

FORMULATION, EVALUATION, WOUND HEALING STUDIES OF BENZENE-95 % ABSOLUTE ETHANOL EXTRACT OF LEAVES

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The aim of present study was to assess the wound healing activity of Formulation, Evaluation, Wound Healing Studies of Benzene-95 % Absolute Ethanol Extract of Leaves of *Momordica charantia* leaves. of *Momordica Charantia* fruit was examined for wound healing potential in the form of 10 %w/v solution in the excision wound created on the dorsal side of experimental animals, the 15% w/v extract should considerable difference in wound models and the result were compatable to that of the standard drug betadine (10% w/w) in terms of wound contracting ability and wound closure time. Antibacterial activity of methanol 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 8th, 12th, and 12th, wounding days. The extract treated animals showed tastes epithelisation of wound (27.10 ± 0.19) then the control. The period of epithelisation 25.11 ± 0.01 in case of standard drug 15% betadine ointment.

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Key words: Formulation, Evaluation, Momordica charantia, Wound, Antibacterial activity

1. Introduction

Momordica charantia (kerala) is important vegetable in India. The role of dirty compound preventing cancer is an important area of research. Several study suggested that momordica plays vital roll in prevention of cancer. In numerous studies, at least three different groups of constituents found in all parts of *momordica* have clinically demonstrated hypoglycemic properties or other actions of potential benefit against diabetes mellitus[1]. These blood sugar lowering chemicals include a mix of steroidal saponins (known as charantins), insulin-like peptides, and alkaloids. Charantin is composed of a mixture of beta-sitosterol-beta-D-glucoside and 5,25 stigmadien-3-beta-ol glycoside. This hypoglycemic effect is more pronounced in the fruit of bitter melon where these chemicals are found in greater abundance. To date, close to 100 *in vivo* studies have demonstrated the blood sugar lowering effect of this bitter fruit. This fruit has also shown the ability to enhance cells' uptake of glucose, to promote insulin release, and to potentiate the effect of insulin [2]. *Momordica charantia* (MC), a member of the Cucurbitaceae family, is known as bitter melon, bitter gourd, balsam pear, karela, and pare. It grows in tropical areas of the Amazon, East Africa, Asia, India, South America, and the Caribbean and is used traditionally as both food and medicine. The plant is a climbing perennial with elongated fruit that resembles a warty gourd or cucumber. The unripe fruit is white or green in color and has a bitter taste that becomes more pronounced as the fruit ripens. The seeds, fruit, leaves, and root of the plant have been used in traditional medicine for microbial infections, sluggish digestion and intestinal gas, menstrual stimulation, wound healing, inflammation, fever reduction, hypertension, and as a laxative and

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emetic.³ Clinical conditions for which *M. charantia* extracts (primarily from the fruit) are currently being used include diabetes, dyslipidemia, microbial infections, and potentially as a cytotoxic agent for certain types of cancer.⁴⁻⁷

2. Experimental

2.1 Preparation of extract-

The leaves and bark of *Madhuca longifolia* were collected from Indore, Madhya Pradesh. 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 five days with alcohol with frequent shaking separately. On the five day it was filtered and the benzene 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 (500 g) were extracted with Benzene-95 % Absolute Ethanol (50%) for 48hrs using a Soxhlet extractor. This ethanolic extract was concentrated to dryness under reduced pressure and controlled temperature (65-70°C) to yield solid masses.

2.2 Phytochemical analysis of the extract

The extract was screened for the presence of various constituents employing standard screening test.⁸ Conventional protocols for detecting the presence of glycosides, saponins, flavonoids, tannins etc. was used. Several phyto constituents like flavonoids and glycosides are known to promote wound healing process due to their antioxidant and antimicrobial activities.⁹

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.¹⁰

3. Evaluation of wound healing activity

The rats of either sex weighing 150-200 g were selected. Animals were depilated at dorsal thoracic region before wounding. They were housed individually with free access to food and water, the basal food intake and body weight to the nearest gram was noted. Under light chloroform anaesthesia, wounding was performed aseptically. Excision, incision and dead space wound models in rats were selected for assessing the wound healing activity. The extract was suspended in Tween 85 (5% w/v) and administered orally at a dose of 250 mg/ kg body weight for a period of fifteen days to the animals in the incision and dead space models. The animals in excision model received daily doses in the same manner for a period twenty days or till the excised wound healing which ever was earlier and the animals in the first group, which served as a control received the vehicle only.

Incision wound

The rats were wounded under light Benzene-95 % 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 15th post wounding day by continuous constant water flow technique.

4. Wound healing activity

Excision wound models was used to evaluate the wound – healing activity of *Momordica charantia* fruit.

5. Excision wound

In the excision wound model¹¹⁻¹² rats were anaesthetized lignocaine prior to creation of the wounds. A full thickness of the excision wound of 254 mm² was created by using toothed forceps, a surgical blade and pointed scissors. The entire wound 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 8th, 12th, and 12th,¹³⁻¹⁶

The animals were divided into 3 different groups as follows:-

Total no. Of animals for each group: - 6mice (Swiss albino)

50% benzene *Momordica* extract: - 10% w/v (Topical application)

Betadine Ointment (5%)

Group	Treatment	No. of animals
Control	Untreated	6
Standard	15 % Betadine	6
Test	50% benzene <i>Momordica</i> ext.	6

Study Parameters:-

- ❖ Wound closure
- ❖ Epithelialisation Time

6. Results and discussion

Significant wound healing activity was observed in animals treated with momordica extract compared with those who received the standard and control treatment. In excision wound modal, *Momordica* extract treated animals showed a significant reduction in wound area and period of epithelisation.

The mean percentage wound closure was calculated on the 8th, 12th, and 12th, wounding days. The extract treated animals showed faster epithelisation of wound (2.16±0.01) then the control. The period of epithelisation was 12.15±0.34 in case of standard drug 5% Betadine ointment.

7. Conclusion

In our study, 1%w/v of the 50% benzene-95 % absolute ethanol *Momordica* fruit extract 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 *Momordica* fruit extract may be responsible for promoting the wound healing activity. New method developed

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