be useful for wound healing in the traditional system of medicine. These plant remedies are used since ancient times even if the mechanism of action and efficacy of very few of them have been evaluated scientifically[3].Wounds are the physical injuries that result in an opening or breaking of the skin and appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin[4]. In other words wound is a break in the epithelial integrity of the skin and may be accompanied by disruption of the structure and function of underlying normal tissue and may also result from a contusion, haematoma, laceration or an abrasion[5]. Healing of wounds starts from the moment of injury and can continue for varying periods of time depending on the extent of wounding and the process can be broadly categorized into three stages; inflammatory phase, proliferate phase, and finally the remodeling phase which ultimately determines the strength and appearance of the healed tissue[6]. Wound healing process holds several steps which involve coagulation, inflammation, formation of granulation tissue, matrix formation, remodeling of connective tissue, collagenization and aquisation of wound strength[7]. A lot of research work has been carried out to develop better healing agents and it has been a challenging task to the researchers to keep up the pace with problems encountered. Presently scientists are keen to evaluate drugs from plant origin. It is due to their specific healing property and nontoxic action. Medicinal herbs are an indispensible part of traditional medicine. The *Ichnocarpus frutescens* (L.) W. T. Aiton finds an important place in indigenous medicine used as in rheumatism, asthma, cholera, fever, tumour, snake bite, protection of liver cell in acetaminophen over dose, analgesic, anti-inflammatory and correct hyperlipidemia in diabetic rats and it has also capacity to improve glucose tolerance in diabetis, lower fasting glucose. However to the best of our knowledge a systematic study on wound healing activity of *Ichnocarpus frutescens* has not been undertaken. Hence, the present study was undertaken to evaluate the wound healing property of hydro-alcoholic extract of *Ichnocarpus frutescens* leaves, stems and roots and to study comparision influence wound healing on animal wound models in Wistar rats[8].

MATERIALS AND METHODS

Plant material

The plant species for the proposed study material is *Ichnocarpus frutescens* have been collected in the month of October 2011 from the adjoining area of Barpali Dist. Bargarh, Odisha, India. The plant species was identified and authenticated by Botanical survey of India, Central National Herbarium Howrah, Kolkata. The plant authentication no. (CNH/II(5)/2009/Tech.II/35).

Preparation of plant extract

The shade dried leaves, stems and roots of *Ichnocarpus frutescens* were crushed into small pieces and powdered. The powder was loaded into soxhlet extractor in 8 batches of 250 g each and was subjected to extraction for about 30–40 h with hydro-alcoholic (Ethanol 70:water 30). After extraction the extracts were dried using a rotary vacuum evaporator (BUCHI, GERMANY). Then it was stored in the dessicator. The yield was about 13.3%.

Evaluation of Physical parameter

Physical parameters like Total ash, Water soluble ash, Acid insoluble ash, Moisture content, Water soluble, Alcohol soluble and P^H of powder drug were analyzed as per the standard procedure mentioned in Indian Pharmacopoeia and recorded in table -1

Table 1: Physical parameters of *Ichnocarpus frutescens* whole plant

S. N.	Constant	Whole plant (%)
1	Total ash	7.0±0.02
2	Water soluble ash	4.1±0.05
3	Acid insoluble ash	3.1±0.07
4	Moisture content	7.96±0.01
5	Water soluble	21.2±0.10
6	Alcohol soluble	13.2±0.33
7	P^H of 1% solution	6.15
8	P^H of 10% solution	5.93

Animals and animal grouping

Animals

This was done as per the guidelines set by the Indian National Science Academy New Delhi, India. Twelve-week-old healthy Wistar rats (150–200 g) of either sex bred locally in the animal house of TPC, Barpali were selected for the study. They were housed under controlled conditions of temperature of 23 ± 2°C, humidity of 50 ± 5% and 10–14 h of light and dark cycles respectively. The animals were housed individually in polypropylene cages containing sterile paddy husk (procured locally) as bedding throughout the experiment and had free access to sterile food (animal chow) (M/s Hindustan Lever Ltd.) and water *ad libitum*. Animal were allowed to recover and housed individually in a plastic cages containing sterilized paper cutting[9,10].

Preparation of ointment

Hydro-alcoholic extracts ointments were prepared separately by incorporating dried extract in simple ointment base USP for topical use[6].

Animal grouping

In the experiment, the rats were divided into five groups and in each group consisting of six animals, where group- I was kept as control, group-II animals were locally applied with povidone iodine ointment, group-III were locally applied with ointment of extract of *Ichnocarpus frutescence* leaves, group-IV animals with locally applied with ointment of extract of *Ichnocarpus frutescence* roots and group-V animal were locally applied with ointment of extract of *Ichnocarpus frutescence* stems[9,10].

Wound healing activity

The excision wound models were used for study of wound healing activity of plant extracts.

Excision wound model

An excision wound model was used for studying wound healing activity as describes according to Rashed *et al.*, 2003 and Nagappa *et al.*, 2001 with some modification. Animals were anesthetized prior to and during creation of the wounds with 1 ml of intravenous ketamine hydrochloride (10 mg/kg body wt). Hair was removed by shaving the nape of the back of all the rats. Ethanol (70%) was used as antiseptic for the shaved region before making the wound. A full thickness of the excision wound of uniform 2 cm. diameter circular area was created along the markings using toothed forceps, scalpel and pointed scissors. The wound was left undressed to the open environment and no local or systemic anti-microbial agents were used. The rats were distributed in groups randomly and each rat was placed in a separate cage. The albino rats were divided into groups and extracts were applied once times daily starting from the excisions till complete epithelization. Contractions, which contribute for wound closure in first 2 week was studied by tracing the raw wound. Wound area was measured by retracing the wound on a centi meter scale. The degree of wound healing was calculated using formula: 1− (wound area on corresponding day/wound area on zero days)×100. The number of days for complete epithelization was noted (Nagappa *et al.*, 2001)[3,9-19]

Statistical data analysis

Results were expressed as mean ± SEM. Statistical comparison were made by using Student's t-test analysis and difference were considered statistically significant when P-value were <0.05.

 ISSN: 2250-1177 CODEN (USA): JDDTAO

Table 2: Effect of applied *Ichnocarpus frutescence* extracts on excision wound model in rats. Contraction of excision wound area (cm^2) after days.

Group	Animal Treatment	1st Day	5th Day	9th Day	14th Day
Group-I	Control (White soft paraffin)	2.5±1.5	2.3±1.6	1.9±1.6	1.2±1.0
Group-II	Standard (povidone iodine)	2.5±1.5	1.9±1.0	1.2±0.6	0.5±0.2
Group-III	Hydro-alcoholic extract of leaves	2.5±1.5	1.4±0.8	1.2±0.9	0.2±0.0
Group-IV	Hydro-alcoholic extract of stems	2.5±1.5	1.1±0.6	0.7±0.4	0.0±0.0
Group-V	Hydro-alcoholic extract of roots	2.5±1.5	1.5±0.7	1.3±0.8	0.7±0.4

Values are mean ±SEM (n=6) statically significant difference in comparison with control group: P< 0.05.

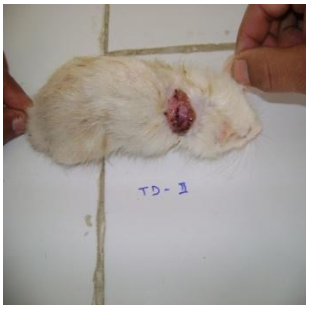
Once a day, for 14 day; control, no treatment.

Day 1:-

White soft paraffin

Povidone iodine

***Ichnocarpus frutescence* leaves**



***Ichnocarpus frutescence* stems**

***Ichnocarpus frutescence* roots**

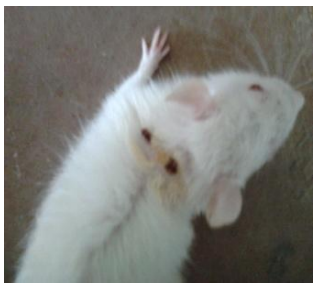
***Ichnocarpus frutescence* leaves**

***Ichnocarpus frutescence* stems**

***Ichnocarpus frutescence* roots**

Day 9:-

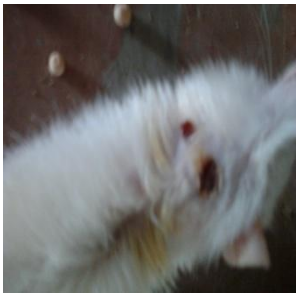
White soft paraffin



Povidone iodine

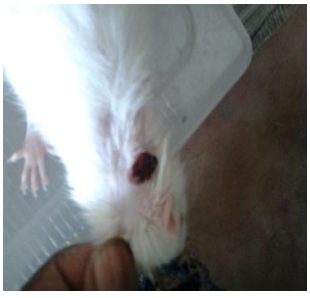
Day 5:-

White soft paraffin



Povidone iodine

***Ichnocarpus frutescence* leaves**



***Ichnocarpus frutescence* stems**

 ISSN: 2250-1177 CODEN (USA): JDDTAO

***Ichnocarpus frutescence* roots**

Day 14:-

White soft paraffin

Povodone iodine

***Ichnocarpus frutescence* leaves**

***Ichnocarpus frutescence* stems**

***Ichnocarpus frutescence* roots**

RESULT AND DISCUSSION

Plant extracts have been used for many thousand years, in food preservation, pharmaceuticals,alternative medicines and natural therapies.it is necessary to investigate those plant scientifically which have been used in traditional medicine to improve quality of health care[5]. The extraction of biologically active compound from plant material depend upon the solvent used in extraction procedure. The most commonly used solvents for investigations of wound healing activity in plants are ethanol and methanol. Most of the antibacterial active compound that have been identified were soluble in polar solvent such as ethanol and methanol instead of water[(4)]. Plant extracts are potential source of novel wound healing agents. Wound healing and repair is a natural process of regenerating dermal and epidermal tissue and may be categorized into 3 phases Inflammation, proliferation and remodeling phase. The capacity of wound to heal depends ,in part, on its depth, as well as on the overall health and nutritional and bundles form between cells[(12)]. On the basis of the results finding in the present investigation, it is concluded that the hydro-alcoholic extract of stems of plant *Ichnocarpus frutescence* has highest wound healing activity. Also the hydro-alcoholic extract of leaves of *Ichnocarpus frutescence* shows remarkable wound healing activity in compare to control and standard. Wound healing activity of the hydro-alcoholic extract may be due to the individual or combined effect of the above phytochemicals. Comprehensive evaluation on the plants with wound healing activity on the basis of traditional medicine may possibly give new compounds that could be used as prominent drugs in wound healing therapy. Further investigations are needed for identification of active principles responsible for the wound healing activity. The present investigation offers a scientific support to the traditional healer account in use of the plant *Ichnocarpus frutescence* for treatment of cuts and wounds. Further study on the analysis of the most active extracts using NMR, Mass spectroscopy,LC-MS,HPLC,etc will help in isolation of the therapeutic agents.

ACKNOWLEDGEMENT

The authors are thankful to The pharmaceutical college Barpali ,Odisha, for providing facilities and technical assistance.

CONFLICT OF INTEREST

There is no conflict of interest in relation to the publication on manuscript file.

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 ISSN: 2250-1177 CODEN (USA): JDDTAO