# **Original Research Article**

# Phytochemical screening and assessment of wound healing activity of the fruits of *Mimusops elengi* Linn.

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#### ABSTRACT:

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. The aqueous extract of the fruits of Mimusops elengi Linn., was investigated to determine its total yield, phytochemical constituents and wound healing in groups of Wistar rats by excision model. Bioactive constituents like alkaloids, glycoside, terpene, saponin, tannins and flavonoids occur in abundance in the crude aqueous extract. Three test doses of ointment applied to study wound healing i.e 100mg/kg, 200mg/kg and 400mg/kg. The parameters studied included rate of % wound contraction and days of complete wound healing. The result of the study relative to wound healing was very interesting. There was a progressive decrease in wound area with time, indicating an efficacy of the formulations in healing the induced wounds. By the 15th day, The % mean wound contraction area in day  $18^{\text{th}}$  were  $27.2\pm 0.06$  (control),  $1.2\pm 0.02(100 \text{ mg/kg})$ ,  $3.21\pm 0.04$  (200 mg/kg) and  $1.21\pm 0.18(400 \text{ mg/kg})$ , and  $0.90\pm0.00$  (povidone iodine ointment). A better healing pattern with complete wound closure was observed rats treated with 100mg/kg aqueous fraction within 18 days while it took 24 days in control rats. There was a significant increase ( $p \le 0.05$ ) in wound contraction from day 4 onwards in all the treated rats except those treated with 200mg/kg aqueous fraction in day 4. The present study thus provides a scientific rationale for the traditional topical application of the ointment of fruits of Mimusops elengi Linn., on wounds.

Keywords: Mimusops elengi Linn., wound-healing activity, Phytochemical, aqueous extact and excision.

#### INTRODUCTION

The classical systems of Indian Medicine especially Ayurveda, Siddha and Unani employed a large number of medicinal plants for treatment of skin diseases which includes cuts, wounds and burns. Medicinal plants have been used since time immemorial for treatment of various ailments of skin and dermatological disorders especially cuts, wounds and burns<sup>1</sup>. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of the indigenous medicinal plants are used as spices and food plants. They also sometimes added to foods **39** www.earthjournals.org Volume 4 Issue 1 2014

meant for pregnant women and nursing mothers for medicinal purposes<sup>[1,2,3]</sup>. Wounds are generally classified as, wounds without tissue loss (e.g. in surgery), and wounds with tissue loss, such as burn wounds, wounds caused as a result of trauma, abrasions or as secondary events in chronic ailments eg: venous stasis, diabetic ulcers or pressure sores and iatrogenic wounds such as skin graft donor sites and derma  $abrasions^{[4]}$ .

A large number of plants are used by traditional medical practitioners in many countries for the treatment of wounds and burns. These natural agents induce healing and regeneration of the lost tissue by multiple mechanisms. The herbal extracts and fractions effectively arrest bleeding from fresh wounds, inhibit microbial growth and accelerate wound healing<sup>[5]</sup>.

Fresh fruits of *Mimusops elengi Linn.*,were collected in a street in Eluru, west godavari district, Andhra pradesh, India. In july and authenticated by department of botony, Acharya Nagarjuna university, guntur, India. A herbarium is maintained in sir crr College of Pharmacy, Eluru, Andhra Pradesh , India.It belongs to the Order; *s and* Family: Sapotaceae. It is commonly called Bakul tree, Telugu - Pogada, Bogad, vakula, Tamil - Mahilam, English : Spanish -Cherry, West Indian Medlar and Hindi - Bakul, Bolsari, Maulsarau, Maulser, Maulsari<sup>[6]</sup>.

The plant *Mimusops elengi Linn.*, (Sapotaceae ) are proved to shown the antibacterial activity<sup>[7]</sup>, antioxidant<sup>[8]</sup>, acute anti-inflammatory activity<sup>[9]</sup>, neuropharmacological activities<sup>[10]</sup>, analgesic and antipyretic<sup>[11]</sup>. A survey of literature revealed that no systematic approach has been made to study wound healing activity of this plant. The fruits of *Mimusops elengi Linn.*, contains Quercitol, ursolic acid, dihydro quercetin, quercetin,  $\beta$  - d glycosides of  $\beta$  sitosterol, alpha-spinasterol<sup>[12]</sup>. In this study we assess the phytoconstituents and the wound healing ability of The fruits of *Mimusops elengi Linn.*,

#### MATERIALS AND METHODS

#### Plant material

Fruits of *Mimusops elengi Linn*, were collected from local areas of west godavari district during august 2013 dried and were authenticated by Acharya Nagarjuna university, Andhra Pradesh.

#### Plant extract preparation

Cold extraction method was followed. Portions (100g) of the fresh and dry samples were weighed into 1000 ml conical flasks and 1000ml of water was added and left for 48 h. The mixtures were filtered under vaccum pressure and the filtrates were concentrated using rotary evaporator and subjected for the various activity studies<sup>[13]</sup>.

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All standard chemicals used in this study were of analytical grade. The drugs povidone iodine was obtained from WIN Medicare Pvt. Ltd., Hyderabad .

#### Phytochemical Analysis

The different extracts thus obtained were qualitatively tested for the presence of various phytochemical constituents<sup>[14]</sup>.

#### 1. Detection of Alkaloids:

(Wagner's test) Wagner's test: to the acidic solution, Wagner's reagent (iodine in potassium iodide) was added. Brown precipitate indicates the presence of alkaloids. (I2=1.27gm, KI=2gm+5ml H2O final makeup 100ml)

#### 2. Detection of Saponins :

A drop of sodium bicarbonate was added in a test tube containing about 50ml of an aqueous extract of samples. The mixture was shaken vigorously and kept for 3min. a honey comb like froth was formed and it shows the presence of saponins.

#### **3. Detection of Glycosides:**

A small amount of alcoholic extract of samples was dissolved in 1ml water and then aqueous 10% sodium hydroxide was added. Formation of a yellow colour indicated the presence of glycosides.

#### 4. Detection of Carbohydrates (molish test) :

To 2ml of the extract, add 1ml of  $\alpha$ -napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet color at the junction of the two liquids reveals the presence.

#### 5. Test for Tannins:

Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

#### 6. Detection of Flavonoids (Ferric chloride test):

A few drops of neutral ferric chloride solution were added to one ml each of above alcoholic solution. Formation of blackish red colour indicates the presence of flavonoids.

#### 7. Detection of Steroids (Salkowski's test):

About 100 mg of dried extract was dissolved in 2m l of chloroform.Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface was an indicative of the presence of steroidal ring.

**8. Test for Proteins (Biuret test):** Add 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution till a blue color is produced, and then add to the 1ml of the extract. Formation of pinkish or purple violet color indicates the presence of proteins.

#### 9. Test for Terpenes (Noller's test) :

Dissolve two or three granules of tin metal in 2ml thionyl chloride solution. Then add 1ml of the extract into test tube and warm, the formation of pink color indicates the presence of triterpenoids.

#### **10. Test for Gums:**

Add about 10ml of aqueous extract slowly to 25ml of absolute alcohol with constant stirring. Filter the precipitate and dry in air. Examine the precipitate for its swelling properties and for the presence of carbohydrates.

Preparation of ointment formulation of aqueous extract :

Ingredients list: 1.wool fat-1gm 2.hard paraffin-1gm 3.cetostearyl alocohol-1gm 4.yellow soft paraffin-17gm 5.methylparaben-0.04gm

6. *Mimusops Elengi Linn.*, fruits aqueous extract 100mg/kg, 200mg/kg and 400mg/kg Method of preparation:

By using fusion method ointment formulation is done. one by one all ingredients are melted according to their melting points. After melting the product is cooled in water bath. Finally aqueous extract was incorporated<sup>[15]</sup>.

#### EXPERIMENTAL

Animals:

Male *Wistar* rats (220-260 g) were used for all experiments. Animals were housed under conditions of controlled temperature ( $25\pm1$  °C) with relative humidity of 45-55% and lighting (lights on: 6 am to 6 pm) and had free access to food and water and the animals were acclimatized for a period of dark/light cycle. The animals were acclimatized for a period of two weeks and were kept under pathogen free conditions.

#### Wound models

The animals were starved for 12h prior to wounding. Studies were carried out using ether-anaesthetized rats. The rats were divided into five groups (n = 6). Animals were depilated at the dorsal thoracic region before wounding. The first group served as control similarly second group served as standard (povidone iodine ointment) third, fourth and fifth groups received 100mg/kg, 200mg/kg and 400mg/kg aqueous extract by topical application according to body weight excision of wound model and for 20 days in the excision wound model.

#### **Experimental protocol:**

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Animals were selected, weighed (200–250g) and devided into five groups (n=6), namely control, three groups belonging to aqueous extract ointment of *Mimusops Elengi Linn*.,

Groups of six animals in each groups as follows and the treatment was done topically in all the cases:

- Group I- Control animals: Received injury for wound formation but did not receive any ointment or drug treatment
- Group II- standard animals: Received injury for wound and treatment with povidone iodine ointment
- Group III- Drug treated animals: received injury for wound formation and treatment with *Minusops Elengi Linn.*, fruits aqueous extract ointment (given 100 mg/kg)
- Group IV- Drug treated animals: received injury for wound formation and treatment with *Mimusops Elengi Linn.*, fruits aqueous extract ointment (given 200 mg/kg)
- Group V- Drug treated animals: received injury for wound formation and treatment with *Mimusops Elengi Linn.*, fruits aqueous extract ointment (given 400 mg/kg)

### Excision Wound Model

Excision Wound healing was used to evaluate the wound healing activity of extract. Circular wounds were inflicted on the cleared skin by cutting under ether topical anesthesia. The areas of the wounds was measured (sq. mm) immediately by using vernier callipers. This was taken as the initial wound area reading. The formulation ointment and standard drug were applied daily until the complete healing. In this model, wound contraction was monitored. The wound areas were measured while the animals were under an aesthesia on the 1, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 day after surgery<sup>[16]</sup>. (Table 2)

*Statistical analysis* : The data were analyzed using one way analysis of variance (ANOVA) and data subjected to LSD post hoc test. Differences in mean between paired observations were accepted as significant at  $P \le 0.05$ 

#### RESULTS

This study has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals such as terpenoids, reducing sugar, flavonoids, alkaloids and phlobatannins were present in the samples. The result of the phytochemical analysis shows that the ten plants are rich in at least one of alkaloids, flavonoids, terpenoids, glycosides and phlobatannins. Plant *Mimusops elengi Linn.*, having all these phytochemicals. The phytochemical screening and qualitative estimation of medicinal plant studied showed that the fruits were rich in phlobatannins, terpenoid, flavonoids, alkaloids and glycosides (Table 1).

#### WOUND HEALING ACTIVITY

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In this study, topical application of the Aqueous extract of fruits of *Mimusops elengi Linn.*, incorporated into an ointment base on the excision wound in rats caused a significantly (P<0.05) higher rate of wound healing. Application of the ointment batch containing the concentrations of *Mimusops elengi Linn.*, extract (100 mg/kg, 200 mg/kg and 400 mg/kg in 20g of ointment) produced the highest rate of wound healing, reducing the wound healing area to 15 days compared to the blank ointment treatment with wound healing area of 18 days (Tables 2). Wound healing is a natural process of regenerating dermal and epidermal tissues. Whenever there is a wound, a set of overlapping events takes place to repair the damage. On comparison of three doses of tested ointment *Mimusops elengi Linn.*, extract of 100 mg/kg and 400 mg/kg showed good contraction of wounds compared with standard drug povidone iodine ointment

The result of this work shows that formulating fruits of *Mimusops elengi Linn.*, extracts into an ointment is effective in wound repairs and encourages the harnessing of the extracts in the formulation of commercial dermatological ointments

Phytoconstituents	Aqueous extract				
ALKALOIDS	PRESENT				
Saponins	Present				
Glycosides	Present				
Carbohydrates	Absent				
Tannins	Present				
Flavonoids	Present				
Steroids	Present				
Proteins	Absent				
Terpenoids	Absent				
Fats	Absent				
Gums and Mucilages	Absent				

TABLE NO.1 QUALITATIVE PHYTOCHEMICAL ANALYSIS OF AQUEOUS EXTRACT OF FRUITS OF Mimusops elengi Linn.,

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#### TABLE- 2

The effect of *Mimusops Elengi Linn.*, fruits aqueous extract ointment on excision wound healing in rats

	Dose	Wound area in mm <sup>2</sup>								
Group		2 <sup>nd</sup> day	4 <sup>th</sup> day	6 <sup>th</sup> day	8 <sup>th</sup> day	10 <sup>th</sup> day	12 <sup>th</sup> day	14 <sup>th</sup> day	16 <sup>th</sup> day	18 <sup>th</sup> day
С	-	225.3 ±0.12	210.3± 0.09	190.5± 0.17	90.5± 0.09	80.30± 0.10	65.3± 0.11	55.2± 0.09	33.5± 0.12	27.2± 0.06
S	0.5mg/kg	225.3 ±0.12	186.44± 0.07	120.44± 0.04	47.22± 0.03	13.81± 0.09	6.61± 0.11	5.51± 0.07	2.31± 0.06	0.7± 0.02 <sup>**</sup>
	100 mg/Kg	225.3 ±0.12	187.44± 0.07	125.44± 0.04	50.22± 0.03	15.81± 0.09	8.61± 0.11	6.51± 0.07	2.31± 0.06	1.2± 0.02**
AQ EX	200 mg/Kg	225.3 ±0.12	215.8± 0.11	187.44± 0.07	148.84± 0.06	123.21± 0.03	73.71 ± 0.04	12.71± 0.02	6.51± 0.10	3.21± 0.04
	400 mg/Kg	225.3 ±0.12	161.04± 0.04	136.64± 0.09	104.04± 0.03	57.51± 0.08	50.41 ± 0.06	20.91± 0.05	3.41± 0.10	1.21± 0.18 <sup>**</sup>

C means control, S means standard and AQ EX means aqueous extract \*\*p < 0.05

#### Figure .1

Photographs of rats showing various phases of wound healing. A



A: Control; B:Standard C: *Mimusops Elengi Linn.*, fruits aqueous extract 100mg/kg; D: *Mimusops Elengi Linn.*, fruits aqueous extract 200mg/kg; E: *Mimusops Elengi Linn.*, fruits aqueous extract 400mg/kg

**Statistical analysis:** The data were analyzed using one way analysis of variance (ANOVA) and data subjected to LSD post hoc test. Differences in mean between paired observations were accepted as significant at  $P \le 0.05$ 

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#### **GRAPH NO-2**

THE EFFECT OF *MIMUSOPS ELENGI LINN.*, FRUITS AQUEOUS EXTRACT OINTMENT ON EXCISION WOUND HEALING IN RATS.



#### DISCUSSION

The wound-healing property of *Mimusops Elengi Linn.*, fruits may be attributed to the phytoconstituents present in the plant and the faster process of wound healing could be a function of either the individual or the additive effects of the phytoconstituents. Tannins are astringent and antimicrobial in property, hence it can be inferred that the woundhealing activity of the fruits extract of *Mimusops Elengi Linn.*, observed is due partly to its tannin and flavonoids contents, which seems to be responsible for wound contraction and increased rate of epithelization<sup>[17, 18]</sup>.

Wound healing involves various phases. Initially involves acute inflammatory phase followed by the synthesis of collagen and other extra cellular macromolecules, which are later removed to form a scar<sup>[19]</sup>. In excision wound model the increased rate of wound contraction and decrease in period of epithelization in the animals treated with aqueous extract of *Mimusops Elengi Linn.*, may be attributed to their broad spectrum antibacterial activity. Significant increase in skin breaking strength and hydroxyl proline content which was a reflection of increased collagen levels by increased cross linking of collagen fibres. In addition, increase in dry granulation tissue weight indicated the presence of higher protein content<sup>[20]</sup>. The breakdown of collagen liberates free hydroxyl proline and

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its peptides and elevated level of hydroxyl proline is the index of increased collagen turnover.

#### CONCLUSION

The results obtained in the present study clearly indicate that the *Mimusops elengi Linn.*, fruits aqueous extract ointment are having significant wound healing activity in rats. The wound healing effect of *Mimusops elengi Linn.*, fruits aqueous extract may be due to the presence of more than one active principles mentioned above. Further pharmacological and biochemical investigation will clearly elucidate the mechanism of action and will be help full in projecting this plant as an therapeutic target in wound healing and other diseases

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