

PHARMACOLOGICAL EVALUATION OF MORINDA TINCTORA AND BOMBAX CEIBA FOR WOUND HEALING ACTIVITY

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Abstract: Medicinal plants or traditional remedies have been used for infection, disease, and injury over millennia. Humans have learned to identify and transform the botanical resources from the immediate environment, and with the development of trade, as food and medicine from prehistory. A great many of these “ancient” and traditional medical plants have been validated to confer therapeutic benefits, albeit not always in controlled clinical trials. *Morinda tinctoria* Roxb. (Family: Rubiaceae) is very well known for its therapeutic benefit in Indian systems of medicine including Ayurveda and Siddha and in other forms of traditional Medicine worldwide for the treatment of several ailments. *Ayurveda*, that *Bombax ceiba* possesses proven medicinal properties and is the ingredient of many products. Approximately each part of this plant is used as drug, and its ancestry and flowers are used for therapeutic the highest number of illnesses.

Keywords: *Morinda Tinctora*, *Bombax Ceiba*, Pharmacological study, Wound Healing activity, Ayurvedic work

Introdcution: Wound healing is a dynamic and highly regulated process consisting of cellular, humoral and molecular mechanisms. The normal cutaneous wound healing process is a temporal process involving a complex series of overlapping events, which can be divided into key stages including: hemorrhage and fibrin clot formation, inflammatory response, reepithelialization, granulation tissue formation, angiogenic response, connective tissue contraction, and remodelling [1]. Our skin is the key to our

survival, sensing the environment, maintaining physicochemical and thermal homeostasis, acting as a reservoir of essential nutrients, providing passive and active defence, and responding to trauma and injury. Maintaining these critical functions requires robust and effective mechanisms to protect it from trauma and insult and to repair and replace critical skin functions when damaged or lost. Humans have been treating their wounds for millennia [2]. Traditional wound management is limited by what is immediately at hand or can be acquired locally, such as water, soil, and plant and animal products, and is frequently complemented with ceremony and ritual as an added measure. For millions of people across Asia, Africa, the Middle East, and Latin America, traditional medicines derived from local plants, animals, and natural products are the mainstay of wound care; for some, it is the only source of wound care. Traditional medicine is often described using terms such as “alternative,” “nonconventional,” “indigenous,” and “complementary,” when in fact many of the techniques and practices of “modern” medicine are little deferent from traditional practices when it comes to wounds [3]. Traditional approaches depend almost entirely upon natural resources, such as water, plants, animals, and minerals, and continue to be valued and widely practiced by a majority of the world’s population. Fascinatingly, of the new anticancer drugs developed between 1940 and 2002, approximately 54% were derived from natural products. Another study has determined that of all current pharmaceutical products, about 73%, include ingredients derived from natural products. [4] The therapeutic activity of many traditional medicines are conferred by natural ingredients produced within the plant; consequently, the efficiency of Traditional medicine preparations can vary widely and are determined by the genotype, environmental, and growing conditions encountered by each source plant. Urbanization and industrialization of pharmaceutical engineering have increased demand for “off the shelf” Traditional medicine products with consistent composition, quality, and clinical efficacy. Concomitantly, industrialization has also introduced rigorous product testing for evidence of biological activity and clinical efficacy [5]. *Morinda tinctoria* Roxb. (Family: Rubiaceae) is commonly known as Indian mulberry or Aal in India. This plant is very well known for its therapeutic benefit in Indian systems of medicine including Ayurveda and Siddha and in other forms of traditional Medicine worldwide for the treatment of several ailments. Almost all parts of

this plant have been explored for its medicinal uses [6]. *Bombax ceiba* is an important medicinal plant of tropical and subtropical India usually identified as Silk Cotton Tree. It is the tall deciduous tree, with straight buttressed trunk and broad spreading branches. It has been claimed in Ayurveda, that *Bombax ceiba* possesses proven medicinal properties and is the ingredient of many products. Approximately each part of this plant is used as drug, and its ancestry and flowers are used for therapeutic the highest number of illnesses [7]. Medicinal plants or traditional remedies have been used for infection, disease, and injury over millennia. Humans have learned to identify and transform the botanical resources from the immediate environment, and with the development of trade, as food and medicine from prehistory. A great many of these “ancient” and traditional medical plants have been validated to confer therapeutic benefits, albeit not always in controlled clinical trials. One unexpected outcome from validation studies is just how many medical plants synthesize equivalent or closely related compounds. Consequently, it is not surprising that many biological properties are also shared by unrelated species. Wound healing is a dynamic and highly regulated process consisting of cellular, humoral and molecular mechanisms [8]. The normal cutaneous wound healing process is a temporal process involving a complex series of overlapping events, which can be divided into key stages including: hemorrhage and fibrin clot formation, inflammatory response, reepithelialization, granulation tissue formation, angiogenic response, connective tissue contraction, and remodelling. On the basis of previous reported research work and traditional uses, the present study was aimed to evaluate the *Morinda tinctora* and *Bombax ceiba* for wound healing activity.

Experimental work

Procurement and Authentication of Plant material (*Morinda Tinctoria* and *Bombax Ceiba bark*): The pharmacognostic study of plant material was support for confirmation and determination of identity, purity and quality of a crude drug. The *Morinda Tinctoria* and *Bombax Ceiba* bark were collected and authenticated. Plant material were powdered to 60# separately and stored in airtight containers and used for phytochemical and pharmacological studies [9].

Macroscopical study: The macroscopical description of different parts of *Morinda tinctoria* and *Bombax Ceiba* include size, shape, nature of outer and inner surfaces, types of fracture, and organoleptic characters like color, odour, taste etc. were studied.

Physicochemical Evaluation: Physicochemical analysis helps to set up certain standard for dried crude drugs in order to avoid batch-to-batch variation and also to judge their quality. Their studies also give an idea regarding the nature of phytoconstituents present. Proximate analysis of *Morinda Tinctori* and *Bombax Ceiba* bark powders were carried out using methods prescribed in the Ayurvedic pharmacopoeia of India by subjecting them to various determinations like Total Ash, Acid insoluble ash, Water soluble ash, Alcohol soluble extractive value, Water soluble extractive value, Loss of moisture content and Swelling index [10].

Extraction process of plant: Extraction involve separation of bioactive portion of the plant tissues from the inactive components by using selective solvents in standard extraction procedures. All selected plant drugs were extracted sequentially with petroleum ether, and methanol using continuous hot extraction method i.e. soxhlet extraction. The completely dried plant material was coarsely powdered and then defatted with non polar solvent petroleum ether till completion of extraction. The exhausted powder extracted with methanol till completion of extraction. The obtained extract was concentrated under reduced pressure using rotary evaporator. The methanol exhausted powder of *Hibiscus* macerated with water, filtered and concentrated to get aqueous extract [11].

Chemical Tests: The extracts were then subjected to various qualitative test using as per following to determine the presence of various phytoconstituents such as alkaloids, glycosides, flavonoids, carbohydrates, aminoacids, saponins, sterols and terpenoids, cardiac glycosides, coumarins, carotenoids, tannins, phenolic compounds, fixed oils and fats etc.

Preparation of formulations: The ointment of plant extracts were prepared to evaluate its efficacy with ointment base or vehicle for local activity of wound healing. Ointment base was prepared by boiling in a beaker at 65°C water bath using wool fat 5 g, cetostearyl alcohol 5 g and soft white paraffin 90 g as the ingredients. The plant extract (5

g and 10 g) was incorporated in 100 g of the base. Povidone marketed preparation obtained from bhopal was used as standard drug for comparing the wound healing potential of extract in different animal models [12].

Group of animals: The excision and incision wound models were used to evaluate the wound-healing activity of methanol and water extract of Herbal ointment formulation. The wistar rats were divided into various groups, each group containing six animals, for excision and incision wound models. 5% and 10% extract containing formulated ointments were applied topically to each animal once a day.

Group I: The animals of received ointment base (control)

Group II: Treated group with a marketed povidone formulation

Group III: Methanol extract of Morinda Tinctoria (MMT 5 %) ointment

Group IV: Methanolic extract of Morinda Tinctoria (MMT 10 %) ointment

Group V: Methanolic extract of Bombax Ceiba fruit (TDME 5 %) ointment

Group VI: Methanolic extract of Bombax Ceiba fruit (TDME 10 %) ointment

Excision wound model: The animals were divided into three groups with six in each were anaesthetized by open mask method with anesthetic ether before wound creation. The particular skin area was shaved 1 day prior to the experiment. An excision wound was inflicted by cutting away a 300 mm² full thickness of skin from a predetermined shaved area (Saha et al., 1997). The wounds were left undressed to the open environment. The ointment base, standard drug ointment (0.1% silver sulfadiazine) and methanolic extract of TDR ointment (5%, w/w) was applied topically to the control group, standard group and treated group respectively, till the wound was completely healed. In this model, wound contraction and epithelialization period was monitored. Wound contraction was measured as percent contraction in each 2 days after wound formation. From the healed wound, a specimen sample of tissue was collected from each rat for histopathological examination [13-14].

Incision wound model: In incision wound model (Ehrlich and Hunt, 1968), all the animals of each group were anaesthetized under light ether anesthesia. Two full thickness paravertebral long incisions were made through the skin at the distance of about 1 cm from midline on each side of the depilated back of rat. After the incision was made the both edges of skin kept together and stitched with black silk surgical thread (no. 000) and a curved needle (no. 11) was used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. After stitching, wound was left undressed then ointment base, standard ointment and extracts ointment were applied daily up to 10 days; when wounds were cured thoroughly the sutures were removed on the day 10 and tensile strength of cured wound skin was measured using tensiometer [15].

Result And Discussion

The pharmacognostic study of plant material was support for confirmation and determination of identity, purity and quality of a crude drug. *M. tinctoria* is an evergreen shrub or small tree growing to 5 - 10 m tall. The leaves are 15 - 25 cm long, oblong to lanceolate; blade 4 - 8, narrowed into petiole 1.3 - 2.5 cm long. The bark of the *Bombax ceiba* tree, also known as the kapok tree or red silk cotton tree, has a distinct odor and taste. The bark is corky, grey-brown in color, and has sharp spines

Physicochemical parameters: Physiochemical values for chosen plant sedate were distinguished by the official techniques. The different parameters incorporate, for example, debris esteem and extractive qualities were resolved. The WHO rules on quality control strategies for restorative plant materials was streams for assessing the above parameters (Table 1). Total ash, Acid insoluble ash and Water solvent ash, Extractive qualities and Loss on drying were resolved for *Morinda Tinctora* and *Bombax Ceiba*. The accompanying physicochemical examination was resolved for the powder medicate.

Table 1: Physicochemical parameters of *Morinda tinctoria* bark and *Bombax Ceiba* bark

S. No.	Physicochemical parameter	values (% w/w)	
1	Total ash	5.8	4.8
2	Water solvent ash	3.4	3.45
3	Acid insoluble ash	2.1	1.23
4	Foreign organic matter determination	1.1	0.9
5	Alcohol-soluble extractive	10.52	11.02
6	Water-soluble extractive	12.78	13.08
7	Loss on drying (%)	1.98	1.82

Extraction of the Drugs: The coarse powder of *Morinda tinctoria* and *Bombax Ceiba* were subjected to successive solvent extraction using solvents of ascending polarity. After extraction the percentage yield of each extract was calculated with reference to the air dried drug used for the study (Table 2).

Table 2: Physicochemical parameters of *Morinda tinctoria* bark and *Bombax Ceiba* bark

S. No.	Extract	Yield (% W/W)	
1	Petroleum ether	3.1	2.9
2	Methanol	10.5	11.5
3	Water	9.6	10.6

Preliminary Phytochemical Screening: The qualitative phytochemical screening of the extracts of *Morinda tinctoria* and *Bombax Ceiba* were carried out. Preliminary phytochemical analysis of *M. tinctoria* investigated for the presence of various secondary metabolites using different solvents. Aqueous extract of *M. tinctoria* fruits showed the

presence of carbohydrate, protein, alkaloids, saponin, glycosides, tannins, flavonoids and steroids. methanol extract showed the presence of all the tested metabolites except saponins. Proteins were not identified in the methanol extract. In ethyl acetate extract, alkaloids were not detected, Carbohydrates, proteins, tannins and steroids were present in chloroform extract, while flavonoids, saponins, glycosides and alkaloids were absent. Preliminary phytochemical analysis of *Bombax Ceiba* investigated for the presence of various secondary metabolites using different solvents. Aqueous extract of *M. tinctoria* fruits showed the presence of carbohydrate, protein, alkaloids, saponin, glycosides, tannins, flavonoids and steroids. methanol extract showed the presence of all the tested metabolites except saponins. Proteins were not identified in the methanol extract. In ethyl acetate extract, alkaloids were not detected, Carbohydrates, proteins, tannins and steroids were present in chloroform extract, while flavonoids, saponins, glycosides and alkaloids were absent.

Acute toxicity studies of *Morinda tinctoria* and *Bombax Ceiba* extracts as per OECD

guideline: The objective of this Acute Toxicity Study of *Morinda tinctoria* and *Bombax Ceiba* on rats was to assess the toxicological profile of the test drug when given orally once (single dose) to the test system (rats) and monitor the vital signs for 14 days. This study will provide information on the possible health hazards likely to arise from single oral exposure of the aqueous and methanol extracts of the plant. Acute toxicity studies of methanol and aqueous extracts of *Morinda tinctoria* and *Bombax Ceiba* were performed in Albino rat dose levels of 50, 300 and 2000 mg/kg as per OECD guide lines. The treated animals did not demonstrate any significant changes in behavioral pattern and exhibited normal activity. Also there were no clinical signs of tremors, convulsions, exophthalmos, salivation, diarrhea and lethargy. There was no significant difference in the mean body weights between treated groups and control group and the rats exhibited normal body weight gain during the study. No lethal effects or mortality was observed in animals throughout the test period following single oral administration at all selected dose levels of all extracts. The animals were examined for long term toxicity (14 days) (Table 3 and Figure 1).

Table 3: Effect of plant extract ointment on % of wound contraction of excision wound models in rats

Group	0 Days	3 Days	6 Days	9 Days	12 Days	15 Days	18 Days	21 Days	24 Days
Group I	0	27.34	34.28	47.64	52.4	75.15	84.43	92.76	100
Group II	0	52.24	74.32	98.15	100				
Group III	0	21.14	41.24	61.02	69.24	78.17	97.24	100	
Group IV	0	47.68	68.61	81.41	98.82	100			
Group V	0	19.12	39.14	58.21	65.11	71.02	92.02	100	
Group VI	0	34.21	51.24	72.61	84.45	100			

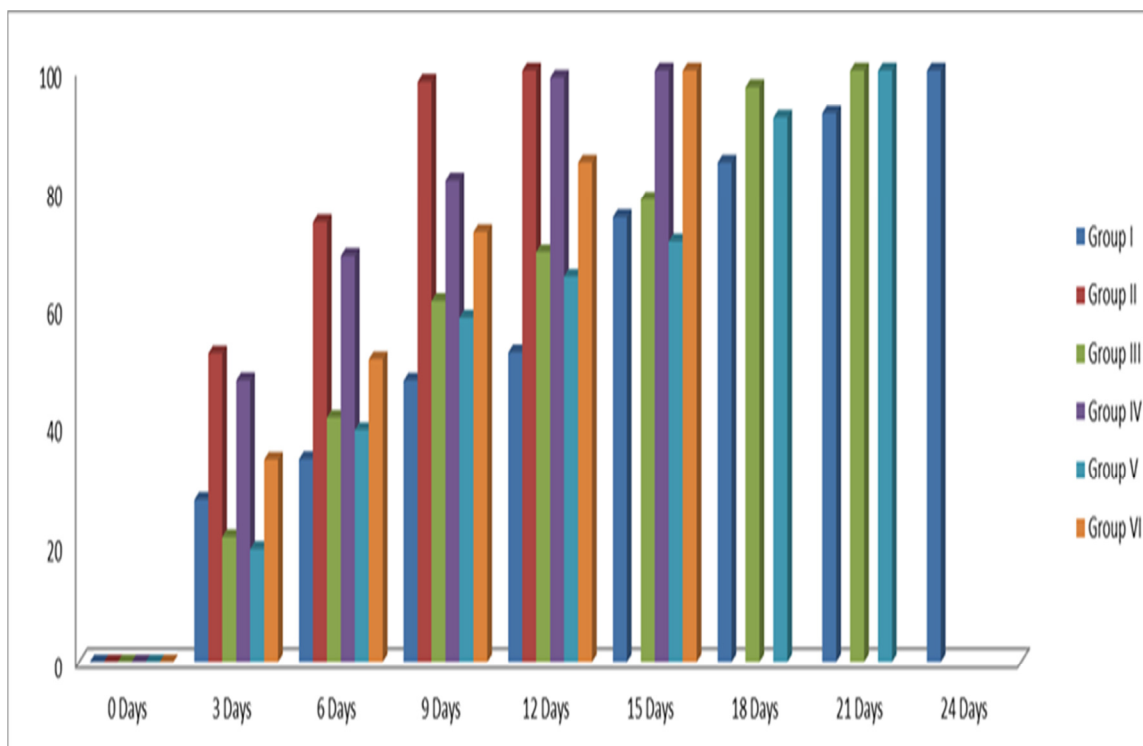


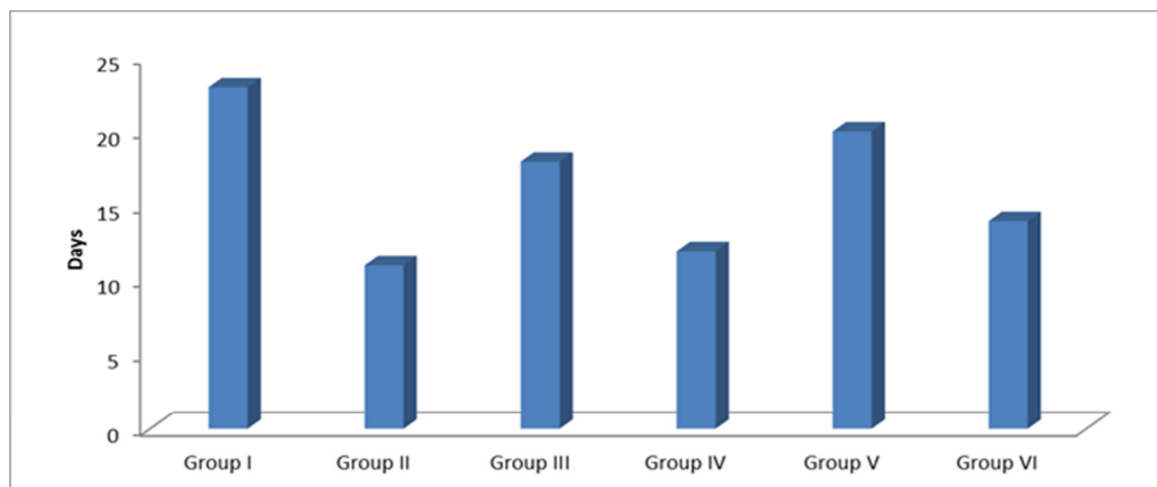
Figure 1: Effect of plant extract ointment on % of wound contraction of excision wound models in rats

Wound contraction and Epithelialization time of excision wound models in rats: A better healing pattern with complete wound closure was observed in standard and treated group within 10 and 12 days respectively while it was about 24 days in control rats. Group IV treated with methanol extract of *Morinda tinctoria* (10%) showed complete wound closer in 12 days while other group takes more time for complete wound closer. Group VI treated with methanol extract of *Bombax Ceiba* (MBC 10 %) ointment showed complete wound closer in 14 days. The result concluded that methanolic extract of *Morinda tinctoria* (10%) showed best effect than other extract and significant with treated group (Table 4 & Figure 2).

Hydroxyproline content and tensile strength of incision wound models in rats: Treated group showed significant increase in hydroxyproline content when compared to control group ($P < 0.01$). The Hydroxyproline content and tensile strength in incision model estimated at Group IV treated with *Morinda Tinctoria* result was showed best result than *Bombax Ceiba* extract and significant with treated group.

Table 4: Effect of plant extract ointment of excision wound models in rats

Group	Epithelialization time (Days)
Group I	23
Group II	11
Group III	18
Group IV	12
Group V	20
Group VI	14

**Figure 2: Effect of plant extract ointment on Epithelialization time of excision wound models in rats****Table 5: Effect of plant extract ointment on incision wound models in rats**

Group	Hydroxyproline (mg/g tissue)	Tensile strength (g/mm ²)
Group I	24.11 ± 0.01	392.21 ± 3.21
Group II	71.22 ± 0.03	596.04 ± 4.15
Group III	41.02 ± 0.02	458.31 ± 3.13

Group IV	60.13 ± 0.04	516.21 ± 2.24
Group V	34.27 ± 0.03	446.11 ± 1.08
Group VI	58.22 ± 0.05	502.01 ± 4.51

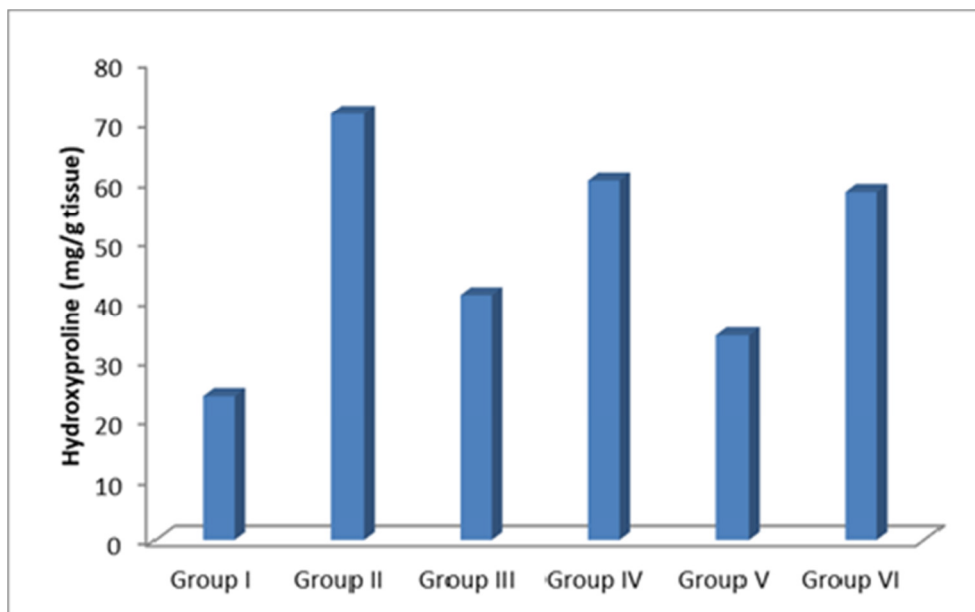


Figure 3: Effect of plant extract ointment on Hydroxyproline on incision wound models in rats

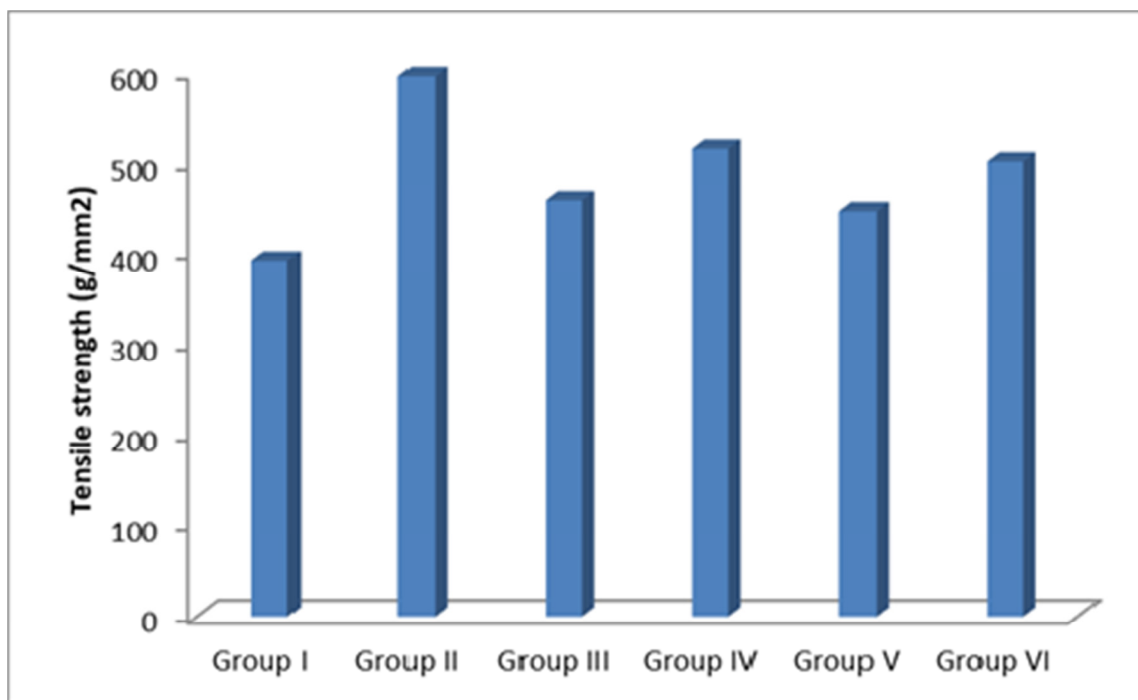


Figure 4: Effect of plant extract ointment on Tensile strength on incision wound models in rats

Summary & Conclusion: Natural products and secondary metabolites from different plant sources have been used to treat wounds for centuries, worldwide. The most common mechanisms behind the phytochemical-mediated optimization of the wound healing process are their antioxidant, anti-inflammatory, and antimicrobial activities. In this review, we can find the most current reports, both in vitro and in vivo, which provide evidence to support the premise that natural products, belonging to different groups of phytochemicals, exert a potential for wound healing and the underlying molecular mechanisms; These compounds can be involved in the four stages of the wound healing process, since they interact at the intracellular level, for example, in the modulation of the generation of ROS, increasing the response of immune cells to decrease the inflammation and regeneration of the tissue, while reducing the probability of infection by helping to eliminate invading pathogens from the wound. A better healing pattern with complete wound closure was observed in standard and treated group with methanol extract of *Morinda tinctoria* (10%) and methanol extract of *Bombax Ceiba*. The result concluded that methanolic extract of *Morinda tinctoria* (10%) showed best effect than other extract and significant with treated group.

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