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Preparation of external herbal formulation for wound healing activity

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Abstract

Wound healing is a tedious process involving multiple parameters. Wounds happen during trauma, sports, skin burning etc. Skin is the largest organ in terms of surface area. Skin is composed of triple helical collagen tissue which is giving strength to skin. In this experiment attempt was made to determine the wound healing activity of selected medicinal plants mainly *Acorus calamus*, *Hibiscus rosasinensis*, *Mimosa pudica* which are available in our locality. The plants were extracted with solvents selected from previous research activities. The preliminary phytochemical screening was performed to know the major phytochemical in each plant extract.

The creams were prepared using oleaginous base, absorption base, preservative and flavouring agent as prescribed by official books. Accelerated Stability study of six months was performed on creams and best stable cream was used for further activity. Acute toxicity study was performed to know the therapeutic dose and toxic dose of extracts.

Determined Wound healing activity using major two models excision wound model and incision model, which are used to identify percentage, wound closure and tensile strength of newly synthesized skin. Further the major marker amino acid compound Hydroxyproline was determined as a supporting factor to the wound healing activity.

By this study we come to conclusion that the selected medicinal plants are good wound healing herbs if prescribed in correct dosage form and appropriate formulation suitable to the better skin compatibility.

Keywords: *Acorus*, *mimosa*, *hibiscus*, wound healing, cream, tensile strength etc.

Introduction

Medicinal plants are used for curing innumerable diseases. In terms of medicinal uses mixture of constituents found in extracts of plants are more effective than isolated compounds. Many herbs in nature possess tissue regenerating property as they possess pharmacologically active compound in minute quantity along with energy boosting molecules such as carbohydrates, lipids and proteins.

The plants possess various therapeutic activities which should be brought to the notice of the scientific field for the systematic evaluation. Hence an attempt was made to select the plants possessing wound healing activity. A wound occurs when the integrity of tissue is compromised example when skin breaks, muscle tears, burns or bone fractures. Wounds are caused by cuts or scrapes. The main objective of wound healing is to minimize the possibility of infection and scarring. The four phases of wound hearing are inflammatory phase, proliferative phase, granulation and wound contraction. Wounds are two types open wounds and closed wounds.

The creams are topical preparations usually applied on skin. Creams are semi-solid emulsions that are mixture of oil and water. Two types of creams i.e., Oil in water and water in oil creams. The three plants were selected for determination of wound healing activity as they are well known plants used to treat wounds obtained from ethno botanical review from material medica. As per the literature survey the studies showed that methanol extract of *Acorus calamus* rhizome & aqueous extract of *Hibiscus rosasinensis* leaves having anti-inflammatory activity as well as wound healing activity & aqueous extract of *mimosa pudica* root showed wound healing activity with the strong support of literature available, the creams were prepared with different proportions of the respective extracts and further suggested to carryout wound healing activity.

Methodology

Collection and Authentication of Plants

The *Acorus calamus* plant collected from cultivators Gubbi road, Tumkur. The *Mimosa pudica* plants collected from rural areas of Bangalore. The *Hibiscus rosasinensis* leaves collected from Madhugiri, Tumkur District. The plants and their parts authentication done at FRLHT Bangalore.

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Extraction**Aqueous extract of *Mimosa pudica***

Mimosa pudica plant was dried at 50 °C in hot air oven for 5 hours, powdered and sieved to obtain coarse powder. Mimosa powder was macerated with water for three days with often stirring and filtered concentrated and dried to obtain the semisolid Mimosa extract.

Aqueous extract of *Hibiscus rosa sinensis*

Hibiscus rosasinensis leaves were dried in hot air oven at 50 °C for 5 hours and powdered sieved to obtain powder. The powder was macerated with water for 3 days with stirring, filtered, concentrated & dried to obtain extract.

Methanol extract of *Acorus calamus*

Acorus calamus rhizomes were obtained from local market in Bangalore, powdered, sieved to obtain coarse powder. The powder was extracted with methanol using Soxhlet apparatus for six hours, concentrated extract dried to obtain extract.

Preliminary Phytochemical screening (Qualitative analysis of extracts)

The chemical test was performed for compounds such as Steroids, Triterpenes, Alkaloids, Saponins, Tannins, Flavonoids, Carbohydrates, proteins, fixed oil and resins

Method of preparation of cream

Various ingredients were weighed accurately. The fatty bases such as yellow Besswax, Stearyl alcohol, PEG 4000 were melted in a beaker. The oily phase Tween 20, Tween 80, Tween 40 were added and maintained at 75 °C. The extract was dissolved in required amount of water and filtered. To the filtrate methyl paraben, glycerol were added and maintained at 75°C. When the temperature of both the phases was 75°C, the aqueous phase was added gradually into oily phase with continuous stirring and left at room temperature to obtain the required product. Fatty phase-yellow bees wax, stearyl alcohol, PEG 4000, stearic acid and cetostearyl alcohol. Humectants-Glycerol, surfactants, Tween 20, 40, 80. Preservatives- Methyl paraben. Active herb extract- Mimosa, Hibiscus and Acorus extract. Flavouring agents- Pine oil or Lavender oil. Aqueous phase- Distilled water.

The single plant extract 5% and 10% creams prepared and mixture of plant extract 5% (2% Acorus calamus, 1% Mimosa pudica, 2% Hibiscus rosasinensis extracts used) and 10% creams (3% Acorus calamus, 3% Mimosa pudica, 4% Hibiscus rosasinensis extracts) were also prepared

Creams batches were evaluated physically by Spreadability, Excrudability and accelerated stability studies. Stable batches were used for study of wound healing activity.

Acute toxicity study was performed on experimental rats. Small incision wound was made on back of rats and 1%, 5%, 10% creams of plant extract and mixture extracts was applied once and observed for any deaths of animals. No toxicity reported.

Wound healing activity of creams**Excision wound model (Dr Pulok k Mukherjee, Quality control of herbal drugs, 546-47, Pharmaceutical Publishers)**

Ten groups of Wister Albino rats containing six in each group were anesthetized by Diethyl ether. The rats were depilated on the back. One excision wound was inflicted by cutting away 400mm² thickness of skin of a predetermined area. The rats were treated with reference standard Soframycin cream, simple

cream control, test creams were applied till the wound was completely healed. This model was used to monitor wound contraction and epithelization time. The progressive change in healing of wound was monitored planimetrically by tracing the wound margin on a graph paper every alternate day (0, 2, 4, 6, 8, 10, 12, 14, 16, 18 days). The wound area was expressed as mm² unit.

Incision wound model: (Dr Pulok k Mukherjee, Quality control of herbal drugs, 546-47, Pharmaceutical Publishers)

Ten groups of wister albino rats containing six in each group were anesthetized and one paravertebral long incision of 6cm length was made through the skin and cutaneous muscles at a distance of about 1.5cm from midline on each side of the depilated back of rat. All the groups were treated in the same manner as mentioned in case of excision wound model. After the incision was made the parted skin was kept together and stitched with black silk by 0.5cm apart. Surgical thread (No:000) and curved needle (No:12) was used for stitching. The continuous thread on both wound edges was tightened for good adoption of wound. The wound was left undressed. The test creams. Soframycin cream and simple cream were applied to the wound once daily until recovery of animals.

Biochemical estimation of marker: (Determination of hydroxyproline content during wound healing)(Robert E, Neumann, Milan K Logan E. The determination of Hydroxyproline. The Journal of Biological Chemistry. 2009:299-306.)

1. At the end of 10th day of wound healing, collagen tissue of excision wound was removed.
2. The tissue was dried at 108°C for 16 hours & weighed.
3. Collagen tissue was placed in volumetric flask and 6N H₂SO₄ was added and autoclaved for 3 hours at 50 pounds pressure after sealing.
4. Hydrolyzed solution was filtered to remove cell debris.
5. Required volume was pipetted to separate tube.
6. To each tube 1ml of 0.01M CuSO₄, 1ml 2.5N NaOH & 1ml 6% H₂O₂ solution was added.
7. Shake vigorously for every 5 minutes on water bath at 80°C for 5 minutes (Reason: Heating & Shaking will destroy excess of peroxide since traces of peroxide will decrease color formaton.)
8. Tubes were chilled in ice water bath.
9. To the above solution 4ml of H₂SO₄ was added with vigorous shaking.
10. p-Dimethylamino benzaldehyde (1ml) was added to above solution to obtain orange color which was measured at 559nm in UV-Visible Spectrophotometer.
11. Same procedure was repeated for determination of absorbance of known concentration of hydroxyproline which was compared with the absorbance of hydrolyzed tissue hydroxyproline.

Results and Discussion

All the plant extracts were soluble in water, dilute HCL and ethanol. Hibiscus and acorus extracts were brown colour and mimosa extract reddish brown colour.

In preliminary phytochemical screening shows aqueous extract of *Mimosa* contain Terpenoids, Alkaloids, Saponins, Tannins, Carbohydrates, Proteins, Fixed oil and Resins. The aqueous extract of *Hibiscus* contains Saponins, Carbohydrates, Proteins, Fixed oils and Resins. The *Acorus* extract contains Terpenoids, Proteins, Fixed oils and Resins. All the extracts

devoid of Flavonoids. Stable herbal cream batch selected based on accelerated stability study

All three plant extract 5% and 10% cream batches were prepared and mixture cream batch (2% acorus extract+1% Mimosa extract+ 2% Hibiscus extract), 10% mixture formulation (3% Acorus +3%Mimosa+4%hibiscus extract).

Wound healing activity of all 10 batch creams (3 batch single herb 5%extract, 3 batch single herb 10% extract, control batch, 5% and 10% mixture batches, Standard Soframycin cream batch) performed 6 rats in each batch used.

Two method of wound healing activity was performed. Excision wound model and incision wound model. Further Hydroxyproline content as a marker in newly regenerated collagen tissue was determined.



Initial Wound



After Stitching



Incision Wound Healing Photo



Tensile Strength Determination Method

The result of excision wound model indicate that first 2 days there is no significant increase in percentage wound closure compared to control group. The result of 10th day indicates that

there is significant increase in wound closure in the all herbal creams and soframycin cream compared to control group.

Excision wound model

Sl no	cream	Percentage wound closure on 14 th day
1	10% Mimosa cream	98.25
2	5% Mimosa cream	97.29
3	10% Hibiscus cream	97.75
4	5% Hibiscus cream	98.3
5	10% Acorus cream	98.0
6	5% Acorus cream	96.51
7	10% herbal mixture cream	96.15
8	5% herbal mixture cream	99.41(maximum)
9	1% Soframycin cream	96.7

Wound Healing activity of Herbal cream formulation: Excision Wound Model

Cream	Hibiscus cream (10%)	Hibiscus cream (5%)	Acorus cream (10%)	Acorus cream (5%)	Mimosa Cream (10%)	Mimosa Cream (5%)	Mixture Cream (4%H+3%M+3%A)	Mixture Cream (2%H+1%M+2%A)	Standard Cream (1%)	Control cream
Days	% Wound Closure±S.E(Standard Error)									
0	0	0	0	0	0	0	0	0	0	0
2	14±0	21.85±0	14.43±0	25.78±0	18.92±0.033	12.02±0	12.43±2.43	19.51±0	18.375±10.5	7.5±1.13
4	44.56±0	35.46±0	32.0±0.3	34.53±0	34.95±0.137	19.25±0	37.625±6.25	32.68±0	37.25±5.25	14.35±0.466
6	55.875±0	40.20±0	55.0±0	47.16±0	56.687±0	45.31±0	52.0±0	46.43±0	57±0	37.5±0
8	65±0.12	68.78±0	61.312±0.5	61.72±0	70.5±0.067	63.63±0	60.5±0	68.48±0	70±0	59.5±0
10	79.675±0	88.56±0	87.75±0.3	82.49±0	86.43±0	84.88±0	81.93±0	88.78±0	84.75±0	72.875±0
12	88.75±0.033	94.75±0	94.0±0.03	92.57±0	91.875±0	93.54±0	90.875±0	95.46±0	93.9±0.18	83.43±0
14	97.75±0	98.30±0	98.0±0.83	96.51±0	98.25±0.43	98.25±0.43	96.1575±1.187	99.41±0	96.7±2.46	94.125±0.75
16	100	100	100	100	100	100	100	100	100	98.0±0.16
18										100

Incision wound model

SI No	Treatment	Tensile strength (g/cm) 10% cream	Tensile strength (g/cm) 5% cream
1	Blank (control cream)		400.0±0
2	Hibiscus cream	408.3±2.9	486.75±0
3	Mimosa cream	354.3±0.577	420.7±0.05
4	Acorus cream	534.3±9.2	541.75±0
5	10% herbal mixture cream	437.6±0.11	-----
6	5% herbal mixture cream	-----	652.0±0(maximum)
7	Soframycin cream (1%)		469.0±0

N= 6 animals in each group

The treated groups are compared by student t test with the control group.

P<0.0001

Hydroxyproline content in different cream batches

SI No	Treatment	Concentration of Hydroxyproline (µg/mg collagen tissue) 10% cream	Concentration of Hydroxyproline (µg/mg collagen tissue) 5% cream
1	Blank (control cream)	18.96±0.3	
2	Hibiscus cream (10%)	116.5±0	26.12±0
3	Mimosa cream (10%)	102.8±1.32	31.6±6.193
4	Acorus cream (10%)	82.0±0	33.57±0
5	10% herbal mixture cream	102.65±0.03 (maximum)	---
6	5% herbal mixture cream	----	28.33±0.003
7	Soframycin cream (1%)	66.15±0.137	

N=6 animals in each group,

The treated animal were compared by student t test with the control group

P<0.001

Conclusion

Creams were prepared from selected medicinal plants collected from literature review. Preliminary phytochemical screening was performed to know the major phytochemical group. Various excipients were used to formulate a stable cream. Stability of cream batch's was determined by accelerated stability study, pH, Spreadability and Excrudability etc.

The temperature maintenance and trituration are the major parameters should be monitored cautiously during cream formulation. The 5% herbal mixture cream containing the three extracts shown better wound contraction, tensile strength compared with 5 and 10% of individual plant extracts. The 5% Acorus, Mimosa and Hibiscus creams shown better tensile strength than the respective 10% creams.

The percentage wound closure of 5% herbal mixture cream (99.41±0) containing the all three plant extracts was better than the single plant extract cream and soframycin cream (96.7±2.46) The tensile strength of 5% herbal mixture cream (652.0±0), 5% Acorus cream (541.75±0), 10% Acorus cream(534.3±9.2) and 5% Hibiscus cream (486.75±0) was better than other creams and 1% soframycin cream.

Hydroxyproline concentration was maximum with 10% creams, minimum with control group, soframycin cream

(66.15±0.137) group hydroxyproline is in between 5% & 10% creams.

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