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PHYTOCHEMICAL INVESTIGATION AND EVALUATION OF WOUND HEALING ACTIVITY OF *EUPATORIUM ODORATUM LINN.*

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ABSTRACT

Eupatorium odoratum Linn is found in the tribal area of Baipariguda (Dist: Koraput) and extensively used traditionally by the tribal people as anthelmintic, antibacterial, antifungal and wound healing agent. The present study is an attempt to preliminary investigation of phytochemical constituents and to explore the wound healing property of the ointments prepared from solvent extracts (Petroleum ether, ethanol and chloroform). The phytochemical studies indicated that the tests for alkaloids, tannins and phenolic compounds were positive while the tests for anthraquinone glycosides, proteins, amino acids and saponins were negative for all extracts. Other chemical tests exhibited varying results. The various doses of ointment extracts were evaluated for their wound healing activities on healthy Wistar rats by excision wound models. All extracts showed wound healing activity and activities are well compared with the standard drugs (neosporin and betadine). When the dose of the extract is increased, a gradual increase in wound healing activity was observed. Among all the extracts (ointments), ethanolic extract containing ointment showed better wound healing activity over the others and even in comparison to standard drugs.

Keywords: *Eupatorium odoratum*; *wound healing; phenolic compounds*.

INTRODUCTION

Wound may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue[1]. Wound healing is the process of repair that follows injury to the skin and other soft tissues. It is a complex phenomenon involving a number of processes, including induction of an acute inflammatory process, migration and proliferation of both parenchymal and connective tissue cells, remodeling of connective tissue and parenchymal components, and acquisition of wound strength[2,3]. All these steps are orchestrated in a controlled manner by a variety of cytokines including growth factors[4].

Some of these growth factors like platelet-derived growth factor B (PDGF), transforming growth factor B (TGF-B), fibroblast growth factor (FGF) and epidermal growth factor (EGF) have been identified in self healing wound [5]. In chronic wound, the normal healing method is disrupted due to some unknown reasons, and in such cases, exogenous application of certain growth promoting agents or compounds which can enhance the *in situ* generation of these growth factors is required to augment the healing process. *Eupatorium odoratum* Linn. (Family: *Asteraceae*) is also commonly called as Christmas bush (English). The plant is mostly perennial herb or shrub, sometimes climber. Leaves are opposite. There are 15 to 25 tubular florets per head, white, lavender, or purple colour, cylindrical glandular often hairy. It is a scrambling shrub. The seeds are brownish gray to black achene, 4mm long with a pale brown pappus 5 or 6 mm long[6].

It is used as a traditional medicine in Indonesia. The young leaves are crushed, and the resulting liquid can be used to treat skin wounds. In herbal medicine, the leafy extracts with salt are used as a gargle for sore throats and colds. In the southern part of Nigeria, the leaves are used for wound dressing, skin infections as antimicrobial and to stop bleeding[7]. The literature survey reveals that there are no reports on the wound healing activity of the fresh whole plant extracts of *E. odoratum*. This prompted us to investigate the wound healing activity of *E. odoratum* whole plant extracts including the preliminary phytochemical studies.

MATERIALS AND METHODS

Drugs and Chemicals

Neosporin ointment (Glaxo-Smithkline Pharmaceutical Limited, Bangalore, India), Betadine ointment (G. S. Pharmbutor Pvt. Ltd., Uttarakhanda, India), Petroleum Ether AR (60-80°C, Thomas Baker Chemical Pvt. Ltd.), Chloroform GR (Loba Chemicals), Ethanol AR (Merck Pvt. Ltd. Mumbai) and other chemicals were procured from different suppliers.

Plant material

The fresh whole plant material of *Eupatorium odoratum* Linn. (Family: *Asteraceae*) was collected from local

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area Baipariguda of Koraput district (India) in the month of June 2008. The plant was identified and authenticated by the Biju Pattnayak Medicinal Plants Garden and Research Centre, Dr. M.S. Swami Nathan Research Foundation, Jeypore, Koraput (District), Odisha (Letter no. MR08/DBT/115, 12.06.2008). The leaves were shade dried under normal environmental condition. The dried leaves were powered and stored in a closed container for further use.

Preparation of solvent extracts

For extraction, about 1.5 Kg of powdered whole plant was used for each solvent such as petroleum ether, chloroform, and ethanol. For each solvent, 50 cycles were run. Each extract was filtered and concentrated by distillation of the solvent to obtain the crude extract, followed by drying with rotary evaporator. Successive solvent extraction of leaves resulted in separation of constituents of different polarity range in different solvent extracts.

Phytochemical screening

Chemical tests were carried out for all the extracts for the qualitative determination of phytochemical constituents[8,9]. Total phenolic content was determined using Folin-Ciocalteau reagent[10].

Animals care and handling

This was done as per the guidelines set by the Indian National Science Academy, New Delhi, India. Twelve-week-old healthy Wistar rats (150–180 g) of either sex bred locally in the animal house of Jeypore College of Pharmacy, Jeypore were selected for the present 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 paddy husk (procured locally) as bedding throughout the experiment and had free access to food (animal chow) (M/s Hindustan Lever Ltd.) and water *ad libitum.* The rats were anaesthetized prior to infliction of the experimental wound. The surgical interventions were carried out under sterile conditions ketamine anesthesia (10 mg/Kg). Animals were caged and all operations on animals were done in aseptic condition. Approval for the research work was obtained by the Institutional Ethical Committee of regd. No. HPI/07/60/IAEC/0013 of date. 07-05-2007.

Experimental Method (Wound healing activity)

The rats were inflicted with excision wounds as described by Morton and Malon[11]. The rats were anaesthetized prior to creation of the wounds, with anesthetic ether .The dorsal fur of the animal was shaved with an electric clipper and the area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless stencil. A full thickness of the excision wound of 2.5 cm in width (circular area = 4.90 cm^2) and 0.2 cm depth was created along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound was left open[12,13]. The animals were divided into eleven groups (six animals in each group). The group I animals were treated with oleaginous ointment base (control – used both in case of polar and non polar solvent extracts) which was prepared freshly in every study. Group II & III animals were treated with reference standards Neosporin & Betadine ointments. Groups IV to VI animals were treated with 2.5, 7.5 and 10 % w/w of petroleum ether extract ointments, Groups VII to IX animals were treated with 2.5, 7.5 and 10 % w/w of chloroform extract ointments and Groups X to XII animals were treated with 2.5, 7.5 and 10 % w/w of ethanol extract ointments respectively for 16 days. The extract ointments (2.5, 7.5 and 10 % w/w) at a quantity of 0.5 g were applied once daily to treat different groups of animals. The simple ointment base, Neosporin & Betadine ointments were applied in the same quantities to serve as control and standards respectively. Wound healing potential was monitored by wound contraction and wound closure time. The wound contraction was calculated as percentage reduction in wound area. The progressive change in wound area were monitored planimetrically using transparent paper and a permanent marker on 1^{st}, 4^{th}, 8^{th}, 12^{th} & 16^{th} days. The extent of wound healing areas was measured with graph paper.

Statistical analysis

The data on biological studies were reported as mean ± Standard deviation (n = 5). For determining the statistical significance, standard error mean and analysis of variance (ANOVA) at 5 % level significance was employed. P values < 0.05 were considered significant[14].

RESULTS AND DISCUSSION

The phytochemicals study revealed that tests for alkaloids and tannins were positive in all extracts. Tests for saponins, proteins, amino acids and anthraquinone glycosides were negative in all extracts. Triterpenoids, sterols & steroids, flavons & flavonoids are present in petroleum ether and chloroform extracts but absent in ethanolic extracts. Cardiac glycosides are present in ethanolic extracts but absent in petroleum ether and chloroform extracts. Gum mucilage is present in petroleum ether and chloroform extracts but absent in ethanol extracts. The phytochemicals detected in the extracts are playing a major role to possess medicinal properties.

The effects of the different ointments, Neosporin, Betadine ointment (standard) and simple ointment base (control) in the excision wound model were assessed by measuring the wound areas or wound contraction areas respectively. The present investigation revealed that the test extracts in varying concentrations in the

ointment base were capable of producing significant wound healing effect as presented in Table 1. The result indicating that the ethanol extract was endowed with potent wound healing property as compared to other extracts. The petroleun-ether and chloroform extracts also possess significant activities. The data also indicate that the extent of activity depends on the concentration of each extract. Potency of the extracts was found to be inversely proportional to the time taken for healing of wound. It was also observed that the activities were comparable with the reference drugs (Neosporin and betadine). The above findings justify the wound healing properties of the plant. By employing one-way ANOVA, all data were verified and found to be statistically significant at 5 % level of significant ($p<0.05$).

Table 1: *Wound healing activity of extract of E. odoratum and percentage of wound contraction in days.*

Groups	Wound area (mm²/Rat) (X±S.E.M.)				
	0th day	4th day	8th day	12th day	16th day
I	454.8±0.7 (0)	391.2±1.6 (1.3)	389.5±1.2 (15.46)	285.3±0.5 (37.49)	152.7±0.8 (66.42)
II	456.2±0.8 (0)	310.7±1.2 (31.88)	196.5±0.9 (56.91)	54.38±1.1 (88.08)	Healed (100)
III	456±0.86 (0)	330.2±1.4 (27.51)	209.1±1.06 (54.17)	58.14±0.43 (87.24)	9.41±0.44 (97.95)
IV	455.7±0.8 (0)	375.2±1.7 (18.56)	253.8±1.1 (44.22)	76.3±0.5 (82.96)	19.5±0.8 (95.71)
V	456.5±0.5 (0)	349.2±1.4 (23.43)	218.5±1.6 (52.14)	62.6±1.1 (85.91)	14.2±0.6 (96.89)
VI	455.8±0.8 (0)	343.5±1.1 (24.17)	213.8±1.3 (53.16)	57.8±0.7 (87.38)	10.3±0.4 (97.74)
VII	456.16±0.6 (0)	365.3±1.5 (20.86)	247.7±0.8 (46.7)	70.1±1.2 (85.73)	14.2±0.6 (98.14)
VIII	456.2±0.6 (0)	344.5±1.8 (25.45)	208.5±0.9 (49.19)	51.3±1.1 (91.52)	9.0±0.54 (98.78)
IX	456.5±0.5 (0)	341.5±1.2 (26.22)	199.0±1.3 (51.16)	43.3±1.1 (96.3)	6.7±0.3 (100)
X	456.5±0.4 (0)	361.3±0.9 (19.85)	243.1±0.96 (45.67)	64.1±1.1 (84.63)	8.4±0.5 (96.88)
XI	456.5±0.4 (0)	340.3±1.2 (24.5)	227.2±1.0 (54.30)	38.6±1.0 (88.75)	5.55±0.5 (98.02)
XII	457.0±0.4 (0)	336.3±1.4 (24.61)	223.1±0.96 (56.35)	16.3±0.69 (90.49)	Healed (100)

Values are expressed as mean ± Standard error of mean, n=6. Numbers in parenthesis indicate the percentage of wound healig. Data are found to be significant by testing through one way ANOVA at 5 % level of significance ($p < 0.05$).

Group I – Control (Simple ointment base), group II – Standard - 1 (Neosporin), group III – Standard - 2 (Betadine), groups IV to VI – 2.5 %, 7.5 % and 10 % w/w of Pet-ether extract ointments, groups VII to IX – 2.5 %, 7.5 % and 10 % w/w of chloroform extract ointments and groups X to XII – 2.5 %, 7.5 % and 10 % w/w of ethanol extract ointment.

CONCLUSION

The healing effect of the extract ointments, Neosprin & Betadine ointment (standard) and simple ointment base (control) in the excision wound model was assayed by measuring the wound area and wound contraction respectively. It is concluded that the ethanolic extract exhibit the most potent wound healing activity. The results of this study support the use of these plants in human and animal therapy and reinforce the importance of the ethno botanical approach as a potential source of bioactive substances. Further studies are required to identify the actual chemical constituents that are present in the crude extracts of this plant which are responsible for wound healing activity and to establish the effectiveness and pharmacological rationale for the use of *Eupatorium odoratum* as a wound healing drug.

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