

## WOUND HEALING ACTIVITY AND FORMULATION OF ETHER-BENZENE-95% ETHANOL EXTRACT OF HERBAL DRUG *MADHUCA LONGIFOLIA* LEAVES IN ALBINO RATS

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The aim of present study was to assess the wound healing activity of ethanolic extracts of leaves and bark of *Madhuca longifolia*. Ethanolic extract of leaves and bark of *Madhuca longifolia* was examined for wound healing potential in the form of 5% w/w ointment in the excision wound created on the dorsal side of experimental animals, the 5% w/w extract should considerable difference in wound models and the result were compatible to that of the standard drug Betadine (5% w/w) in terms of wound contracting ability and wound closure time. Antibacterial activity of ethanolic extract of the plant was also carried out as a supporting evidence for its wound healing potential. The mean percentage wound closure was calculated on the 8<sup>th</sup>, 11<sup>th</sup>, 13<sup>th</sup>, 15<sup>th</sup> and 19<sup>th</sup> wounding days. The extract treated animals showed faster epithelisation of wound ( $17.86 \pm 0.19$  and  $14.81 \pm 0.67$ ) bark and leaves respectively then the control. The period of epithelisation  $11.8 \pm 0.37$  in case of standard drug 5% betadine ointment.

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*Keywords:* Madhuca longifolia, Betadine, Epithelisation period

### 1. Introduction

Mahua (*Madhuca longifolia*, family- Sapotaceae) is an important plant having vital socio-economic value. Mahuwa Flower is edible and is a food item of tribal. Flowers are used to make syrup for medicinal purposes. The flowers of Mahuwa tree are fomented to produce an alcoholic drink called Mahuwa, country liquor. Mahua oil is used in medicine as emollient, cure of skin diseases, rheumatism, headache, as laxative, in piles and haemorrhoids<sup>[1]</sup>. The flowers are fleshy, off white in color, and emit attractive sweet fragrance when the plant is in full bloom. The fruit is a kind of berry, egg shaped. Mature seeds can be obtained during June to July. Mahua is a slow growing species, attains a mean height of 0.9 to 1.2 m at the end of the fourth year. The coppice crop may attain a mean height of 9.19 m and girth 0.5 m in 30 years<sup>[2]</sup>.

### 2. Experimental

#### 2.1 Preparation of extract

The leaves and bark of *Madhuca longifolia* were collected from Indore, Madhya Pradesh. The plant authenticated by comparing with the herbarium voucher specimen deposited at C.D.R.I. Lucknow. The leaves were dried under shade, powdered and passed through 40 meshes and stored in closed vessel for further use. The dried powdered material macerated for nine days with alcohol with frequent shaking separately. On the nine day it was filtered and the alcohol extract was concentrated in vacuum under pressure using rotary flash evaporator. The material was air dried

under shade, pulverized by a mechanical grinder and passed through a 40 mesh and then stored in airtight containers. The powdered leaves (250 g) were extracted with ethanol (50%) for 24 h using a Soxhlet extractor. This ethanolic extract was concentrated to dryness under reduced pressure and controlled temperature (50-60°C) to yield solid masses.

Table 1. Wound healing properties of the Ether-Benzene-Absolute Ethanol Extract

Drug	Percentage contraction (Sq mm $\pm$ SEM)			Incision Model
	8 <sup>th</sup> day	11 <sup>th</sup> day	19 <sup>th</sup> day	
Control	23.51 $\pm$ 5.91, 34.09 $\pm$ 2.61, 62.2 $\pm$ 0.81			321 $\pm$ 16.8
Extract	32.12 $\pm$ 2.03, 51.2 $\pm$ 2.31, 79.1 $\pm$ 3.01*			431.61 $\pm$ 32.91

\* Significant at  $P < 0.001$ , P-value was calculated by comparing with control by ANOVA test, Values are expressed as mean  $\pm$ SEM (n=6)

## 2.2 Phytochemical analysis of the extract

The extract was screened for the presence of various constituents employing standard screening test.<sup>3</sup> Conventional protocol for detecting the presence of glycosides, saponins, flavonoids, tannins etc. was used. Several phytoconstituents like flavonoids and glycosides are known to promote wound healing process due to their antioxidant and antimicrobial activities.<sup>4</sup>

## 2.3 Toxicity Studies

Toxicity studies of alcoholic extract were carried out in oral doses of 150 to 250 mg/ kg-body weight using albino rats. After test extract administration, animals were observed 72 hr. period. The number of deaths was expressed as a percentile and the LD50 was determined by probate a test using the death percentage versus the log dose.<sup>5</sup>

## 3. Wound healing activity

Excision wound models were used to evaluate the wound – healing activity of leaves and bark extract of *Madhuca longifolia*

## 4. Excision wound

In the excision wound model<sup>6, 8</sup> <C:\Documents and Settings\user\Local Settings\Temporary Internet Files\acrous\rwer.htm - ref16> rats were anaesthetized with lignocaine prior to creation of the wounds. A full thickness of the excision wound of 314 mm<sup>2</sup> was created by using toothed forceps, a surgical blade and pointed scissors. The entire wound was left open. The groups were treated in the same manner as mentioned in the animal experimentation. The ointment was topically applied once in a day. The progressive changes in wound area were monitored planimetrically by tracing the wound margin on graph paper on wounding day, followed by 8<sup>th</sup>, 11<sup>th</sup>, 13<sup>th</sup>, 15<sup>th</sup> and 19<sup>th</sup> wounding days<sup>7-8</sup>. The animals were divided into 3 different groups as follows:- Total no. of animals for each group: - 4 rats (Wistar)  
50% Ether-Benzene-Absolute Ethanol *M.longifolia* extracts (leaves and bark): - 5% w/w ointment

#### Study Parameters:-

- ❖ Wound closure
- ❖ Epithelialisation Time

#### 4.1 Incision wound

The rats were wounded under light Ether-Benzene-Absolute Ethanol anaesthesia method. Two vertebral incisions of 6 cm were made through the entire thickness of the skin, on either side of the vertebral column with help of straight rounded bodied needle. Sutures were removed on 8th post wounding day and the tensile strength was determined on 10th post wounding day by continuous constant water flow technique.<sup>9</sup>

### 5. Result and discussion

Significant wound healing activity was observed in animals treated with *Madhuca longifolia* extract compared with those who received the standard and control treatment. In excision wound modal, *Madhuca longifolia* extract treated animals showed a significant reduction in wound area and period of epithelisation. The mean percentage wound closure was calculated on the 8<sup>th</sup>, 11<sup>th</sup>, 13<sup>th</sup>, 15<sup>th</sup> and 19<sup>th</sup> wounding days. The extract treated animals showed faster epithelisation of wound ( $17.86 \pm 0.19$  and  $14.81 \pm 0.67$ ) bark and leaves respectively then the control. The period of epithelisation was  $11.8 \pm 0.37$  in case of standard drug 5% Betadine ointment.

### 6. Conclusions

In our study, 5%w/w ointment of the Chloroform, 50% Ether-Benzene-95 % Absolute Ethanol *Madhuca longifolia* extract of leaves and bark significantly increased the rate of wound closure and rate of epithelisation. The wound was healed in very efficient manner that is very close to the standard Betadine. The drug has equally shown better and similar wound healing property as compared with Betadine. The constituents present in the *Madhuca longifolia* extract of leaves and bark may be responsible for promoting the wound healing activity.

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