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Original Article

Wound Healing Efficacy of *Mimusops elengi* L. Flowers in Albino Wistar rats using Excision wound model

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ABSTRACT

Introduction: *Mimusops elengi* L. (Sapotaceae), known as *Bakul* in Ayurveda, is an evergreen tree found throughout India. *M. elengi* flowers (MEF) are reported to possess immunomodulatory, hepatoprotective, skin whitening, anti-anxiety and wound healing activities which may be attributed to its phytochemical constituents including triterpenoids, alkaloids, phenolics, volatile oils, etc. Although the flowers have been traditionally used as a wound healing agent, there is a paucity of scientific data in support of its efficacy. **Methods:** An ethanolic extract of MEF was evaluated for its wound healing potential in male Albino Wistar rats using an excision wound model. The ethanolic extract of MEF was standardized in terms of its gallic acid content using a validated HPTLC technique. The antioxidant activity of MEF was also evaluated *in vitro*. The wound healing potential of the ethanolic extract of MEF was studied on the basis of wound contraction data, epithelialization period, the effect on biochemical parameters from the granulation tissue and histopathological observations. The results were compared with the traditional drug *Jatyadi Taila* (JT) and the modern drug Betadine (BTD). **Results:** Topical application of the standardized ethanolic extract of MEF on excision wounds caused a significantly faster reduction in the wound area as compared to JT and BTD. Similarly, it also showed the significant increase in the tissue hydroxyproline, hexosamine and protein biochemical parameters when compared with the untreated control. **Conclusion:** The findings of the present study provide data on wound healing and antioxidant potential of MEF and supports their traditional therapeutic claim.

Key words: Ayurveda, Antioxidant activity, gallic acid, *Mimusops elengi* L. flowers.

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INTRODUCTION

The wound is defined as a disruption of cellular, anatomical and functional continuity of a living tissue.[1-2] Wound healing, the body's natural process of regenerating dermal and epidermal tissue[3-4] involves different overlapping phases and processes including inflammation, wound contraction, re-epithelialization, tissue regeneration, and formation of granulation tissue with angiogenesis.[2] Inappropriate progress in the phases of wound healing may lead to a chronic wound or pathological scarring.[2]

Though a large number of pharmacological preparations are used to treat or reduce the inflammation in wound healing process, they do not directly cause the repair of damaged tissues.[5] The available drugs are either bacteriostatic or bactericidal, and in these cases healing may be by natural phenomenon.[6] Therefore, the presence of various life-sustaining constituents in plants has urged scientist to determine their wound healing properties.[2] The classical systems of Indian Medicine employed a large number of medicinal plants for the treatment of skin diseases[7] which have been used by tribal and folklore in many countries for the treatment of wounds and burns.[2]

In Ayurveda, plants like *Azadirachta indica*, *Rubia cordifolia*, *Curcuma longa*, *Berberis aristata*, etc have been described as wound healing agents.[8-9] *Mimusops elengi* L. (Sapotaceae), known as *Bakul* in Ayurveda is another such plants.[10-11] Almost all the morphological parts of *M. elengi* are traditionally used for the management of a wide range of disorders.[12] *M. elengi* flowers (MEF); show cooling and astringent effect to the bowels and are reported to possess immunomodulatory, hepatoprotective, analgesic, anti-asthmatic and wound healing activities.[10-11] Different extracts of MEF have also been scientifically evaluated for their neuroprotective effect, in the management of brain-related disorders,[13-14] skin whitening ability, and for their antioxidant properties.[15] MEF have also been reported to possess bioactive markers including ursolic acid, β-sitosterol, lupeol, gallic acid, quercetol, eugenol, etc which have antioxidant and wound healing properties.[10,11,16]

A thorough literature survey revealed that, although MEF have been used in the management of wounds traditionally, there is a dearth of scientific data on the evaluation of their wound healing potential. Various quality defining parameters for MEF have been already reported by our group.[10] In continuation to this, an attempt has been made to evaluate the wound healing activity of the ethanolic extract of MEF using an excision wound model. In addition, the antioxidant activity of the ethanolic extract of MEF was evaluated *in vitro*.

MATERIALS AND METHODS

Plant materials

MEF were collected from Alibaug, and their taxonomic identification was confirmed by Agharkar Research Institute, Pune (Authentication no. 14-004). The flowers were shade dried for a week followed by drying in an oven preset at 37°C for four days. The sample was powdered in a mixer grinder, sieved (85 mesh, BSS) and stored in an airtight container.

Chemicals and reagents

Gallic acid (98% purity, Figure 1), DPPH, and Galvinoxyl were procured from Sigma-Aldrich Chemical Company, (Steinheim, Germany). Glycol stearate, 1, 2-propylene glycol and liquid paraffin used to prepare vehicle base for the ointment and other chemicals of analytical grade were purchased from Merck Specialties Pvt. Ltd., Mumbai. Topical antibiotic ointment formulation BTD (G. S. Pharmbutor Pvt. Ltd., Uttarakhand) and traditional drug JT (Shree Baidyanath Ayurved

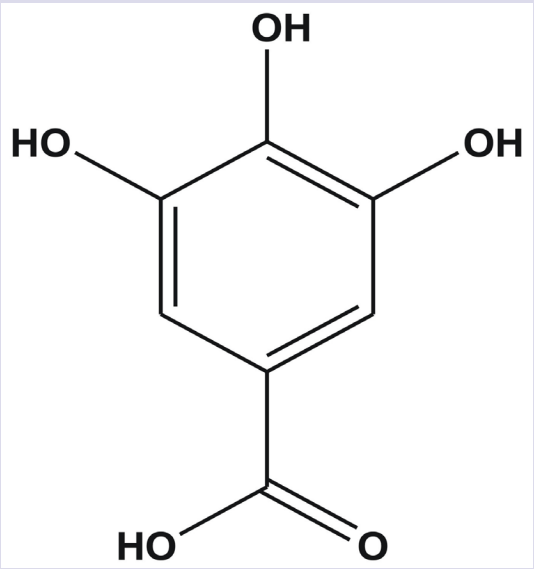

Figure 1: Structure of gallic acid

Bhawan Pvt. Ltd., Nagpur) purchased from market were used as positive controls for the wound healing evaluation. LOX 10% Spray (Lidocaine Topical Aerosol, Neon Laboratories Ltd., Mumbai) was used as a local anesthetic agent during the wound healing study. AR grade ethanol was procured from Jiangsu Huaxi International Trade Co. Ltd., China.

Preparation of the ethanolic extract from MEF and standardization

Accurately weighed powdered MEF (500 g) was extracted with ethanol (5 l × 3) under reflux for 6 h. The mixture was filtered through Whatman filter paper no. 1 and evaporated under reduced pressure at 40°C. Chemical standardization of the extract was carried out by performing preliminary phytochemical tests as per the reported method.[17] In addition, the extract was subjected for evaluation of its total phenolics content as per standard methods.[18] The extract was also standardized in terms of its gallic acid content (a phenolic compound with wound healing potential)[19] using a validated HPTLC technique.[10,20]

Antioxidant activity

Antioxidant activity of the ethanolic extract of MEF was evaluated *in vitro* using two stable free radicals, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH)[21] and galvinoxyl.[18] The total ferric chloride reducing power of the extract was also determined.[22] Ascorbic acid was used as a reference standard.

Experimental animals

Acute oral toxicity and wound healing activity of the ethanolic extract of MEF was studied in male Albino Wistar rats (200-250 g) while female New Zealand Albino rabbits (1.3-1.7 kg) were used for the skin irritation study. The animals were procured from Haffkine Biopharmaceuticals, Mumbai; housed in polypropylene cages under standard experimental conditions, fed standard pellet diet (Amrut laboratory animal feed, India) and provided water *ad libitum*.

Formulation of the extract and evaluation of safety

The white colored base of semi-solid consistency (vehicle) was prepared by heating the mixture of glycol stearate; 1, 2- propylene glycol and liquid paraffin (3:6:1 w/v/v) at 60°C as described previously.[23] The ethanolic extract of MEF was triturated with the vehicle using a mortar pestle to prepare three different doses as low dose 2.5% (MEL), mid dose 5% (MEM) and high dose 20% (MEH). Ethanolic extract of MEF was evaluated for safety as per OECD guidelines no. 404 (skin irritation test of MEH in female healthy rabbits)[24-25] and OECD test guidelines no. 420 (acute oral toxicity study at 2 g/kg body weight in male Albino Wistar rats)[26] as described previously. The studies were approved from the Institutional Animal Ethics Committee (CPCSEA/315, DG-130624-01/02).

Wound healing activity (excision wound model)

The dorsal side of each rat was depilated and local anesthesia was given using 10% lidocaine. Circular wounds of uniform size (1 cm diameter) were made with a punch-biopsy tool on the depilated region. One wound each was made towards both flanks and the wounds were kept open. The wounding day was considered as day zero. The rats were divided into six groups of six animals each and housed in groups of three/cage. The test ointments were applied topically on the wound site. In all the groups, the left-side wound was considered as untreated control while the right-side wound were treated as follows; group I: Vehicle base (0.5 g/wound); group II-IV: MEL, MEM and MEH, respectively (0.5 g/wound); group V: JT (0.75 mL/wound); group VI: BTD (0.5 g/wound). The study was approved from the Institutional Animal Ethics Committee (CPCSEA/315, DG-130624-03).

Wound area and wound contraction

Application of the test samples was done on each day in the morning until the last day of the treatment. Daily evaluation of wound area with the photographic records was done from day one until the day of complete healing. Progressive changes in the wound area were measured planimetrically by tracing wound margin on graph paper every day.[27-28] Wound contraction was calculated as a percent reduction in the wound area. A parallel group of animals were wounded with excision wounds and treated similarly as described above. From this parallel group of treated animals, the granulation tissue was removed on the 5th day post-wounding from respective patches. Biochemical and histopathological evaluations were made using the granulation tissue samples.

Biochemical and histopathological evaluation

Hydroxyproline and hexosamine content of granulation tissue was determined as per reported methods.[29,30] The protein content of granulation tissue was estimated by using Biuret method.[31] The number of days required for falling off the dead tissue remnants without any residual raw wound was considered as the period of epithelialization.[1] The excised granulation tissue was embedded in paraffin wax and serial sections (5 µm thickness) were cut. These sections were stained with hematoxylin and eosin and were examined under light microscope. Ulceration, necrosis, epithelialization, congestion, edema, polymorphonuclear leukocytes (PNL), mononuclear cells, fibroblasts, and vascularization were qualitatively graded.

Statistical analysis

GraphPad Prism software 5 version 5.03 (GraphPad Software, Inc., California, USA) was used to statistically evaluate the results. The statistical significance of the results has been evaluated using one-way ANOVA and Tukey's test. All the differences were considered statistically significant if $p<0.05$.

RESULTS AND DISCUSSION

There have been several studies on wound healing activity of plant extracts.[23,32-34] Due to the paucity of data in support on the traditional

Table 1: Antioxidant activity of the standardized ethanolic extract of MEF expressed in IC_{50} (µg/mL) for DPPH and galvinoxyl free radical assay and EC_{50} (µg/mL) for reducing power

Sample	DPPH radical	Galvinoxyl radical	Reducing power
Ethanolic extract of MEF	53.41±0.024	66.37±0.033	514.44±0.126
Ascorbic acid	2.29±0.004	1.54±0.002	31.43±0.012

MEF: *M. elengi* flowers

Table 2: Effect of the standardized ethanolic extract of MEF on epithelialization period and biochemical parameters (hydroxyproline, hexosamine and protein content) of granulation tissue

Groups	Parameters			
	Hydroxyproline content (mg/g)	Hexosamine Content (mg/g)	Protein content (mg/g)	Epithelialization period (in days)
NC	20.035±0.5607	2.195±0.0716	22.347±0.2584	12.08±0.239
VC	21.908±0.3653	2.328±0.1436	23.137±0.1914	11.92±0.239
MEL	36.765±0.4294[a]	3.197±0.1531[a]	42.753±0.3869[a]	10.17±0.167[a]
MEM	41.963±0.4442[a]	4.133±0.1315[a]	47.253±0.3154[a]	10.00±0.183[a]
MEH	61.703±0.5157[a]	7.565±0.1260[a]	77.650±0.3599[a]	8.08±0.154[a]
JT	64.313±0.5270[a]	7.425±0.1748[a]	69.413±0.3221[a]	10.00±0.183[a]
BTD	63.063±0.4432[a]	7.030±0.0976[a]	72.753±0.3131[a]	10.08±0.154[a]

MEF: *M. elengi* flowers, NC: Normal/untreated control, VC: Vehicle control (base), MEL: Low dose of the standardized ethanolic extract of *M. elengi* flowers (2.5%), MEM: Mid dose of the standardized ethanolic extract of *M. elengi* flowers (5%), MEH: High dose of the standardized ethanolic extract of *M. elengi* flowers (20%), JT: *Jatyadi Taila* (traditional drug), BTD: Betadine (modern drug). Data expressed as mean ± SE, n=6. [a]p<0.001 when compared with control group (NC).

claim of MEF as wound healing agent, in this study, the ethanolic extract of MEF has been standardized (in terms of total phenolics and gallic acid- a phenolic compound with wound healing properties) and evaluated for its wound healing potential in Albino Wistar rats. The findings have also been supported by evaluating the antioxidant property of the extract.

Standardization of the ethanolic extract of MEF

Extraction of MEF using ethanol gave a dark brown colored residue (yield: 8.40%). During qualitative preliminary phytochemical evaluation, flavonoids, alkaloids, glycosides, resins and phenolics were found to be present in the ethanolic extract of MEF. The extract showed total phenolics content of 69.0 ± 0.12 mg gallic acid equivalent/g of dried extract. The content of gallic acid was found to be 6.94 ± 0.009 mg/g of dried extract (determined by our recently published validated HPTLC technique). Similarly, the ethanolic extract of *Emblica officinalis* fruits[35] and *Agaricus bisporus*[36] has also been standardized in terms of gallic acid content prior to their pharmacological evaluation. The content of gallic acid in the ethanolic extract of MEF was found to be more when compared with these published reports.

The standardized ethanolic extract of MEF as an antioxidant agent

ROS (reactive oxygen species) produced at the site of wound against invading bacteria can induce severe tissue damage, leading to neoplastic transformation, and even decrease in the healing process by damaging the cellular membranes, DNA, proteins and lipids.[4] Thus, the overall role of antioxidants appears to be significant in the successful treatment of wounds. Various plants including *Acacia senegal, Centella asiatica, Curcuma longa, Ficus lutea,* etc.[4] have been reported to possess both wound healing and antioxidant properties which clearly supports that the wound healing properties of plants, in most cases, are associated with their significant antioxidant activities.

DPPH and galvinoxyl radicals are the most commonly used substrates to evaluate the antioxidant activity.[37] In this study, the IC_{50} value of the ethanolic extract of MEF was found to be less for DPPH free radical when compared with galvinoxyl free radical (Table 1). Similarly, the extract reduced the Fe^{3+} complex to Fe^{2+} form (EC_{50} value 514.44 ± 0.126 µg/mL). This clearly suggests that the extract could transfer its protons to both the free radicals and donate electrons to ferric ions which finally terminate the free radical reaction.[38] This may be attributed to the phenolic compound gallic acid and other groups of phytochemical constituents present in it[36,39]. Similar results have been reported for the ethanolic extract of *Terminalia glaucescens* flowers,[40] different plant parts of *Lepidium sativum*,[41] and *Mollugo nudicaulis*.[42]

Safety of the standardized ethanolic extract of MEF

The skin irritation test of the ethanolic extract of MEF (20%) on rabbits showed no signs of dermal irritation after the topical application. The primary irritation index of the ethanolic extract of MEF was found to be 0.00 (appendix 1: Supplementary material). Thus, it was confirmed that the ethanolic extract of MEF has an adequate safety margin to be used on rabbit skin. In addition, oral administration of the ethanolic extract of MEF to rats did not cause any mortality as well as no significant change in the body weight, food and water intake was observed when compared with the animals of control group. The cage-side observations were also appeared normal (appendix 2: Supplementary material). Thus, the data obtained from the skin irritation study and acute oral toxicity study may ensure an adequate safety margin of MEF for their intended use.

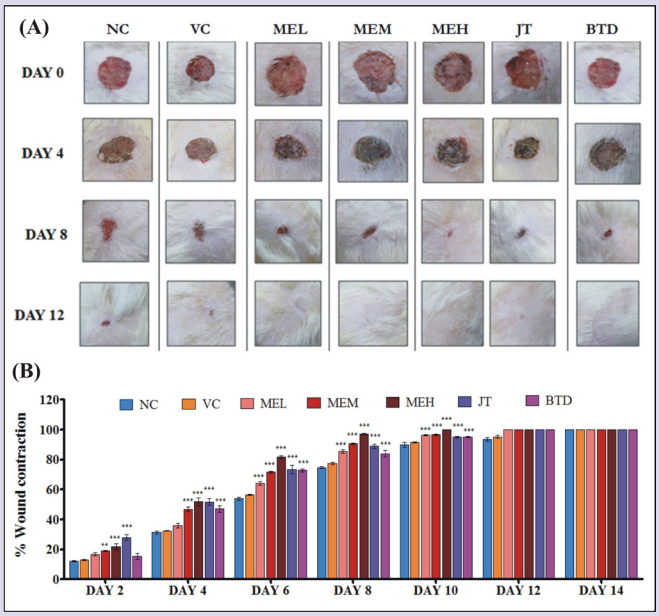

Figure 2: Effect of MEF on wound contraction in excision wound rat model: photographic record (A) and percent wound contraction (B). MEF: *M. elengi* flowers, NC: Normal/ untreated control, VC: Vehicle control (base), MEL: Low dose of the standardized ethanolic extract of *M. elengi* flowers (2.5%), MEM: Mid dose of the standardized ethanolic extract of *M. elengi* flowers (5%), MEH: High dose of the standardized ethanolic extract of *M. elengi* flowers (20%), JT: *Jatyadi Taila* (traditional drug), BTD: Betadine (modern drug). Data expressed as mean ± SE, n=6. **p<0.01 and ***p<0.001 compared to the control group (NC) on respective day.

Wound healing potential of the standardized ethanolic extract of MEF

The topical application of the ethanolic extract of MEF at three different doses significantly increased (p<0.001) the rate of wound contraction when compared with animals of control groups (Figure 2a and 2b). The highest dose of the ethanolic extract of MEF (MEH-20%) showed the fastest rate of wound closure with complete healing on 10th day post-wounding. The complete wound closure by the other two doses of plant extract (MEL-2.5% and MEM-5%), JT and BTD was observed on 12th day post-wounding (Figure 2a and 2b). Although, the animals in these treatment groups showed complete healing of the wound on the same day, the rate of contraction was higher in the animals treated with JT and BTD. During the study, no significant change in the body weight, food and water intake of all the animals was observed. As per the observations made, the study indicated dose-dependent nature of the ethanolic extract of MEF to induce cellular proliferation, rapid epithelialization, and collagenization, and dose of 20% may be considered potentially useful to obtain significant wound healing activity.

Epithelialization is a process of restoring the epidermis and involves proliferation and migration of keratinocytes. Cell proliferation is an essential event during re-epithelialization, so proliferating keratinocytes ensure an adequate supply of cells to migrate and cover the wound.[43] In the present study, it was observed that the epithelialization period was not significantly different when animals from normal (untreated) and vehicle control groups were compared (Table 2). On the contrary, the epithelialization period was significantly reduced in the animals treated with the highest dose of the ethanolic extract of MEF when compared with the animals from the control group. This suggests that the extract may exert a positive effect towards cellular proliferation.[32] Animals from the other treatment groups also showed significant reduction in the epithelialization period (p<0.001) when compared with the animals of the control group (Table 2). Thus, the ethanolic extract of MEF exerts wound healing potential in rats with excision wounds by significantly increasing the rate of wound contraction and reducing the period of epithelialization.

Hydroxyproline, hexosamine, and protein are the biomarkers which indicate the rate of wound healing and collagen turnover.[27,44] Collagen plays a role in hemostasis and provides strength and integrity of the wound matrix. It is also essential for re-epithelialization and cell-cell/ cell-matrix interactions. The strength of the repaired wound tissue is a result of the regeneration of collagen and the formation of intra-and inter-molecular cross-linking to form fibers.[32] Wounds treated with the highest dose of the ethanolic extract of MEF showed significant increase in the content of hydroxyproline, hexosamine and protein of the granulation tissue (scab) as compared with control wounds, (Table 2, p<0.001). The significant increase in hydroxyproline content of scab tissue after application of the ethanolic extract of MEF may be attributed to the increase in collagen concentration and stabilization of fibers.[45] Increase in the hexosamine content of the granulation tissue in the animals treated with the ethanolic extract of MEF indicate the active synthesis of ground substances (mucopolysaccharides) by fibroblasts, on which the collagen can be laid.[45] Significant increase in the protein content of dry granulation tissue from the wounds treated with the ethanolic extract of MEF indicates the cellular proliferation and also suggests an increase in the synthesis of collagen, which is the predominant extracellular protein in the granulation tissue of wounds.[32]

Skin is the important external defense organ of the body in living organisms. The exposure of skin to environmental factors such as UV light leads to the degradation of collagen in a rapid rate and results in aging, hyper-pigmentation, inflammation etc. The continuous exposure to various such environmental factors leads to alterations in the connective tissue via the formation of enzymes and reactive oxygen species leading to the impairment of the tensile strength of the skin. Furthermore, MEF have also been reported to possess anti-tyrosinase activity.[15] Therefore, the results of the present research work indicate that the ability of the ethanolic extract of MEF to increase the collagen concentration and antioxidant potential shown by the extract may also support its use as anti-aging and skin whitening agent.

Newly synthesized collagen at the wound site increases the tensile strength of the wound tissue. Histopathological evaluation of wound tissue after the treatment of excision wounds with the ethanolic extract of MEF revealed increased cellular proliferation and marked collagenization which might have significantly contributed to the healing process (Appendix 3: Supplementary material). Epithelialization was evident in all the groups including normal/untreated control group but with a marked recovery of tissue in animals treated with the highest dose of the ethanolic extract of MEF. None of the treatment regimens induced necrosis on the wound area. Similar results have been reported for the ethanolic extract of *Acacia caesia* bark,[43] *Calotropis procera* bark,[46] *Curcuma longa* rhizomes,[47] *Tectona grandis* leaves,[48] and *Achyranthus aspera* leaves.[49]

Findings of this study confirm that the rate of wound contraction was significantly higher and the period of epithelialization was shorter in rats treated with the ethanolic extract of MEF. Therefore, the topical administration of the ethanolic extract of MEF accelerates and promotes various stages of wound healing including fibroplasia, collagen synthesis, wound contraction, and epithelialization. The biochemical evaluation of the granulation tissue from the excision wound animal model revealed the significant increase in the hydroxyproline and hexosamine content in the treated wounds, which indicates cellular hyperplasia. Similarly there is an increase in the total protein content representing the active synthesis

Appendix 1: Skin irritation studies of the standardized ethanolic extract of MEF showing Primary Irritation Index

Rabbit ID No.	Total erythema + edema						Average as primary irritation index
	24 h		48 h		72 h		
	Control	Test	Control	Test	Control	Test	
R-01	0	0	0	0	0	0	0
R-02	0	0	0	0	0	0	0
R-03	0	0	0	0	0	0	0
Mean	**0**	**0**	**0**	**0**	**0**	**0**	**0**

MEF: *M. elengi* flowers

Appendix 2: Daily body weight record of the animals administered with distilled water (control) showing the percent mean difference between consecutive days

Animal no.	Body weight on different days (g)														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Mean (n=5)	222.6	223.8	224.4	223.8	228.0	227.2	228.4	230.2	229.4	229.4	230.8	232.2	233.0	232.8	234.8
SE	2.52	2.33	3.01	2.75	2.88	2.06	2.50	1.85	2.18	1.66	2.08	1.85	2.17	1.93	2.58
% Mean difference	–	0.54	0.27	-0.27	1.88	-0.35	0.53	0.79	-0.35	0.00	0.61	0.61	0.34	-0.09	0.86

Daily body weight record of the animals administered with the standardized ethanolic extract of MEF at 2000 mg/kg body weight (test) showing the percent mean difference between consecutive days

Animal no.	Body weight on different days (g)														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Mean (n=5)	227.2	226.4	226.8	228.0	229.4	231.2	231.4	232.6	232.6	233.0	234.4	235.0	236.2	236.8	237.8
SE	2.58	2.69	2.22	2.83	2.62	2.76	2.04	2.38	2.06	1.82	1.72	1.58	1.39	1.20	1.71
% Mean difference	–	-0.35	0.18	0.53	0.61	0.78	0.09	0.52	0.00	0.17	0.60	0.26	0.51	0.25	0.42

MEF: *M. elengi* flowers

Daily food intake record of the animals administered with distilled water (control) and the standardized ethanolic extract of MEF at 2000 mg/kg body weight (test) showing the percent difference between consecutive days

Treatment group	Parameters	Different days													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	**Food intake (g)***	62.5	65	64	62.5	65	67.5	70	67	66.5	68	70	71.5	70	70
	% Difference		4.00	-1.54	-2.34	4.00	3.85	3.70	-4.29	-0.75	2.26	2.94	2.14	-2.10	0.00
Test	**Food intake (g)***	72.5	70.5	72	75	76.5	75	76.5	77.5	74.5	76.5	78.5	77.5	79	80.5
	% Difference		-2.76	2.13	4.17	2.00	-1.96	2.00	1.31	-3.87	2.68	2.61	-1.27	1.94	1.90

*As animals of respective groups were kept in separate cages, record of food intake was cumulative for all the animals (n = 5) of respective group.
MEF: *M. elengi* flowers

Daily water intake record of the administered with distilled water (control) and the standardized ethanolic extract of MEF at 2000 mg/kg body weight (test) showing the percent difference between consecutive days

Treatment group	Parameters	Different days													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	**Water intake (mL)***	95	95	90	95	90	95	90	95	100	105	100	105	110	110
	% Difference		0.00	-5.26	5.56	-5.26	5.56	-5.26	5.56	5.26	5.00	-4.76	5.00	4.76	0.00
Test	**Water intake (mL)***	105	110	105	100	100	95	100	105	100	105	110	115	120	115
	% Difference		4.76	-4.55	-4.76	0.00	-5.00	5.26	5.00	-4.76	5.00	4.76	4.55	4.35	-4.17

*As animals of respective groups were kept in separate cages, record of food intake was cumulative for all the animals (n = 5) of respective group.
MEF: *M. elengi* flowers

Data on mortality record, mortality latency and cage side observation during acute oral toxicity study of the standardized ethanolic extract of MEF for fourteen days

Treatment group	Sample details	D/T	Mortality latency*	Symptoms of toxicity**
Control	Administered with distilled water	0/5	--	No toxic symptoms during the observation period
Test	Administered with the standardized ethanolic extract of MEF at 2000 mg/kg body weight	0/5	--	

MEF: *M. elengi* flowers

D/T = Dead/treated rats. *Time to death (in h) after oral administration. **Changes in skin and fur, teeth, eyes and mucous membrane (nasal) and also autonomic changes (salivation, lacrimation, perspiration, piloerection, urinary volume, breathing abnormalities, abdominal distension and defecation) and alterations to the central nervous system (ptosis, drowsiness, gait, tremors and convulsion).

Appendix 3: Comparative qualitative histopathological evaluation of the granulation tissue excised on 5th day post wounding

Parameters	Groups						
	NC	VC	MEL	MEM	MEH	JT	BTD
Fibroplasia	+	+	+	++	+++	+++	+++
Collagenization	-	-	++	++	+++	+++	+++
Neovascularization	-	-	-	-	-	-	-
Leucocytic infiltration	+	++	++	++	+++	+++	++
Epithelialization	+++	+++	+++	+++	+++	+++	+++
Hemorrhage	+	+	+	+	+	+	+

Minimal (+), Mild (++), Moderate (+++), Marked (++++). NC: Normal/ untreated control, VC: Vehicle control (base), MEL: Low dose of the standardized ethanolic extract of *M. elengi* flowers (2.5%), MEM: Mid dose of the standardized ethanolic extract of *M. elengi* flowers (5%), MEH: High dose of the standardized ethanolic extract of *M. elengi* flowers (20%), JT: *Jatyadi Taila* (traditional drug), BTD: Betadine (modern drug).

and deposition of matrix proteins in the granulation tissues which is also supported by the observations made during the histopathological evaluation of granulation tissue. Thus, results obtained in this study validate the traditional claim on wound healing efficacy of MEF. Indeed, it is equally essential to evaluate these flowers using some other wound models such as incision wound, burn wound etc.

CONCLUSION

Many traditional plant remedies are known in folk medicines with reported wound healing property and some of them have been validated by scientific studies to actually support their biological action. This study, therefore, provides a basis to the traditional use of MEF as wound healing agent. The present work is the first attempt in evaluating the wound healing potential of the standardized ethanolic extract of MEF as well as evaluation of its antioxidant potential. MEF, an Ayurvedic medicinal plant, shows great potential to be developed into an ointment or gel for topical use. The findings of the present study also provide the baseline data for designing further investigations on the therapeutic action of the standardized ethanolic extract of MEF, especially to evaluate wound healing efficacy in diabetic as well as in chronic ulcers.

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CONFLICTS OF INTEREST

Authors have no conflicts of interest to declare.

ABBREVIATION USED

ANOVA: Analysis of variance; **BSS:** British Standard Sieve; **BTD:** Betadine; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **DNA:** Deoxyribonucleic acid; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **EC_{50}:** Half maximal effective concentration; **GAE:** Gallic acid equivalent; **HPTLC:** High Performance Thin Layer Chromatography; **IC_{50}:** Half maximal inhibitory concentration; **JT:** Jatyadi Taila; **MEF:** *M. elengi* flowers; **MEH:** High dose of the standardized ethanolic extract of *M. elengi* flowers (20%); **MEL:** Low dose of the standardized ethanolic extract of *M. elengi* flowers (2.5%); **MEM:** Mid dose of the standardized ethanolic extract of *M. elengi* flowers (5%); **NC:** Normal control; **OECD:** Organization for Economic Co-operation and Development; **PNL:** Polymorphonuclear leukocytes; **ROS:** Reactive oxygen species; **SE:** Standard error; **UV:** Ultra violet; **VC:** Vehicle control.

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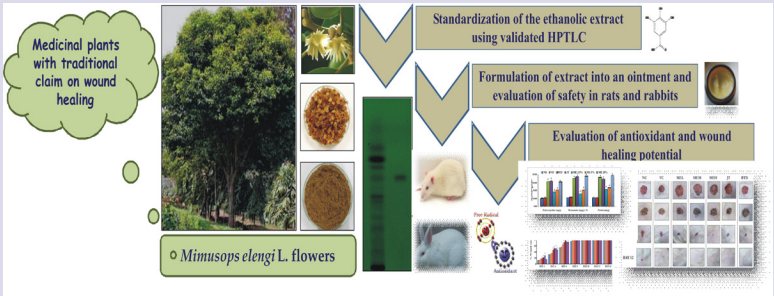
SUMMARY

- Traditionally known wound healing effect of an Ayurvedic medicinal plant; *Mimusops elengi* flowers might be mediated in part through their scientific evaluation (*in vivo* excision wound model).
- The ethanolic extract of *Mimusops elengi* flowers was non-toxic up to 20% for topical administration and 2000 mg/kg body weight for oral administration in animal models.
- *Mimusops elengi* flowers in conjugation with promising antioxidant activity show great potential to be developed into an ointment or gel for its topical use as wound healing agent.

PICTORIAL ABSTRACT



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