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Research Article

WOUND HEALING ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACT OF *HOUTTUYNIA CORDATA* LEAVES IINDIGENOUS TO NORTH-EAST INDIA

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ABSTRACT

The present study objective are to investigate indigenous plant used in wound healing in northeast India, we hereby reported our findings related to wound healing activities of plant leaves extracts from *in vitro* and *in vivo* model in rats. The aqueous and ethanol extracts of the leaves of *H. cordata* was tested for phytochemical constituents and wound healing activity against rats. The animals were divided into four groups with six rats in each group. Topically applied 10% w/v of plant *H. cordata* leaves extracts in saline taking 0.2% w/w nitrofurazone, ointment as standard. The ethanol extract of *H. cordata* leaves on topical application significantly reduced the epithelization period as well as decrease in the scar area. The tensile strength and hydroxyproline content of plant extract treated group significantly increased when compared with control and standard group animals. The finding of studies revealed that the plant has potential wound healing activity and to justify the traditional use in wound healing in India.

Keywords: Excision; Incision; *Houttuynia cordata*; Epithelization period; Scar area

1. Introduction

North-East India is a mega bio-diversity centre and origin of many important cultivated plant species. The people belonging to this region have been using different traditional medicinal plants for the treatment of various diseases such as liver damage, dysentery, stomach disorder wound healing etc., and thus they have achieved much knowledge regarding the use of different ethnomedicinal plants. In India, the uses of 2416 plants of ethnomedical purposes has already been documented, and out of these 1953 plant species are used by different ethnic groups of the North East region of the country alone (Sajem and Gosai, 2006).

Wound healing defined as loss destruction or breaking of anatomic and cellular or functional integrity of leaving tissues. Wound healing process involves sequential steps, which involves coagulation, inflammation, formation of granulation tissue, matrix formation, remodeling of connective tissue, collagenization and aquisation of wound strength. In formation of new tissue, endothelial cells proliferate and form new blood vessels. Different types of synthetic drugs are available which improve the wound healing activity. Villagers traditionally use the medicinal plant based paste prepared from different crude extracts to treat a variety of skin ailments including wounds. There are various researches and reports stating that the extracts of several plants, used for wound healing. (Diwan et al., 1982; Udupa et al., 1989; Suguna et al., 1996; Saha et al., 1997; Sunil kumar et al., 1998; Rasik et al., 1999; Shukla et al., 1999; Mukherjee and Suresh, 2000; Park and Chun, 2001; Nagappa and Cheriyan, 2001; Govindarajan et al., 2004; Perez Gutierrez and Vargas, 2006; Stephen et al., 2010; Verma et al., 2011, 2014)

The scientific community and research on wound healing agents through the plants is major developing areas in modern biomedical sciences. Traditionally many plants are used for wound healing has recently more attention to researcher (Houghton et al., 2005). The wound healing properties in the traditional Indian and Chinese system of medicine having plant remedies (both single plant and multi-herbal reparations) are used since ancient times but few of them have been scientifically evaluated the efficacy and mechanism of action.

Houttuynia cordata Thunb (locally known as Masandari) belongs to the family-Saururaceae. Traditionally in Chinese indigenous systems of medicine plant has been used for the treatment of inflammatory diseases such as

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ulcerative colitis (Jiang et al., 2004), cough, leucorrhea and ureteritis so on (Ji and Zhao, 2003; Zhou et al., 2003; Sun et al., 2004), antiviral and antibacterial Hayashi et al., 1995; Lu et al., 2006), antiallergic (Lee et al., 2008; Li et al., 2005), antioxidant (Chen et al., 2003; Toda et al., 2005) and antimutagenic activities. (Chen et al., 2003) Fresh plants steam distillate of *Houttuynia cordata* Thunb. possessed direct inhibitory activity against herpes simplex virus type 1 (HSV-1), influenza virus and human immunodeficiency virus type 1 (HIV-1) without showing cytotoxicity (Hayashi et al., 1995). In china thus, *H. cordata* plant has been used as an Oriental medicine for the therapy of inflammatory diseases. (Jiang et al., 2004)
The plant *H. cordata* having major constituents of essential oil included methyl nonyl ketone, β-myrcene, β-pinene, α-pinene, α-terpineol and n-decanoic acid and the occurrence of the essential oil in this plant was associated with a possible role in HIV and antiinflammatory effects were also demonstrated (Hayashi et al., 1995; Lu et al., 2006).

Recently, the plant extract is reported to be efficacious in treating cancer and atopic dermatitis. (Kim et al., 2007; Lim at al., 2009)

In India, plant leaves are taken as salad and leaf juice is considered as tonic it is taken for stomachache, cholera, dysentery and liver diseases. The plant leaves paste is applied in measles, gonorrhoea and skin troubles. Traditionally the people of northeast India also used plant leaves as treatment in wounds.

Thus, the objective of the present study was an attempt to assess and explore the wound healing activity of *H. cordata* leaves and justify the traditional use.

2. Materials and Methods

2.1 Plant materials and Preparation of Extracts

The fresh tender leaves of *Houttuynia cordata* were collected from their natural habitats from nearby areas of Dibrugarh University, Assam. The plant was identified by Prof. P. J. Handique, Department of Biotechnology, Gawahati University, Guwahati, India. Voucher specimen was deposited at the Herbarium of the Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, India.

The collected plant materials were dried in shade and made course powder for the preparation of extract. The powdered plant materials were subjected to successive extraction with different solvent petroleum ether, ethyl acetate, water and ethanol and the water and ethanol extract was used for the study.

2.2 Phytochemical Analysis

The presence of phytoconstituents viz., glycosides, saponins, flavonoids, tannins, flavonoids (Shinoda-Pew test) and polyphenolic compounds **(**Folin-Ciocalteu's test) in the aqueous extracts of plants were done by usual methods prescribed in standard texts. (Liu, 2011; Harbon 1973; Wall et al., 1952)

2.3 Animals

Healthy Wistar albino rats either sex (180-200 g) were purchased from M/S Chakroborty Enterprises, 3/1 D, Grish Vidyaratan Lane Kolkata (WB) India were used in the experiment. These rats aged between 2 and 2.5 months were housed in animal house of department of pharmaceutical sciences in well ventilated stainless-steel cages at room temperature (25±2ºC) in hygienic condition under natural light and dark schedule with not more than four animals per cage. Animals were allowed free access to standard laboratory food diet and water were given *ad libitum*. All the experimental studies were approved from Institutional animal ethical committee (IAEC) and performed in accordance with the National Institute of Health's guideline for Survival Rodent Surgery (1985)

2.4. Animal groping

In present experiment, the rats were divided into four groups consisting six animals in each group. Group-I the control group animal applied simple ointment base (BP). Group-II standard group animals were locally applied 0.2% w/w nitrofurazone ointment (a standard antimicrobial agent in topical wound dressing). Group-III and Group-IV treated group animal applied 10% v/v aqueous extract (AEHC) and ethanol extract (EEHC) of *Houttuynia cordata* topically on rat wounds respectively. Wounds were created on the dorsal back of rats and treatments were continuing daily until the wounds completely healed.

2.5. Wound healing activity

The wound healing activity was studied by excision wound model and incision wound model mainly these two models were used for evaluation of potential wound healing activity.

2.5.1 *Excision wound model*

An excision wound model was used according to Rashed *et al.,* (2003) and Nagappa *et al.*, (2001) with slightly modification. An expression 1 cm away from vertebral column and 5 cm

away from ear using a round seal of 2.5 cm diameter biopsy punch was made on the dorsal thoracic region in anesthetized animals with 1 ml of intravenous ketamine hydrochloride (10 mg/kg body wt) prior to and during

creation of the wounds. The impression areas of skin was excised to the full thickness to generate a wound area of about 500 mm^2. Haemostasis was achieved by blotting the wound left undressed to the open environment with cotton swab soaked in normal saline. Randomly distributed the rats in different groups and each rat were placed in a separate cage. Contractions, which contribute for wound closure in first 2 weeks, were studied by tracing the raw wound and with the help of millimeter scale graph paper wound area were measured by retracing the wound.

The degree of wound healing was calculated using formula: 1− (wound area on corresponding day**/**wound area on zero days) ×100. Hydroxiproline content of collagen was measured using spectrophotometer at 557 nm. according to Shukla *et. al.* (1999) and the number of days for complete epithelization was noted.

2.5.2 *Incision wound model*

The incision wound model used according to Udupa *et al.,* (1995) Govindarajan *et al.,* (2004) Perez Gutierrez *et al.,* (2006) with little modification. The animals were divided into four groups (n=6). Rats were anesthetized and two para vertebral straight long incisions of 6 cm length each were made through the entire thickness of the skin, on either side of the vertebral column with the help of a sharp scalpel. After complete haemostasis the wound were closed by means of interrupted sutures placed with the help of surgical thread (No.000) and curves needle (No.11) and continuous threads on both wound edges were tightened for good wound closure. The tensile strength measured on 10^{th} day after removing sutures with the help of Tensile Testing Machine TKG-20 from the following equation:

$$\text{Tensile strength} = \frac{\text{total breaking load}}{\text{cross - sectional area}}$$

The mean tensile strength of the two para vertebral incisions on both sides of the animals was taken as the measures of the tensile strength of the wound for an individual animal. The tensile strength of treated group wounds was compared with the control and standard group (nitrofurozone ointment treated) wounds tensile strength. After tensile strength determination, further measured the epithelization period and scar area daily for 20 days.

2.6 Statistical data analysis

Results were expressed as mean ± SEM. Statistical comparison were made by One-way Analysis of Variance (ANOVA) followed by Bonferroni's multiple comparison tests using Graph Pad Prism software and P-value $P<0.05$ were considered statistically significant.

3. Results and Discussions

The preliminary phytochemical investigation of the plant extracts showed positive results for the presence of important secondary metabolites alkaloid, glycosides, carbohydrates, carbohydrates, tannins, polyphenols flavonoids and essential oil (Table-1).

Phytochemical work of ethanol extract of *Houttuynia cordata* leaves reveal that plant contains tannins, polyhenolic compound and flavones. In various researches, implied that tannin and flavonoids is one of the active compounds, which may be responsible for the antioxidant activity and scavenging effect might be one of the most important components of wound healing activity. Therefore, in present investigation the ethanol extract of plant which containing tannins, polyhenolic compound and flavones may be responsible to support wound healing property through enhance free radical scavenging action as well as enhanced antioxidant enzyme level in granuloma tissues.

Wound healing process involves extreme complex and well-arranged phenomenon in various phases such as granulation, collagenation, collagen maturation, scar maturation, migration and proliferation of both parenchymal and connective tissue cells, formation of extracellular matrix protein, remodeling of connective tissue parenchymal components and acquisition of wound strength which are concurrent but independent to each other. (Reddy *et al.,* 2002)

The single wound healing model is inadequate to confirm the wound healing activity of the drug because the process involved various phases and no *in vitro* experiment exists that collectively present and evaluate various components of wound healing processes. *In vivo* assay are highly suggested to evaluate and confirm the *in vitro* observation. Some of the *in vivo* assays include the determination of hydroxyproline content and tensile strength as an indication of quality of the healing significantly (Perez Gutierrez *et al.,* 2006). The major contribution of wound strength is collagen (protein extracellular matrix component) on breakdown of collagen liberates free hydroxyproline and measurement of hydroxyproline could be used as an index for the collagen turnover and wound healing activity.

The complete wound healing of both extract of *Houttuynia cordata* leaves were studied by two models in the number of treatment days and results are presented in Tables 2 and 3. (Figure 1 & 2) The animals treated with aqueous and ethanolic extract of plant shows no raw wound left after 16^{th} days and also measuring the tensile

strength of incision wound treatment continued upto 20th days.

The present work showed plant ethanolic extract possesses a good wound healing activity; there was a reduction in the epithelization time from 27.42±0.85 to 22.12±0.73 days and the scar area reduced on complete epithelization from 48.88±0.98 to 38.92±0.92 mm^2. The hydroxyproline content and tensile strength of control and ethanolic extract of plant treated group increased from 155.77±7.54 to 269.45±6.55 µg/100mg and from 282.54±7.44 to 351.58±8.28 g respectively are (P< 0.05) significant, results were compared to control and standard group on the 16th day of post healing.

A significant elevation in the hydroxyproline content of the granulation tissue of the animal treated with *Houttuynia cordata* ethanolic extract was recorded and compared with control group, thus indicating positive effect of the *S. spirale* ethanolic extract on collage synthesis hence it indicates wound healing activity. The increase in tensile strength of the granulation tissue indicated enhanced collagen maturation by increase cross-linking and increase in epithelization, as well as the tensile strength could be attributed the increased hydroxyproline content in the wound tissues (Stephen *et al.,* 2010). Thus the ethanolic extract of *Houttuynia cordata* leaves possesses good wound healing activity.

Table 1: Results of preliminary screening of plant extracts

Test for the presence of phytoconstituents	*Houttuynia cordata*
Polyphenols	+
Flavonoids	+
Alkaloid	+
Glycosides	+
Carbohydrates	+
Tannins	+
Essential oil	+

Table 2: Effect of topically applied *Houttuynia cordata* leaves aqueous and ethanol extracts on excision wound model in rats.

Animal Treatment Groups	**Contraction of excision wound area (mm^2) after days**				
	4th Day	**8th Day**	**12th Day**	**16th Day**	**20th Day**
Group-I Control	7.25±0.42	8.37±0.47	9.86±0.38	12.11±0.43	13.68±0.42
Group-II Standard Drug (0.2%w/w nitrofurazone)	8.84±0.46	13.36±0.72	16.67±0.76	17.45±0.62	20.32±0.68
Group-III AEHC treated	7.34±0.36	9.43±0.56	10.24±0.62	18.52±0.87	13.64±0.88
Group-IV EEHC treated	8.84±0.48	11.27±0.28	15.58±0.32	18.62±0.44	19.14±0.44

Values are mean ±SEM (n=6) statically significant difference in comparison with control group and standard group: (*P< 0.05). Once a day, for 20 days treatment on wound healing.

Table 3: Effect of topically applied *Houttuynia cordata* leaves aqueous and ethanol extracts on incision wound model in rats.

Animal Treatment Group	**Epithelization Period (days)**	**Scar area (mm^2)**	**Hydroxyproline content (µg/100mg)**	**Tensile strength (g)**
Group-I Control	27.42±0.85	48.88±0.98	155.77±7.54	282.54±7.44
Group-II Standard Drug (0.2% w/w nitrofurazone)	21.96±0.38	37.87±0.86	250.44±5.88	356.23±7.66
Group-III AEHC treated	25.34±0.63	44.86±0.84	164.34±6.83	286.24±8.12
Group-IV EEHC treated	22.12±0.73	38.92±0.92	269.45±6.55	351.58±8.28

Values are mean ±SEM (n=6) statically significant difference in comparison with control group and standard group: (*P< 0.05). On complete epithelisation day 16th post wound healing.

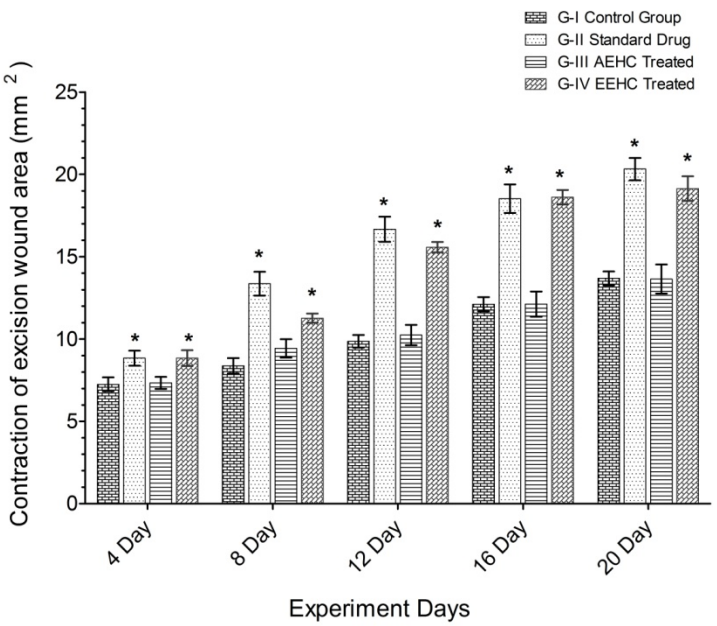

Figure 1: Effect of topically applied *Houttuynia cordata* leaves aqueous and ethanol extracts on contraction of excision wound area (mm^2)

Values are mean ±SEM (n=6) statically significant difference in comparison with control group and standard group: (*P< 0.05). On complete epithelisation day 16th post wound healing.

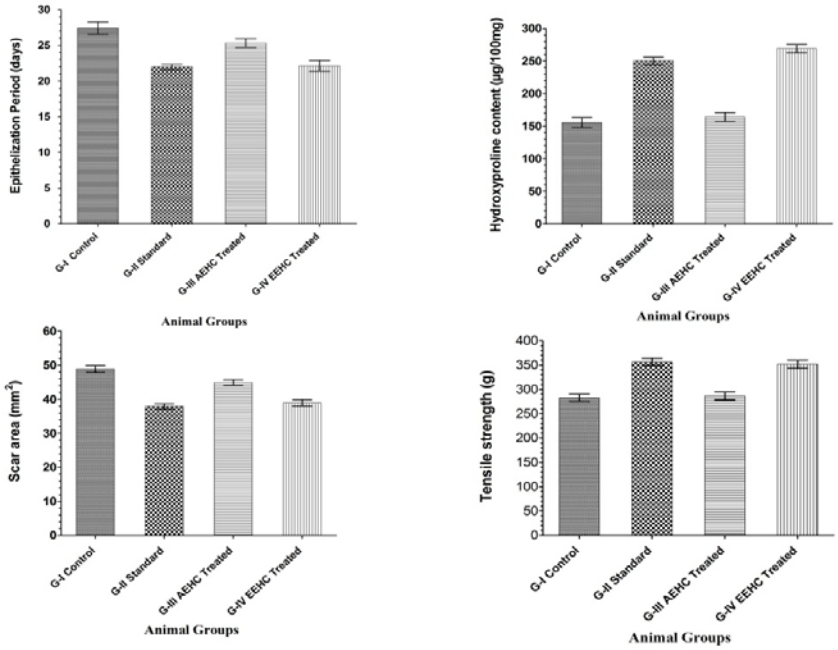

Figure 2: Effect of topically applied *Houttuynia cordata* leaves aqueous and ethanol extracts on different parameters of incision wound model

Values are mean ±SEM (n=6) statically significant difference in comparison with control group and standard group: (*P< 0.05). On complete epithelisation day 16th post wound healing.

4. Conclusion

Finding of the present study suggested, that the ethanolic extract of plant *Houttuynia cordata* leaves has potential wound healing. The wound healing activity of the ethanolic extract may be due to the individual or combined effect of the phytoconstituents present in plant extract. Comprehensive evaluation on the plants with wound healing activity based on traditional medicine may possibly give new compounds that could be used as prominent drugs in wound healing therapy. Further

investigations are needed for isolation and 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 *Houttuynia cordata* leaves for treatment of cuts and wounds.

5. Conflicts of Interest

All the authors have no conflicts of interest.

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