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MANGIFERA INDICA LEAVES EXTRACT CONFERS; WOUND HEALING AND ANTIOXIDANT ACTIVITY

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ABSTRACT

Mangifera indica commonly known as mango tree that are an important source of antioxidant molecules such as phenolic compounds and also it is used as traditional medicine for the treatment of skin irritations diseases. In this present study we carried out the antioxidant and wound healing activity of the extract by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and Superoxide scavenging activity. Evaluation of antioxidant activity was performed at 125, 250, 500 and 1000μg/mL of EEMI concentrations. In DPPH assay EEMI showed the maximum antioxidant effect (68.04%) and Superoxide scavenging activity the extract has showed (74.92%) as compared to the standard (67.83, 97.01%). *In vivo* incision wound healing activity of EEMI ointment concentrations (2.5% (w/w) and 5% (w/w)) were tested in the rats. The wound contraction effect was measured every 2 days of 15 days of post wounding days. The standard drug silver sulfadiazine 10(w/w) showed a complete healing effect on 12th day of wound excision whereas, test extract EEMI 2.5% (w/w), 5% (w/w) showed healing effect on 15th and 14th day respectively. These findings are revealed that ethanol extract of *M.indica* showed variable degrees of antioxidant activity and significantly enhanced the percentage of wound contraction and the period of epithelialization comparable to standard silver sulfadiazine. These results demonstrate a positive effect of EEMI on the wound healing process on wound contraction is possibly due to the antioxidant activity exhibited by phytochemicals in the selected plant extract.

KEYWORDS

Antioxidant, Epithelialization, Superoxide, Wound and Phytochemicals.

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INTRODUCTION

A wound is a kind of damage which occurs relatively quickly in which skin is torn, cut, or punctured, or where blunt force trauma causes a contusion and discoloration. In pathology, wounds are specifically refers to a sharp injury which damages the epidermis of the skin.

(https://en.wikipedia.org/wiki/Wounds (film). Wound healing is an active process involving biochemical and physiological changes occurs that act in a pleasant way in order to guarantee tissue from the injury[1]. Wound healing process is occurs in three stages of inflammation, proliferation, and remodeling. This healing process begins with the blood clotting and is completed with remodeling of the cellular layers of the skin. The process of wound healing may be troubled by the presence of free radicals, which can damage wound surrounding cells, or by microbial infection[2]. Suitable methods of wound healing are essential for the restoration of damaged tissue anatomical continuity and disturbed functional status of the skin[3]. Wound healing agents research is one of the developing areas in modern biomedical sciences and many traditional practitioners across world particularly in countries like India and China have more valuable information of many plants for treating wounds and burns[4].

Many herbal plants have a very significant role in the process of wound healing. Plants are more potent healers because they promote the repair mechanisms in the natural way and also plants are nontoxic compare to other medicaments. The healing process can be physically monitored by assessing the percentage of contraction of the wound in animals[5]. *Mangifera indica* (MI), also known as mango, it has been an important herb in the Ayurvedic and indigenous medical systems for over 4000 years. Mangoes belong to genus *Mangifera* which consists of about 30 species of tropical fruiting trees in the flowering plant family *Anacardiaceae*. According to ayurveda, varied medicinal properties are attributed to different parts of mango tree. Mango possesses antidiabetic, anti-oxidant, anti-viral, cardio tonic, hypotensive, anti-inflammatory properties[6]. The present study was undertaken to evaluate the antioxidant and wound healing activity of ethanolic extract of *M. indica*on circular excision wound model.

MATERIAL AND METHODS

Drugs and Chemicals

Silver sulfadiazine (Rexin pharmaceuticals Pvt. Ltd.), ethanol were used in this study. All substances were prepared immediately before use and the reagents were used as analytical grade.

Collection of Plant Materials

The *Mangifrera indica* leaves were collected from the Kanyakumari District, Tamil and, India. The plant was authenticated by Mr. Chelladurai, Research Botanist (Rtd), CCRAS Tirunelveli, Tamil Nadu.

Preparation of extracts

About 1kg of air-dried leaves of plant was extracted in sox let assembly ethanol 70%. The extract was concentrated by using rotary vacuum evaporator. The extract obtained with each solvent was weighed and the percentage yield was calculated in terms of dried weight of the plant material. The color and consistency of the extract were also noted. All the solvents used for this entire work were of analytical reagent grade (Merck, Mumbai). The yield of the extract was 24.65 % (w/w). In each experiment, the extract was diluted with water to desired concentration.

Preparation of ointment[7]

Simple ointment containing 2.5mg/g, 5mg/g of EEMI were prepared by trituration method in a ceramic mortar and pestle using white soft paraffin base, obtained from S.D. Fine chemicals, India (Cooper and Gunn's, 1987). For this 250/500mg of EEMI was incorporated with 100g of the base. Silver sulfadiazine 10mg/g ointment obtained from Rexin pharmaceuticals Pvt. Ltd. was used as standard drug for comparing the wound healing potential of extract in different animal models.

Animals

Adult male albino rats weighing about 200-250g were used in this study. Rats were maintained in clean, sterile, polycarbonate cages and fed with commercial pellet rat chow (M/S Hindustan lever limited, Bangalore, India) and water ad libitum. Study was approved by CPCSEA and the approval number is (CBLRC/IAEC/11/01 - 2020).

Qualitative chemical tests

The ethanol extract subjected to qualitative chemical analysis to test the presence of alkaloids, carbohydrates, proteins and amino acids, phytosterols, glycosides, saponins, flavonoids, triterpenoids and fixed oils[8,9].

***In vitro* Antioxidant Activity**

DPPH photometric assay[10]

The effect of extract on DPPH radical was assayed using the method of (Mensor *et al*, (2001) A ethanolic solution of 0.5ml of DPPH (0.4mM) was added to 1ml of different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518nm and converted into percentage radical scavenging activity as follows.

$$Scavenging activity(\%) = \frac{A_{518}\,\text{Control} - A_{518}\,\text{Sample}}{A_{518}\,\text{Control}} \times 100$$

Where A_{518} control is the absorbance of DPPH radical + methanol; A_{518} sample is the absorbance of DPPH radical + sample extract/ standard.

Superoxide Scavenging Activity

NBT Dye Reduction Method[11]

In this method, the superoxide is produced by riboflavin. The superoxide anions are subsequently made to reduce nitro blue tetrazolium which yield a chromogenic product, which is measured at 560nm. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne *et al*, (1975). The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5mM NBT) solution, 0.2ml of EDTA (0.1M EDTA), 0.05ml riboflavin (0.12mM) and 2.55ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up wherein DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560nm was measured against the control samples. Ascorbate was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Wound Healing Property

Grouping of animals

Four groups of animals containing six animals in each group were used for the excision wound models. The animals of group G_1 considered as control, group G_2, G_3 were considered as test group, G_4 considered as standard group and were treated respectively.

Wound healing property of extracts by excision wound model[12]

Circular wounds of approximately 10mm diameter were inflicted on the cleared skin cutting under mild ether anesthesia. The areas of the wounds were traced (sq.mm) immediately by placing a transparent paper over the wound. The tracing was then shifted to graph paper, from which the wound area was evaluated. This will be taken as the initial wound area reading.

G_1 Simple ointment I.P applied topically.

G_2 EEMI 2.5mg/g ointment applied topically.

G_3 EEMI 5mg/g ointment applied topically

G_4 Treated with silver sulfadiazine ointment 10mg/g applied topically

All the samples were applied twice in a day for 15 days and wound contraction were measured. The wound area of each animal was measured on 2nd, 4th, 6th, 8th, 10th, 12th, 14th and 15th post wounding measurements of wound area. Day 15 was considered as the end point of the acute healing process. The percentage of wound contraction was calculated by this following formula.

$$\%\ \text{of wound contraction} = \frac{\text{Initial wound size - Specific day wound size}}{\text{Initial wound size}} \times 100$$

RESULTS AND DISCUSSION

M. indica leaves extracts were subjected to qualitative chemical tests for the detection of various phytoconstituents such as alkaloids, carbohydrates, proteins and amino acids, glycosides, flavonoids, tannins, phenolic compounds, saponins. The phytochemical screening results are shown in Table No.1.

In vitro* Anti-Oxidant Activity of *M. indica

DPPH photometric assay

The result percentage of DPPH radical scavenging activity of EEMI *is* presented in Table No.2. The ethanolic extract of *M.indica* showed a better effectiveness when compared with standard rutin compound. The IC_{50} of the ethanolic extract of *Mangifera indica* and Rutin were found to be 280µg/ml and 470µg/ml respectively.

Superoxide scavenging activity

NBT dye reduction method

The percentage scavenging of superoxide anion examined at different concentrations of various extracts of *Mangifera indica* (125, 250, 500, 1000µg/ml) were presented in Table No.3. The IC_{50} values of ethanolic extract of *Mangifera indica* were found to have strong superoxide radical scavenging activity; when compared to that of standard Ascorbate. The IC_{50} of the ethanolic extract of *Mangifera indica* and Ascorbate were found to be 400µg/ml and 50µg/ml respectively.

Wound Healing Effect

Effect of ethanolic extract of *M.indica* on wound contraction

This model was used to evaluate the effect of the test samples on wound closure and contraction and consequently time of epithelialization. The effect was measured every 2 days of 15 post wounding days. The standard drug silver sulfadiazine 10mg/g showed 100% healing on 12^{th} day of wound incision whereas, test extract EEMI 2.5mg/g, 5mg/g showed healing effect on 15^{th} and 14^{th} day respectively. The results observed from this study were presented in Table No.4.

Discussion

Wound healing is a natural response take place in the injured skin and soft tissues, it consists of three interactive phases of inflammation, proliferation, and remodeling. The initial response of the healing period is known as inflammation that act as a defence mechanism of the soft tissue cellular membranes, which provide a resistance to the injured tissues from the microbial infection[13].

Cells can generate free radicals and ROS. Free radicals and ROS causes oxidative damage of biological macromolecules such as deoxyribonucleic acid (DNA), proteins and lipids. It is generally accepted that in a condition of oxidative stress, free radicals and reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet -}$, $OOH^{\bullet}$), hydroxyl ($OH^{\bullet}$) and peroxyl ($ROO^{\bullet}$) radicals are generated. The ROS play an important role in the pathogenesis of various diseases, such as neurodegenerative disorders, cancer, cardiovascular diseases, atherosclerosis, cataracts and inflammation[14].

Epidemiological studies state that intake of fruits and vegetables with high phenol content associated with reduce and prevent cardio, cancer and cerebrovascular morbidity and mortality due to these ailments. Phenolic compounds can produce their biological effects by scavenging of free radicals. Polyphenolic compounds such as flavonoids have been used as major dietary sources that are responsible for cancer protective effect[15]. Plants are the best source of natural antioxidants that might be used for the production of novel drugs. The antioxidant potential of the plant is due to the presence of flavonoids, chlorophyll in, coumarins, dietary fibres, indoles, phytosterols and protease inhibitors and they act as antiradical scavenging mechanism for the treatment of inflammation, gastric ulcer, necrotic and neurodegenerative diseases[16].

In this study the prepared *M. indica* ointment tested experimentally by Excision wound model for the assessment of wound healing activity in rats for 15 days. The results obtained from the present study revealed that, the ointment preparation of EEMI showed a better and fast wound healing effect in rats when compared with control group. G_1 Simple ointment I.P used as a base for the preparation, has shown 52.60± 0.84%wound healing activity on 15^{th} day of treatment. The result revealed the fact that animals treated with both EEMI exhibited a significant (*$p<0.05$) increased the percentage of wound healing power with respect to dose dependent manner when compared to control.

The *In vitro* antioxidant activities of the leaf extracts of *M. indica* was determined by two methods namely, DPPH assay and Superoxide anion scavenging assay, the IC_{50} values of ethanolic extract of *M. indica* were found to have strong superoxide radical scavenging activity; when compared to that of standard Ascorbate. The IC_{50} of the ethanolic extract of *M. indica* and Ascorbate were found to be 400µg/ml and 50µg/ml respectively. In DPPH assay, the ethanolic extract of *M. indica* showed a better effectiveness when compared with standard rutin compound. The IC_{50} of the ethanolic extract of *M. indica* and Rutin were found to be 280µg/ml and 470µg/ml respectively.

These findings are revealed that, wound healing effect of EEMI may be due to presence of flavonoids and other active antioxidant compounds that responsible for the inhibition of lipid peroxidation, which leads to prevention of the cell damage and increase in the viability of collagen fibrils and the wounds were contracted faster in rats[17]. The results of this study suggested that *M. indica* ointment significantly enhanced and improved the rate of wound healing process in wounded rats by excision wound model.

Table No.1: Qualitative Chemical Analysis of Phytoconstituents of the Ethanol Extract of *M. indica* (EEMI)

S.No	Tested Components	*M. indica*
1	Alkaloids	+
2	Carbohydrates	+
3	Glycosides	+
4	Terpenoids	-
5.	Proteins	+
6	Amino acids	+
7	Steroids	+
8	Flavonoids	+
9	Phenols	-
10	Tannins	-
11	Saponins	+

+ = Presence **-** = Absence

Table No.2: Effect of ethanol extracts of *M. indica* on DPPH assay

S.No	Concentration (µg/ml)	% of activity (±SEM)*	
		Sample (EEMI)	Standard (Rutin)
1	125	35.09 ±0.05	17.85 ±0.076
2	250	47.57 ±0.03	21.08 ±0.054
3	500	62.64 ±0.11	51.21 ±0.022
4	1000	68.04 ±0.02	67.83 ±0.014
5		IC_{50}=280µg/ml	IC_{50}=470µg/ml

*All values are expressed as mean ± SEM for three determinations

Table No.3: Effect of ethanol extracts of *M. indica* on Superoxide anion scavenging activity

S.No	Concentration (µg/ml)	% of activity (±SEM)*	
		Sample (EEMI)	Standard (Ascorbate)
1	125	28.51 ± 0.53	72.81 ±0.01
2	250	34.30 ± 0.59	90.31 ±0.01
3	500	57.63 ± 0.67	91.99 ± 0.02
4	1000	74.92 ± 0.93	97.01 ± 0.01
5		IC_{50}= 400 µg/ml	IC_{50} = 50 µg/ml

*All values are expressed as mean ± SEM for three determinations

Table No.4: Effect of ethanolic extract of *M. indica* on wound contraction

Treatment		Percentage of wound contraction (in days)							
Post wounding days	Dose mg/g	2	4	6	8	10	12	14	15
Control	Simple ointment	2.73± 0.03*	09.60± 0.47*	20.55± 0.72*	29.30± 0.48*	34.15± 1.14*	40.74± 0.75*	47.89± 1.26*	52.60±0.84*
EEMI	2.5	3.72± 0.07	12.11± 0.28*	31.70± 0.66*	62.20± 0.96*	74.15± 0.80*	87.08± 0.60*	92.13± 0.94*	100*
EEMI	5	5.55± 0.06*	17.44± 0.34*	38.20± 1.22*	67.90± 0.52*	77.80± 0.92*	90.33± 0.88*	100*	---
Silver sulfadiazine	10	6.40± 0.07*	19.20± 0.56*	35.30± 0.85*	64.70± 0.70*	79.22± 0.42*	100*	---	---

Wounds treated with 2.5 and 5mg/g have shown increased rate of wound contraction Values are expressed as mean ±SEM (n=6). The data were analyzed by one way ANOVA followed by Dennett's multiple comparison test *p<0.05 as compared with control group.

CONCLUSION

Plants and its metabolites are the more potent healers of wounds due it accelerate and stimulate the repair mechanism in an expected way. This study deliberates the importance of conventional medications that are still being used by people of ethnic origin and distinguishes the importance of *M. indica* in wound healing. Further studies are needed to isolate the active principle and assess the effectiveness of the *M. indica* in the treatment of chronic wounds.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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