

## Original Article

# Wound healing activity reported on leaf extract of *Murraya paniculata* (Linn) on albino wistar rats

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### ABSTRACT

*Murraya paniculata* (L) (Family - Rutaceae) is an evergreen shrub or occasionally a small tree, known for its pharmacological activities like anti-inflammatory, antidiabetic and stimulant and analgesic and antioxidant properties etc. The present study was aimed to assess the wound healing potential of crude methanol extract and aqueous extract of *Murraya paniculata* (leaf) using three types of wound models in rats as incision wound, excision wound and dead space wound. The parameters studied include rate of wound contraction and period of epithilization, collagen content in excision wound model. Tensile strength, hydroxyproline estimation and granuloma tissue weight were studied in incision and dead space wound model and assessed alongwith histopathological examination. The treatment of wound with the methanol and aqueous extract was performed in graded dose manner. Higher dose of methanol extract (200 mg/kg b.w.) show significant effect by decreasing period of epithilization, increasing wound contraction rate, increase in tensile strength, hydroxyproline content and granulation tissue weight in the wounded rats and more effective when compared with aqueous extract and control. While aqueous extract (200 mg, 100 mg/kg b.w) show more significant effect than the control. Therefore, *M. Paniculata* leaves accelerated wound healing activity in rats and thus supports its traditional use.

## 1. INTRODUCTION

Nature has provided a complete store-house of remedies to cure all ailments of mankind. From the vast natural resources, the plants are being used for therapeutic purposes from the beginning of the civilization. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine [1].

*Murraya paniculata* (L.) Jack, commonly known as Orange Jessamine, is a tropical, evergreen plant with tiny, white, scented flowers, which is cultivated as an ornamental tree or hedge. It

belongs to the Rutaceae family. This plant is native to areas in Southeast Asia [2], such as Southern China, Vietnam and Malay Peninsula. In China, the ornamental plant *M. paniculata* is widely cultivated on the sides of the road for decorative purposes. In Southeast Asia, it has been used for topical medicinal applications as health food [3]. For example, a solution of the bark of *M. paniculata*, when mixed with other ingredients, is used as an antidote for snake bites. The leaves of *M. paniculata* are known to possess antibiotic activity against *Mycococcus pyogenes* and *Escherichia coli* [4]. Furthermore, the leaves and roots of plant are utilized in folk medicine to treat stomach ache, toothache, gout, diarrhoea, dysentery, rheumatism, cough and hysteria [5, 6]. The use of *M. paniculata* has also been recommended for the treatment of cuts, joint pains and body aches [7]. Based on the

traditional use of the plant for healing wounds the present study was carried out in an experimental animal model to substantiate the folklore claim [8,9]. The aim of the present investigation was to evaluate the wound healing potential of leaf extract of *M. paniculata* using different experimental models.

## 2. MATERIALS AND METHODS

### 2.1 Plant material

Leaves of *M. paniculata* were collected from botanical garden and the plants and their parts were identified and authenticated with the standard samples preserved in the National Botanical Research Institute, Lucknow. A voucher specimen NBRI/CIF/Ref./08/2010/67 was deposited in the herbarium of NBRI, Lucknow.

#### 2.1.1 Preparation of the extract

The fresh young leaves of *M. paniculata* were collected; shade dried and then powdered using a mechanical grinder. 50 grams of pulverized plant part was extracted successively in 500 ml of petroleum ether, methanol and water. (LR grade, Merck, India) using Soxhlet apparatus. At the end of extraction, extracts were filtered under vacuum through a Whatman No. 1 filter paper and the process repeated until all soluble compounds had been extracted. The filtrate obtained was concentrated *in-vacuo* using a Rotavapor. The percentage yield was calculated and was found to be 22.5 % (methanol extract) & 18.6 % (aqueous extract). Both the extracts were stored at 4 °C in air tight bottle until further use.

#### 2.1.2 Phytochemical screening

The alcoholic and aqueous extracts of *M. paniculata* were tested qualitatively for different phytoconstituents using various chemical tests for secondary metabolites like flavanoids, tannins, glycosides, alkaloids, saponins, polyphenols, carbohydrates etc. [10].

### 2.2 Animals

Healthy inbred Albino rats of Wistar strain, weighing about 150-200 g of either sex were used for the present study. All animals were housed, fed and treated in accordance with the in-house guidelines for animal protection. Animals were kept for 2 weeks to be acclimatized prior to the investigation. During this time they were given standard pellet diet and water *ad libitum*. Also, they were periodically weighed before and after experiments. Animals were closely observed for any infection; those which showed signs of infection were separated and excluded from the study. The rats were anaesthetized prior to infliction of the experimental wounds [11]. The experimental procedures and research protocol used in this study were reviewed and approved by Institutional Animal Ethics Committee (1223/ac/05/CPCSEA) constituted as per the guidelines of Committee for Purpose of Control and Safety on Experiments on Animals, India.

### 2.3 Acute toxicity studies

The albino rats weighing 150 -200 g were used for the study. The acute oral toxicity studies of alcoholic and aqueous extract of *M. paniculata* was carried out by using stair case method according to OECD guidelines [12]. Albino wistar rats were divided into 5 groups each divided into six rats each with each group receiving graded dose of 200, 400, 800, 1200 and 1600 mg/kg of b.w of crude methanolic and aqueous extract. The animals were allowed to access to food and water and behavioural changes were observed over a period of 72 h for sign of acute toxicity. The number of mortality caused by the compound within this period of time was observed in order to fix the lethal dose (LD<sub>50</sub>). [13]

### 2.4 Wound healing experimental design

The animals were kept under starvation for 12 hrs prior to wounding. Wounds were made on the animals under light ether anaesthesia. Thirty rats in all were used in the study.

They were divided into five groups containing six animals each as follows:

Group 1: The control group. The animals in this group had their wounds treated with normal saline.

Group 2: Animals in this group had their wounds treated with 200 mg/kg b.w. crude methanol extracts of *M. Paniculata*.

Group 3: Animals in this group had their wounds treated with 100 mg/kg b.w. crude methanol extracts of *M. Paniculata*.

Group 4: Animals treated with 200 mg/kg b.w. crude aqueous extracts of *M. paniculata*.

Group 5: Animals treated with 100 mg/kg b.w. crude aqueous extracts of *M. paniculata*.

Control group of animals were given 1 ml of normal saline; test group animals received the solution of methanolic and aqueous extract of *M. paniculata* by gavage from the day of wounding. The wound healing study was undertaken in excision wound, incision wound and dead space wound models.

#### 2.4.1 Excision wound model

Animals were anesthetized under light ether anaesthesia each animal was secured to operation table in its natural position. An impression was made on the depilated dorsal thoracic central region of the rats, 5.0 cm away from the ears by using a round seal of 2.5 cm diameter. The extract was given everyday up to 16<sup>th</sup> day [14].

#### 2.4.2 Incision wound model

Each animal was secured to operation table in its natural position under light ether anesthesia. Two Para-vertebral straight incisions of 6.0 cm each were made on the depilated back of the animals by cutting through the entire skin with the help of a sterilized scalpel. After complete haemostasis, the wounds were closed (sutured) using 2-zero silk threads as interrupted sutures about 1.0 cm apart with the help of a straight round bodied needle. The sutures were removed on 8<sup>th</sup> post wounding day [15].

### 2.4.3 Dead space wound model (Granuloma studies)

Under light ether anesthesia, dead space wounds were created by subcutaneous implantation of sterilized cylindrical glass piths (2.5 cm × 0.3 cm), one on either side of the dorsal paravertebral surface of rat [16]. The granulation tissues formed on the glass piths were excised on 10<sup>th</sup> post wounding day and the breaking strength was measured. Simultaneously, granulation tissue so harvested was subjected to hydroxyproline estimation and dry granuloma tissue weight study. [17].

## 2.5 Wound healing evaluation parameters

### 2.5.1 Wound contraction and epithelialization time

An excision wound margin was traced after wound creation by using transparent paper and area measured by graph paper. Wound contraction was measured in each 4 days interval, until complete wound healing and expressed in percentage of healed wound area. The percent wound contraction was determined using the following formula. [18]. The period of epithelialization was calculated as the number of days required for falling of the dead tissue without any residual raw wound.

$$[\% \text{ wound contraction} = (\text{Healed area}/\text{total wound area}) \times 100]$$

### 2.5.2 Collagen content from regenerated tissues of excision wound

The regenerated tissue collected from the excision wounds were cut into two pieces. They were washed with 0.5 M sodium acetate and then suspended in ten parts (w/v) of 0.5 M acetic acid and stirred intermittently for 48 hrs. The solution was centrifuged at 5600 rpm for 2 hrs intermittently in the micro centrifuge, and then sodium chloride (5% w/v) solution was added to precipitate the collagen. The collagen so precipitated was filtered using a reweighed Whatman filter paper No.1. The weight of the collagen precipitate obtained was calculated by taking difference between the initial and final weight of the filter paper. The same procedure was followed for the animals of the control and for all the test groups.

### 2.5.3 Measurement of tensile strength

Tensile strength is the resistance to breaking under tension. It indicates how much the repaired tissue resists to breaking under tension and may indicate in part the quality of repaired tissue. Sutures were removed on the day 8 after wound creation and the tensile strength was measured. The skin breaking strength of the 10 day old wound was measured by continuous constant water technique [19]. The skin breaking strength is expressed as the minimum weight (in grams) of water necessary to bring about the gapping of the wound.

### 2.5.4 Hydroxyproline estimation

Tissues were dried in a hot air oven at 60-70°C to constant weight and were hydrolyzed in 6 N HCl at 130 °C for 4 hrs in sealed tubes. The hydrolysate was neutralized to pH 7.0 and was

subjected to Chloramine-T oxidation for 20 min. The reaction was terminated by addition of 0.4 M perchloric acid and colour was developed with the help of Ehrlich reagent at 60°C and measured at 557 nm using a spectrophotometer [20].

### 2.5.5 Dry granuloma weight

The granulomas were excised from the surrounding tissue on 10<sup>th</sup> post wounding day and were dried at 60°C to obtain constant dry weight [21].

### 2.5.6 Histological Study

Granulation tissues obtained on day 10 from the test and control group animals were sectioned for histological study and stained for collagen with Van Gieson's stain.

## 2.6 Statistical analysis

Results, expressed as mean ± SEM were analyzed statistically using the student's t-test to identify the differences between the treated and control. The data were considered at  $p < 0.01$ .

## 3. RESULTS AND DISCUSSION

### 3.1 Phytochemical analysis

The phytochemical analysis of the leaf extract by various chemical test showed the positive result for Alkaloids, flavanoids, phenols, coumarin and tannins in both the methanol and aqueous extract.

### 3.2 Acute toxicity study

The result of acute oral toxicity studies of methanol and aqueous extract were found to be non-lethal up to dose of 2 g/kg body weight. Therefore the LD<sub>50</sub> value was fixed to be 2000 mg/kg b.w. for the test extract. Hence dose (*i.e.* 100 mg/kg and 200 mg/kg, orally) was selected for excision, incision and dead space wound model.

### 3.3 Wound contraction and epithelialization time

Significant wound healing activity was observed in both the group of animals treated with different doses of methanol and aqueous extract. The percentage of closure of wound was significant in the animals treated with 200 mg/kg b.w. of methanol extract (98.82 ± 0.39\*\*\*), (99.48 ± 0.21) as compare to the 200 mg/kg aqueous extract (90.18±1.01\*\*), (97.35±0.30\*\*) on day 16<sup>th</sup> and 20<sup>th</sup> respectively. While in control animals it was (87.51± 0.54) and (93.15 ± 0.33), respectively. Also, the group treated with 100 mg/kg b.w. of methanolic and aqueous extract showed significant value (98.75 ±0.21\*\*\*) & (97.35±0.30\*\*) on 20<sup>th</sup> day in comparison with control group. It was found that the mean time taken for complete epithelialization of the excision wound in 200, 100 mg/kg dose of methanol extract treated group was less than the animals treated with 200, 100 mg/kg of aqueous extract and the data are shown in Table 1.

**Table 1.** The effect of leaf methanol and aqueous extract of *M. paniculata* on excision wound model

Treatment	Percentage of closure of excision wound area					Epithelialization in days
	Day 4	Day 8	Day 12	Day 16	Day 20	
Control	20.12 ± 0.65	61.31 ± 0.53	79.45 ± 0.66	87.51 ± 0.54	93.15 ± 0.33	24.63 ± 0.65
Aqueous extract (100 mg/kg)	27.80 ± 1.8*	65.10 ± 0.22	81.02 ± 0.26**	90.48 ± 0.36	97.35 ± 0.30**	22.01 ± 0.21
Aqueous extract (200 mg/kg)	31.75 ± 1.24**	67.14 ± 0.69**	85.20 ± 0.56**	90.18 ± 1.01**	98.72 ± 0.15**	20.67 ± 0.76
Methanol extract (100 mg/kg)	47.95 ± 2.06**	67.80 ± 1.40**	88.28 ± 0.70*	96.77 ± 0.64	98.75 ± 0.21***	19.33 ± 0.76
Methanol extract (200 mg/kg)	49.74 ± 1.49**	71.45 ± 2.30**	92.79 ± 0.74***	98.82 ± 0.39***	99.48 ± 0.21***	18.00 ± 0.51*

Values are mean ± S.E.; n = 6 in each group. \*P < 0.01 is compared to control

### 3.4 Collagen Content from Regenerated Tissues of Excision Wound

The collagen content was estimated from regenerated tissue for control as well as treated groups. There was a significant increase in collagen content on 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup>, 16<sup>th</sup> and 20<sup>th</sup> day in 200 mg/kg methanol extract treated group as compared to the dose of 100 mg/kg extract and control group. The collagen

content in 200 mg/kg and 100 mg/kg aqueous extract treated group was less significant when compare to methanol extract but more significant than the control group.

Statistical analysis of the results by ANOVA followed by student's t test showed that there was a significant difference between all the groups (p < 0.001) and the ethanol extract was found to be highly effective than aqueous extract (Table 2).

**Table 2.** The effect of leaf methanol extract & aqueous extract of *M. paniculata* on collagen content from regenerated tissues of excision wound (mg/kg)

Treatment	Day 4	Day 8	Day 12	Day 16	Day 20
Control	10.15 ± 0.82	17.79 ± 0.97	23.63 ± 1.32	32.23 ± 0.87	40.97 ± 0.70
Methanol extract (200mg/kg b.w)	22.52 ± 1.68**	31.90 ± 0.96**	39.76 ± 0.66**	45.59 ± 0.94**	51.58 ± 0.64**
Methanol extract (100mg/kg b.w)	19.34 ± 1.29	28.90 ± 0.67*	34.90 ± 0.58*	40.55 ± 0.87*	48.80 ± 0.97*
Aqueous extract (200mg/kg b.w)	15.93 ± 0.65*	22.46 ± 0.90**	30.91 ± 0.66*	33.68 ± 0.35**	43.71 ± 0.75*
Aqueous extract (100mg/kg b.w)	12.45 ± 0.21	19.46 ± 0.81*	27.71 ± 0.52*	30.78 ± 0.86*	41.86 ± 0.54

Values are mean ± S.E.; n = 6 in each group. \*P < 0.01 is compared to control.

### 3.5 Measurement of tensile strength

In incision wound model, significant increase in the tensile strength was observed in animals treated with 200 mg/kg and 100mg/kg of methanol extract as compare to the aqueous extract (200, 100 mg/kg) treated group, indicating the effect of *M. paniculata* leaf extract in maturation of collagen fibres (Table 3). The values were highly significant when compared to control group (p < 0.0001).

### 3.6 Hydroxyproline estimation and granuloma weight

Methanol treated group showed significant increased hydroxyproline in comparison to the aqueous and control group in dead space model (P < 0.01) shown in Table 3. Granuloma dry weight of methanol extract treated animal groups was found to be increased when compared with aqueous and control treated group.

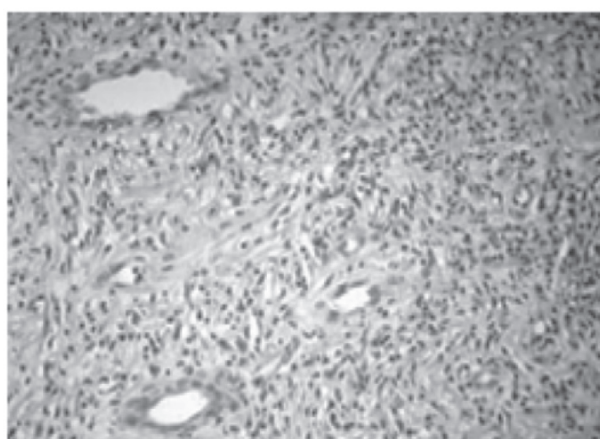
**Table 3.** Effect of methanol and aqueous extract of *M. Paniculata* leaf in incision and dead space wound models

Treatment	Incision model	Dead space model		
	Tensile strength (g)	Hydroxyproline content (µg)	Tensile strength (g)	Granuloma dry weight (mg/100g)
Control	259.20 ± 7.45	6.1 ± 0.1033	256.10 ± 9.14	19.20 ± 0.99
Aqueous extract (100 mg/kg)	281.20 ± 9.20*	6.5 ± 0.1123	280.60 ± 9.50	22.28 ± 2.20*
Aqueous extract (200 mg/kg)	300.70 ± 13.60**	6.917 ± 0.2330*	297.10 ± 12.90	25.27 ± 2.24**
Methanol extract (100 mg/kg)	324.50 ± 11.30***	7.126 ± 0.5640*	331.70 ± 14.70***	27.21 ± 2.30**
Methanol extract (200 mg/kg)	351.40 ± 11.07***	8.917 ± 0.1352**	343.60 ± 5.90***	30.43 ± 2.91***

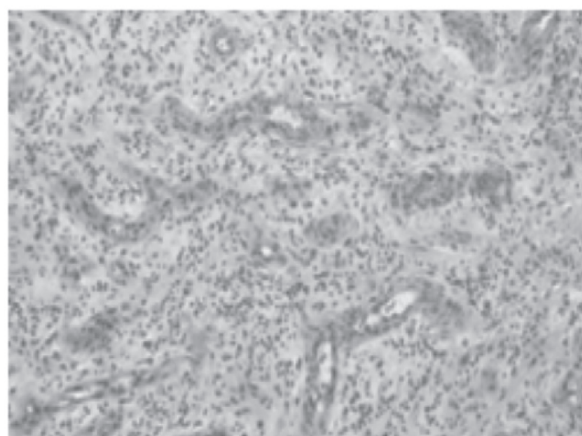
The value are expressed as mean ± SEM, (n = 6). If \* P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 vs. Control

### 3.7 Histopathological study

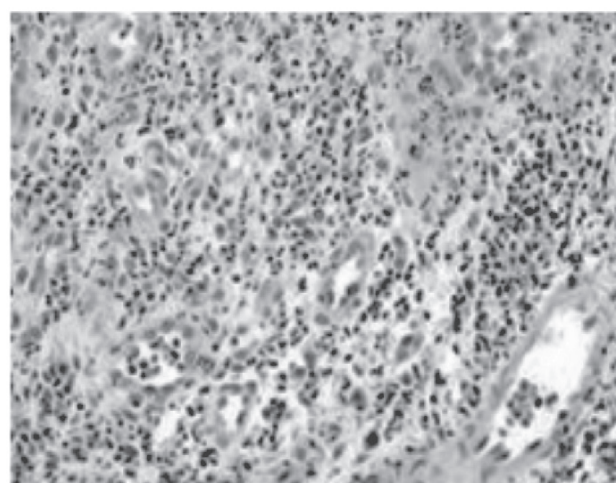
Histological sections of granulation tissue from methanol extract (200 mg/kg) treated rats showed increased and well-organized bands of collagen, more fibroblasts and few inflammatory cells (Fig. 1 c). Granulation tissue sections obtained from control and aqueous extract treated rats revealed more inflammatory cells and less collagen fibres and fibroblasts (Fig. 1(a) & 1(b)).



**Fig. 1 (a):** Control treated group



**Fig. 1 (b):** Aqueous extract (200 mg/kg) dose treated group



**Fig.1 (c):** Methanol extract (200 mg/kg) dose treated group

Wound represents a major health problem both in terms of morbidity and mortality. Wound healing is a fundamental response to tissue injury that results in restoration of tissue integrity. It mainly depends on the repairing ability of the tissue, type and extent of damage and general state of the health of the tissue [22]. A therapeutic agent selected for the treatment of wounds should ideally improve one or more phases of healing without producing deleterious side effects [23]. Wound healing can be discussed in three phases viz. Inflammatory phase, proliferative phase and maturational or remodelling phase. The inflammatory phase is characterized by haemostasis and inflammation. Proliferative phase is followed by epithelialization, angiogenesis and collagen deposition. In the maturation phase, the wound undergoes contraction resulting in a smaller amount of apparent scar tissue [24].

The data from excision model revealed that all the different doses *M. paniculata* leaf extract exhibited significant wound healing promoting activity. However, the effect was found to be concentration related where 200 mg/kg b.w. dose methanol extract resulted with significant wound-healing activity by decreasing period of epithelialization, formation of granulation

tissue, synthesis of collagen and by increased in the rate of wound contraction as compared to the control animals.

The promotion of wound healing activity is also well gazed by its tensile strength of the incision wound. Generally wound-healing agents have the properties to enhance the deposition of collagen content, which provides strength to the tissues and forms cross-linkages between collagen fibres. The tensile strength of the wound treated with methanol extract of higher dose was found to be more significant when compare to control. While aqueous extract was less significant.

Further the hydroxyproline content and granuloma dry weight of the granulation tissue in dead space model of the animals treated with *M. paniculata* leaf methanolic extract (200 mg/kg b.w.) was significantly increased when compared to the control and the group of animals treated with aqueous extract 100 and 200 mg/kg b.w. respectively indicating increased collagen turnover. In addition, increased in breaking strength in dead space model were also indicated the presence of higher protein content.

Histology of the wound tissue of the control animals showed the presence of acute inflammatory cells, fibroblastic connective tissue and very little number of blood vessels. The lesser epithelialization and lesser collagen formation indicated incomplete healing of the wound in control animals (Fig. 1a). The sections of the granuloma tissue of the animals treated with methanolic extract showed increased epithelialization, fibrosis, collagen formation and increased number of blood vessels (Fig. 1c) when compared with the aqueous extract of *M. paniculata* of 200mg/kg b.w doses (Fig. 1b).

The preliminary phytochemical analysis of *M. paniculata* revealed the presence of alkaloids, tannins, flavonoids and polyphenolic compounds. Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Hence, any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibre, preventing the cell damage [25] Studies were revealed that flavonoids are also known to promote the wound healing process mainly due to their astringent and antimicrobial properties which appear to be responsible for the wound healing and increased rate of epithelialisation [26]. Tannins are the main components of many plant extracts and they act as free radical scavengers. [27, 28]

So the study provides a rationale for the use of *M. paniculata* leaves preparations in the traditional system of medicine to promote wound healing. Further the extract did not produce any adverse effect and because of this it is possible to recommend its use in the treatment of wounds.

#### 4. CONCLUSION

The study thus demonstrated the wound healing activity of methanol and aqueous extract of the leaf of *Murraya paniculata* and found to be effective in the functional recovery of the wound healing by dose dependent manner. The result may be attributed to the phytoconstituents present in it which may be either due

to their individual or cumulative effect that enhanced wound healing and provided scientific evidence to the ethnomedicinal futures of the particular plant.

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