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EVALUATION OF WOUND HEALING AND ANTIMICROBIAL ACTIVITY OF *BLEPHARIS REPENS (VAHL) ROTH*

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ABSTRACT

Blepharis repens (vahl) roth is used in Folklore medicine in healing of wounds and eruptions and sealing in secondary infection. There was no scientific evidence justifying the use of whole plant of *Blepharis repens*, therefore the present study was aimed at evaluation of wound healing and antimicrobial activity of the whole plant and In the present study the whole plant ethanolic extract were studied for wound healing activity by incorporating ethanol extract in simple ointment base B.P. in concentration of 2% (w/w) and 4% (w/w The statistical data indicated that the wound with ointment containing 4% w/w ethanol extract exhibited significant (P<0.001) wound contracting ability .The ethanol extract of *Blepharis repens* showed significant antibacterial and antifungal effect against most of the pathogenic organism .phytochemical screening of the extracts showed the presence of a number of bioactive constituents such as alkaloids, tannins, saponins, flavonoids etc.

Keywords: *Blepharis repens*, Incorporating, Phytochemical Screening.

INTRODUCTION

A wound may be defined as a "Disruption of normal tissue structure and function" and can be categorized by its etiology, location, or duration. Wound healing involves a chain of well-orchestrated biochemical and cellular events leading to the growth and regeneration of wounded tissue in a specific manner including clotting, inflammation, granulation tissue formation, epithelization, collagen synthesis and tissue remodelling. India has a rich tradition of plant-based knowledge on healthcare. A large number of plants/plant extracts/decoctions or pastes are equally used by tribals and folklore traditions in India for treatment of cuts, wounds and burns. And medicinal plants represent rich sources of antimicrobial agents. Plants are used in different countries and are a source of many potent & powerful drugs. Since time immemorial man has used various parts of plants in the treatment and prevention of many ailments [1]. Historically all medicinal preparations were derived from plants parts or in the more complex form of crude extracts, mixtures, etc [2].

Blepharis repens (vahl) roth, Acanthaceae is shrub. Several other closely related species also occur in the India, Africa and srilanka. Traditionally these whole plants are used in the healing of wounds and antibacterial activity.

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MATERIALS AND METHODS

Collection of plant material

The *Blepharis repens* (vahl) roth were collected and authenticated from medical plant research centre (PARC) by Prof. P.Jayaraman, Botanist, Chennai- 45. The voucher specimen number is PARC 2012, 2049. The voucher specimen of herbarium sheet is kept in the Plant Anatomy Research Center, West Tambaram, Chennai-45.

Preparation of plant extract

The air-dried crude drug was pulverized to obtain coarse powder. The powdered drug was extracted with ethanol in a soxhlet extractor. The extract thus obtained was concentrated by recovering the solvent by Rotary Flash Evaporator. The concentrated extract was the evaporated to dryness in vacuum oven at neither temperature nor more than 50^0C. The dried extract was stored at 2-8^0C in refrigeration until use for the biological testing and phytochemical screening [3-5].

Preliminary photochemical screening

Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrate, saponins, phenolic compounds, tannins and flavonoids.

Acute oral toxicity study

By following OECD (Organization of Economic Co-operation and Development) guidelines 420 – Fixed Dose Procedure (FDP), acute oral toxicity was evaluated. This involves the identification/calculation of the doses level that becomes evidence of non-lethal toxicity (termed Evident toxicity), which gives clear signs and symptoms of toxicity of a test drug/ substance. When dose where increase to next level of highest fixed dose, which would result in the development of severe toxicity sign or even death. Next highest fixed dose producing, Evident toxicity was assumed and was also calculated on ones experiences. These dose also provide information that lead to a similar classification to that based on the LD value [6].

Animals

Healthy Wistar Rats between 2-3 months of age and weighing 160-200g were used for the study. The experimental protocol was approved by the Institution of ethical committee.

Group 1: Simple ointment treated control group

Group 2: Animals treated with standard (Nitrofurazone 0.2% w/w)

Group 3: Animals treated with WEBR 2% w/w (2g extract in 100g simple ointment, ethanol extract ointment of *Blepharis repens* low dose 2% w/w)

Group 4: Animals treated with WEBR 4% w/w (2g extract in 100g simple ointment, ethanol extract ointment of *Blepharis repens* low dose 4% w/w)

Two types of ointment of the extract were prepared viz. 2% (w/w) ointment, where 2 g of extract was incorporated in 100 g of simple ointment base (Anonymous, 1953); 4% (w/w) ointment where, 4 g of extracts of the whole plant were incorporated in 100 g of simple ointment base B.P. Nitrofurazone ointment (0.2% w/w) obtained from Smith Kline – Beecham Pharmaceuticals Bangalore, India, was used as standard drug for comparing the wound healing potential of the extract in different animal model.

Excision wound model

Male albino rats of (150- 200gm) body weight were selected. Animals were divided individually with free access of food and water. The basal food intake and weight to nearest gm were noted. The animals were starved for 12hrs prior to wounding. Under light ether Anaesthesia wounding was performed. For the excision wound [7-9] each group containing 6 animals were selected. Totally four group. A circular wound of about 2.5 cm diameter was made on depilated thoracic of rats under light ether anaesthesia in aseptic condition and observed throughout the study. The animals were housed individually. The test samples (Herbal extract) were formulated as an ointment in simple ointment base. 0.5gm of the formulated ointment was applied on the wound once daily for 16 days starting from the day of wounding. The observation of percentage wound closure were made on 2^{nd} day, 4^{th} day, 8^{th} day and 16^{th} day post wounding days [10-12].

INCISION WOUND MODEL

Four groups with six animals in each group were anaesthetized and two paravertebral long incisions were made through the skin and cutaneous muscles at a distance of about 6cm from the midline on each side of the depilated back of the rat. Full aseptic measures were not taken and no local or systemic was used for stitching. After the incision was made, the parted skin was kept together and stitched with black silk at 1cm apart; surgical threads (No.000) and a curved needle (no.11) were used for stitching. The continuous threads on both wound edges were tightened for good closures of the wound. The wound was left undressed. Ethanolic extract, Aqueous extract and simple ointment B.P. was applied to the wound twice daily, until complete recovery to the respective groups of animals. They were administered once daily for 9 days. When wounds were cured completely the sutures were removed on the day 9 and tensile strength was measured with a tensiometer [13].

Statistical analysis

The values are represented as mean + S.E.M for six rats. paired t- test was used for reporting the p- value and significance with respect to the control group.

Antimicrobial activity was carried out by the cup-plate method

The antimicrobial activity for the given samples was carried out by Disc Diffusion Technique (Indian Pharmacopoeia 1996, Vol II A-105). The test Gram positive Bacteria *Staphylococcus aureus, Streptococcus fecalis, Bacillus subtilis* and test Gram Negative Bacteria *Escherichia coli, Pseudomonas aeruginosa, Klebsiella aerogenes, Salmonella typhi* and *Fungus Candida albicans, Aspergillus niger* were obtained from National chemical laboratory (NCL) Pune and maintained by periodical sub culturing on Nutrient agar and sabouraud's dextrose medium for bacteria and fungi respectively. The effect

produced by the sample was compared with the effect produced by the positive control (Reference standard Ciprofloxacin 5µg/disc for bacteria and Fluconazole 100 units/disc for fungi).

RESULTS

The antifungal activity offered against *Blepharis repens* by the aqueous extract is more than the Ethanolic extract. Whereas the Antifungal activity against *Blepharis repens* at the concentration of 250µgm/ml is equal in the both extracts. However the Antifungal activity of aqueous extract at the concentration of 50µgm/ml and 150µgm/ml is lesser when compared to activity exhibited by Ethanolic extract. In case of Gram positive bacteria *Staphylococcus aureus*. Aqueous extract of the whole plant *Blepharis repens* exhibited better zone of inhibition. However the zone of inhibition by both extracts reveals that they are sensitive against Staphylococcus aureus. Against the entire test organisms used the aqueous extract of the whole plant exhibited the better zone of inhibition than the Ethanolic extract. In case of *Staphylococcus aureus, Salmonella tphi, Escherichia coli, Pseudomonas aeruginosa* and *bacillus subtilis*. Both the extract exhibited zone of inhibition within the sensitive range.

The Ethanolic extracts manifested Zone of inhibition within the intermediate range when tested against *Klebsiella aerogenes and Staphylococcus faecalis* at 50µgm/ml concentration. In the Aqueous extract the zone of inhibition exerted against *Salmonella typhi* is within the intermediate range at concentration of 50µgm/ml concentration. From the above results reveals that the Aqueous extract of the whole plant *Blepharis repens* possess the appreciable antibacterial activity against organisms used. In the excision wound model the 5% Ethanolic extract sample ointment of *Blepharis repens (vahl)roth* manifested 99% wound contraction on the 16th day which is at par with the standard Nitrofurazone ointment used. The 5% aqueous extract sample ointment of *Blepharis repens* exerted 95% wound contraction on the 16th day which lies at appreciable limit.

The data obtained from both 5%aqueous extract sample ointment and 5% Ethanolic extract sample ointment of the plant *Blepharis repens* confirms the wound healing property of the same. In case incision wound model the Ethanolic extract of *Blepharis repens* offered better results which is considerably good. Whereas the Aqueous extract of the plant *Blepharis repens* exhibited wound healing activity less than the Ethanolic extract obtained within the appreciable limit

The results of wound healing activity by excision wound model were presented in table 1. It was observed that the wound concentration ability of the extract ointment in both concentration were significantly greater than that of the control (i.e. simple ointment treated group). The wound contracting ability of animals treated with ointment containing 4% (w/w) alcoholic extract was found to be highly significant (P<0.001) on day 16 as compared to the control group.

Fig 1. *Blepharis repens* (vahl) roth

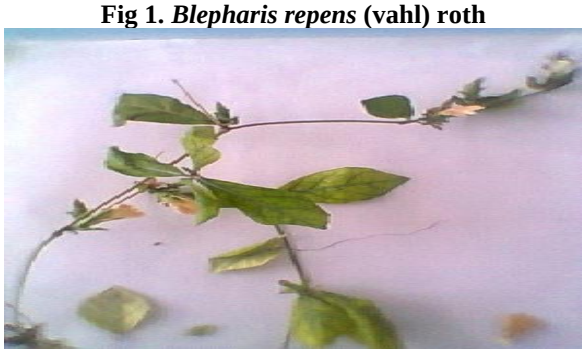

Fig 2. Graphical Represent of *Blepharis repens* (Vahl)Roth Whole Plant Ethanolic Extract On Incision Wound Model.

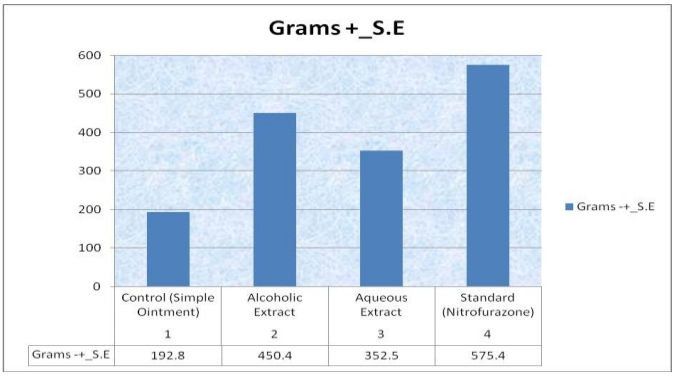

Fig 3. Graphical representation of antibacterial activity on whole plant Aqueous extract of *Blepharis repens* (vahl)roth

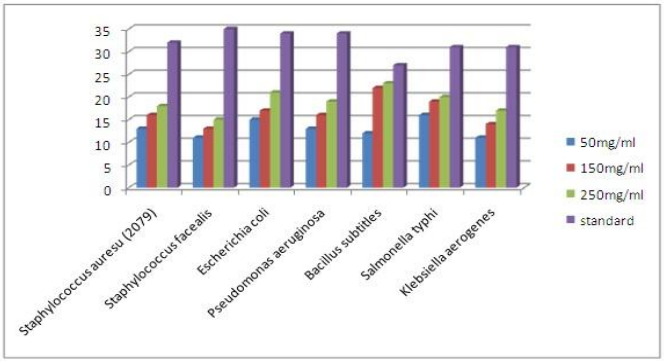

Table 1. Wound healing activity of *Blepharis repens*

drug	Wound contraction (mm2) on day + S.E and percentage of wound contraction.					
	0 day	2nd day	4th day	8th day	12th day	16th day
Simple ointment(control)	505±4.50 (0.0)	455.3±5.61 (11.0)	402.4+-6.1	325.5±3.2 (35.0)	281.4±3.4 (44.5)	235.5±4.0 (53.5)
5% Ethanolic extract samples ointment(W/W)	512.6±3.5 (0.0)	410.6±5.5 (20.0)	325.3±6.0 (36.51)	140.8±4.3 (72.53)	32.5**±1.5 (93.65)	5.1**±0.3 (99.0)
5% Aqueous extract sample ointment (W/W)	507.4±4.7 (0.0)	440.7±7.4 (13.34)	368.8±5.5 (27.29)	180.5±4.1 (64.3)	62.3*±3.7 (87.5)	26.8 **±1.1 (95.0)
Nitrofurazone ointment (0.2%W/W) Standard	510.5±5.1	397.5±5.9 (22.1)	308.3±5.1 (22.2)	123.3±3.4 (75.8)	21.1**±1.4 (95.8)	0.01*** (100)

N=6 animals in each group, Values are mean ± S.E.M of animals in each group P<0.01 Vs Control by student 't' test.

Table 2: Effect of *Blepharis Repens (Vahl)* Roth On Incision Wounds.

S.NO	Drug	Grams ±S.E
1	Control (Simple Ointment)	192.8+-8.4
2	Ethanolic Extract	450.4+-4.2**
3	Aqueous Extract	352.5+-7.2*
4	Standard (Nitrofurazone)	575.45+-8.9***

TAB-2 N = 6 Mean ± S.E.M P> 0.01 Vs. Control by students 'T' test

Table 3. Zone of Inhibition *Blepharis Repens (Vahl)* Roth

S.No	Name of the Micro Organisms	Diameter of Zone of Inhibition in mm at Different Concentration Levels						
		Ethanolic extract			Aqueous extract			Standard Ciprofloxacin 5µgm/disc
		50 µgm/ml	150µgm/ml	250µgm/ml	50µgm/ml	150µgm/ml	250µgm/ml	
1	*Staphylococcus auresu* (2079)	13	16	18	15	19	26	32
2	*Staphylococcus facealis*	11	13	15	12	14	17	35
3	*Escherichia coli*	15	17	21	15	17	22	34
4	*Pseudomonas aeruginosa*	13	16	19	14	17	20	34
5	*Bacillus subtitles*	12	22	23	16	20	22	27
6	*Salmonella typhi*	16	19	20	11	14	17	31
7	*Klebsiella aerogenes*	11	14	17	15	14	18	31

DISCUSSION AND CONCLUSION

Wounds are physical injuries that result in an opening or break of the skin. Proper healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. It is a product of the integrated response of several cell types to injury. This sequence of physiologic events occurs by a process of connective tissue repair. These events involve four phases such as coagulation, which prevents blood loss, inflammation and debridement of wound, epithelial repair, including proliferation, mobilization, migration and differentiation, and tissue remodeling and collagen deposition.

Any agent who accelerates the above processes is a promoter of wound healing. The application of medicinal concoctions from plants to treat skin lesions, in particular, burns and wounds, has had a long tradition. Plants with wound healing activity have been reported and experimentally studied on various animals' models to reveal the most active promising compounds.

Results obtained in the present study suggest that treatment of excision wounds with ethanol extract of *Blepharis repens* has accelerated the wound healing process. Treated excision wounds showed an increased rate of wound contraction, leading to faster healing as confirmed by the increased healed area when compared to the control group. It was observed that nitrofurazone increased the collagen content of the skin ultimately and contributed to wound strength. The results showed that treatment with the ethanol extract of *Blepharis repens* on

the dermal wound healing, and enhancing the wound healing process. The results suggest that treatment with ethanol extract of *Blepharis repens* may have a beneficial influence on the various phases of wound healing like wound contraction, resulting in faster healing. The wound healing potential of the *Blepharis repens* ethanol extract may probably be as a result of the presence of a mixture of phytoconstituents including flavanoids and tannins.

Ointment from the whole plant of *Blepharis repens* exhibited significant prohealing activity when topically applied on rats by affecting various stages of healing process. The results of the present study offers pharmacological evidence on the folklore use of whole plant of *Blepharis repens* for healing wounds. The Ethanolic extracts manifested Zone of inhibition within the intermediate range when tested against *Klebsilla aerogenes* and *Staphylococcus faecalis* at 50µgm/ml concentration. In the Aqueous extract the zone of inhibition exerted against *Salmonella typhi* is within the intermediate range at concentration of 50µgm/ml concentration. From the above results reveals that the Aqueous extract of the whole plant *Blepharis repens* possess the appreciable antibacterial activity against organisms used.

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