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Wound healing activity of ethanolic extract of roots of *Morinda pubescens* J.E. Smith

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Abstract
In folklore medicine, *Morinda pubescens* has been used as a wound healing agent for many years; however, there have been few scientific reports on this activity so far. The ethanolic extracts of roots of *Morinda pubescens* at different concentrations were evaluated for their wound healing activities in mice using excision wound model. The effects of test samples on the rate of wound healing were assessed by the rate of wound closure, period of epithelization. Povidone Iodine 10% w/w ointment was used as reference standard for the activity comparison. The results of the study revealed that the animals treated with different concentrations (10% w/w, 20% w/w) of ethanolic extracts of roots of *Morinda pubescens* showed faster rate of wound healing activity. The extract treated animals exhibit reduction in wound area when compared to controls. The extract treated wounds are found to epithelize faster as compared to controls.

Keywords: *Morinda pubescens*, Excision wound, Povidone iodine ointment, wound healing

Introduction
In recent years, there has been renewed interest in screening higher plants for novel biologically active compounds, particularly those that effectively intervene in human ailments. *Morinda pubescens* J.E. Smith commonly known as Aal or Indian Mulbery is a species of flowering plant in the family Rubiaceae, native to Southern Asia [1].
There were different *Morinda* species found worldwide as *Morinda citrifolia* Linn, *Morinda elliptica* Ridely, *Morinda umbellate* Linn, *Morinda trimera* Hillebr and *Morinda pubescens* J.E Smith (*Morinda tinctoria* Roxb.) of which *Morinda umbellate* Linn, *M. citrifolia* L. and *M. pubescens* J.E. Smith were best known and most widely cultivated in South India and were medicinally used in India and China. Many species of *Morinda* available in India of which *Morinda pubescens* predominantly grows as a weed in vacant agricultural land [2].
Traditionally the leaf juice was given orally to children before food for easy digestion. The charred leaves made into a decoction with mustard were a favourite domestic remedy for infantile diarrhoea. The expressed juice of leaves was externally applied to gout to relieve pain. The leaves are administered internally as a tonic and febrifuge. They were useful in gastropathy, dyspepsia, ulcerative stomatitis, wounds, gout, inflammation, hernia, sarcocele and fever. The roots were styptic, constipating, anti- inflammatory, alexeteric and tonic, and were useful in haemorrhages, dysentery, inflammations, boils and general debility [3]**.** *Morinda pubescens* its medicinal potential has yet to be studied scientifically, and, therefore, this present study was initiated with the aim of investigating the medicinal and therapeutic properties of *M. pubescens* by evaluating its effects on wound healing in injured mice using two different concentrations of ethanolic extract of *M. pubescens*.

Materials and methods
Plant material
The plant specimens (roots) for the proposed study were collected from localities of Kariavattom, Thiruvananthapuram. The collected plants were carefully examined and authenticated in Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram. A voucher specimen (Voucher No.KUBH-5867) has been deposited for future reference.

Animals

The protocol for conducting the wound healing experiments in mice was approved by the Institutional Animal Ethics Committee, Govt. Medical college, Thiruvananthapuram (Regn No: 01/01/2015/MCT). Swiss albino mice of both sexes weighing 20-25 g were used for the study. The animals were kept in the standard condition. The room temperature was maintained 22±2^0 C with food and water *ad libitum.* The animals were transferred to the laboratory at least 1h before start of experiment.

Preparation of *M. pubescens* root extract

The collected roots were washed with running tap water to remove adhering materials and cut into small pieces. Then, the roots were dried at a temperature not exceeding 50°C. These dried materials were cut into small pieces and then pulverized mechanically into coarse powder. The coarse powder obtained by passed through the sieve No. 18. Then this coarse powder was extracted with absolute alcohol by soxhlet apparatus for 3 h. ethanolic extract was obtained in rotary evaporator. The semisolid mass of ethanolic extract was stored in a dessicator.

Chemicals: Povidone Iodine Ointment (10% w/w), Wool fat, hard paraffin, Cetostearyl alcohol, White soft paraffin.

Methods

Procedure

Preparation of medicated ointment base

(a) Preparation of simple ointment: Wool fat-0.5 gm; Hard Paraffin-0.5gm; Cetostearyl alcohol -0.5 gm White Soft Paraffin – 8.5 gm. All the ingredients were mixed, heated gently with stirring then cooled. The base was then packed in a wide mouthed container.

(b) Preparation of 10% ointment: 1 gm of ethanolic extract of root of *Morinda pubescens* was added slowly to the above melted ingredients and stirred thoroughly until the mass cools down and a homogeneous product was formed. The ointment was then packed in a wide mouthed container.

(c) Preparation of 20% ointment: Prepare simple ointment base as above method. 2 gm of ethanolic extract of root of *Morinda pubescens* was added slowly to the simple ointment base and stirred thoroughly until the mass cool down and a homogeneous product was formed. The ointment was then packed in a wide mouthed container [4].

Wound Model: Partial thickness excision wound of 0.8 cm diameter were inflicted on starved animals under ether anesthesia with a sterile scissors. After complete wounding, wound was washed and cleaned with normal saline solution and medicated ointment was immediately applied [5].

Experimental Protocol: For the evaluation of *in vivo* wound healing activity, animals were anesthetized prior and during creation of wounds, under light ether anaesthesia [6]. Animals bearing partial thickness excision wound were distributed into various groups such as control, medicated ointment *(M. pubescens*- ethanolic 10% w/w), medicated ointment (*M. pubescens* –ethanolic 20% w/w) and Povidone- Iodine 10% w/w ointment standard. Group-1, group 11, group 111 and group 1V. Each group has six animals. The wound closure rate was assessed by tracing the wound on days 1, 4, 7, 10, 13, and 16 post wounding days using transparent paper and permanent marker [7]. Changes in the wound area were calculated. The period of epithelization was calculated as the number of days required for falling of dead tissue remnants of the wound without any residual raw wound. The percentage of wound closure was calculated by taking the initial wound as 100% [8].

$$\text{Percentage wound closure} = \frac{(\text{Initial wound area} - \text{Final wound area})}{\text{Initial Wound area}} \times 100$$

Statistical analysis

The statistical analysis was performed by one- way ANOVA followed by Dennett's multiple comparison tests in Graph pad prism Version 6.0 Software. The results were expressed as mean ± SEM to show differences in groups.

Prepared simple ointment and medicated ointment at various concentration

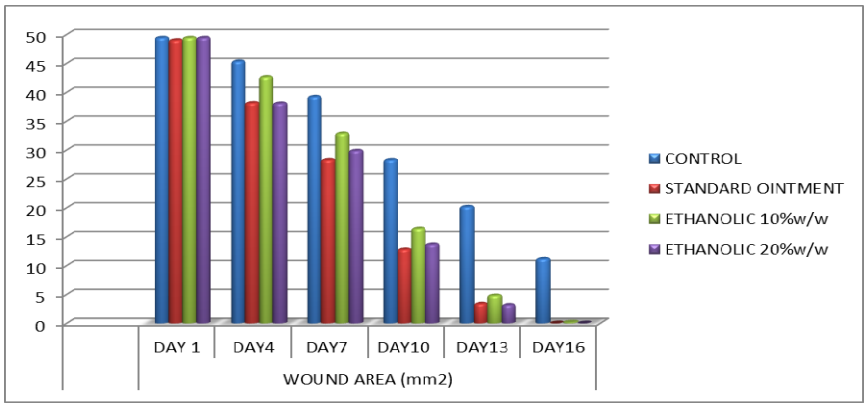
Table 1: Wound areas, % wound contraction and Period of epithelization

Groups	Wound area (mm^2)						Period of epithelisation (days)
	1st day	4th day	7th day	10th day	13th day	16th day	
control	49.41± 0.524*	45.348±0.615** 8.23	39.208±0.469** 13.54	28.26±0.48** 27.90	20158±0.337** 28.69	11.148±0.365** 44.71	21.83±0.477**
Standard Povidone Ointment (10% w/w)	48.99± 0.556**	38.114±0.673** 22.20	28.266±0.486** 25.84	12.7816±0.387** 54.77	3.378±0.279** 73.63	0.0157±0.007** 99.55	14±0.516**
Alcoholic Extract (10%w/w)	49.41± 0.524*	42.614±0.712** 13.76	32.854±0.862** 22.90	16.384±0.441** 50.13	4.793±0.253** 70.75	0.214±0.044** 95.53	16.33±0.0421**
Alcoholic Extract (20%w/w)	49.41± 0.524*	38.044±0.749** 23.50	29.866±0.596* 21.50	13.643±0.403** 54.32	3.150±0.162** 76.90	0.0104±0.006** 99.66	14.166±0.477**

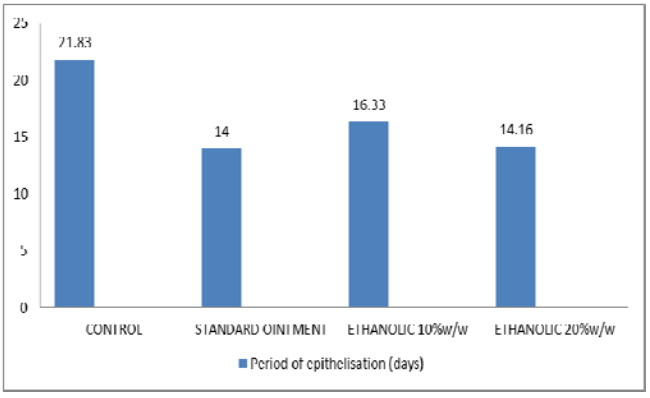
n=6, the results were expressed as Mean ± SEM to show difference in groups. The differences are considered significant when *p<0.01, **P<0.001.

Results

The results of excision wound model were shown in table no. 1. The ethanolic extract exhibited significant wound healing activity as compared to control in excision wound model. From the results obtained it was found that the ethanolic extract 20%w/w is capable of promoting wound healing activity to almost a degree equivalent to standard povidone iodine ointment 10% w/w. Ethanolic 10% w/w ointment also have good wound healing activity. Among the extracts ethanolic 20% w/w showed higher wound healing ability than ethanolic 10%w/w [9].



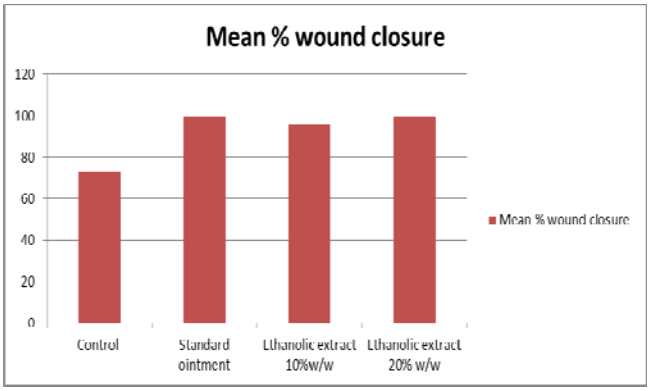
Graph 1: Comparison of Wound areas from 1st day to day 16 on application of standard ointment and ethanolic extracts of *M. pubescens* roots at various concentrations with control



Graph 2: Period of Epithelization (days) of ethanolic extract of *Morinda pubescens* roots at different concentration with Standard and Control

Table 2: Comparison of Percentage wound closure

Sl. No.	Sample	Mean Percentage wound closure
1	Control	73.60%
2	Standard ointment	99.55%
3	Ethanolic extract 10%w/w	95.53%
4	Ethanolic extract 20% w/w	99.66%



Graph 3: Comparison of mean percentage wound closure

Fig 1: Before creation of wound in mice

Treated groups

Control **Standard ointment**

Fig 2: Treatment with control and standard group

Ethanolic 10%w/w Ethanolic20%w/w

Fig 3: Treatment with different concentration of ethanolic extracts

After treatment

Control Standard ointment

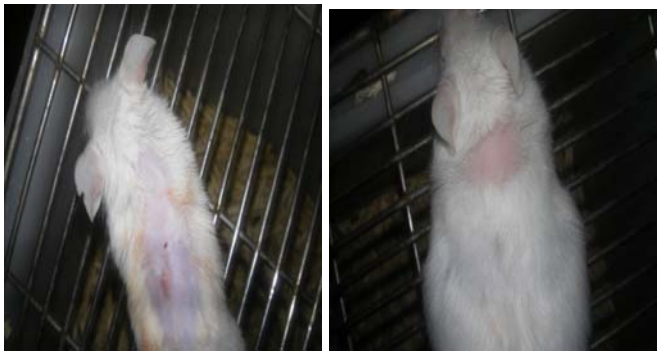


Fig 4: After treatment of control and standard group

Ethanolic 10%w/w Ethanolic 20%w/w

Fig 5: After treatment with different concentration of ethanolic extract

Discussion

Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of area of the wound. It mainly depends on the repairing ability of the tissue, type and extent of damage and general state of the health of the tissue. The granulation tissue of the wound is primarily composed of fibroblast, collagen, edema and small new blood vessels. The undifferentiated mesenchymal cells of the wound margin modulate themselves into fibroblast, which start migrating into the wound gap along with the fibrin strands. Several phytoconstituents like terpenoids, flavanoids and vitamin C were known to promote wound healing process due to their antioxidant and antimicrobial activity. In addition, triterpenoids were reported to possess an ability to increase the collagen content, which is one of the factors promoting wound healing [10, 11].

Furthermore wound healing activity can be attributed to free radical scavenging activity of flavanoids and vitamin C. both these classes of phytochemicals are known to reduce lipid peroxidation not only by preventing and slowing the inset of cell necrosis but also improving vascularity [12, 13]. Lipid peroxidation is an important process in several types of injuries like burns, infected wound, skin ulcer etc. Hence, any drug that inhibit lipid peroxidation is believed to increase the viability of collagen fibrils, which in turn results in an increase in the strength of collagen fibre by increasing the circulation, preventing the cell damage and promoting the DNA synthesis [14, 15].

Conclusion

The results of this showed that the ethanolic extract of roots of *Morinda pubescens* may accelerate wound healing by enhancing epithelization and collagen deposition. The present investigation also offers scientific evidence to the folkloric use of *Morinda pubescens*.

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