

PHYTOCHEMICAL AND WOUND HEALING ACTIVITY OF PROSOPIS JULIFORA BARKS ON DIFFERENT MODELS

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Abstract:

Introduction: The present study provides a scientific evaluation for the wound healing potential of ethanolic (PJOH) extract of Prosopis JuliforaLinn. (PJ) plant.

Materials and Methods: Excision, incision wounds were inflicted upon four groups of six rats each. Group I was assigned as control (ointment base). Group II was treated with 0.5% EtOH extract ointment. Group III was treated with 2% EtOH extract ointment, Group IV was treated with standard cream. The parameters observed were percentage of wound contraction, epithelialization period, tensile strength studies.

Result: It was noted that the effect produced by the ethanolic extract of PJ ointment showed significant ($P < 0.01$) healing in all wound models when compared with the control group. All parameters such as wound contraction, epithelialization period, tensile strength showed significant ($P < 0.01$) changes when compared with the control.

Conclusion: The ethanolic extract ointment of PJ effectively stimulates wound contraction; increases tensile strength of excision, incision wounds.

KEY WORDS: excision injury, incision injury, *PJ* Linn. Wound healing

INTRODUCTION:

Wounds are inescapable events in life. Wounds may be due to physical, chemical or microbial agents. Wound healing involves a complex series of interactions between different cell types, cytokine mediators, and the extracellular matrix. The phases of normal wound healing include hemostasis, inflammation, proliferation and remodeling. Each phase of wound healing is distinct, although the wound healing process is continuous, with each phase overlapping the next. Because successful wound healing requires adequate blood and nutrients to be supplied to the site of damaged tissue^{1,2}.

Plant extracts have been used as wound healing agents since ancient time. The usage of traditional medicinal remedies and plants in the treatment of burns and wounds is viewed as an important mode to improve healing and in the same time to reduce the financial burden in the economically deprived societies of the developing world. Several plants and herbs have been used experimentally to treat skin disorders, including wound injuries in traditional medicine like *Rafflesia Hasseltii*. A wound can be defined as a disruption of the normal anatomical relationships of tissues as a result of injury. The injury may be intentional such as a surgical incision or accidental following trauma. Enoch and Leaper (2008) defined wound that break in the epithelial integrity of the skin and may be accompanied by disruption of the structure and function of underlying normal tissue, may also result from a contusion, hematoma, laceration or an abrasion^{3,4}.

There are several causes or factors, which may interfere with wound healing such as traumatic (mechanical, chemical, physical and surgery), ischemia (e.g. arterial leg ulcer and pressure sore) Normal wound healing response begins as soon as the tissue is injured. Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis begin to increase the collagen production. Later, the epithelial tissue is regenerated. It is accepted that wound repair is an immune-mediated physiologic mechanism, Wound healing or wound repair is an intricate process in which the skin repairs itself after injury. Wound infection is most common in developing countries because of poor hygienic conditions In some part of the world like Malaysia, wounds are mostly due to diabetes. Hence, for infection control, and for the restoration of disrupted anatomical continuity and disturbed functional status of the skin, appropriate method for healing of wounds is essential. Wound can be classified as acute or chronic and as partial thick or fully thick wounds. Acute wounds are defined as wounds that heal in a predictable and expected period of time. Chronic wounds are usually occurs in compromised patients who have an underlying pathology such as poor circulation or diabetes. Partial thickness wounds involve the epidermis and may or may not involve the dermis. These wounds are shallow, moist and painful. They mostly heal first with the initial inflammation response, then re-epithelialization. However, in full thickness wounds, healing begins after injury initiates a series of cellular and biochemical events that occur in coordinated and overlapping phases in the healthy host, which results in healing^{5,6}.

Material and methods:

Prosopis juliflora is one of the most economically and ecologically important tree species in arid and semi-arid zones of the world *Prosopis juliflora* belongs to the family Leguminosae (Fabaceae), sub-family Mimosoideae, and it having 44 species of which 40 are native to the Americas, three to Asia and one to Africa. The tropical Andean region is home to six species and eight species are found in the texas area, seven of them being endemic. These species are having the several properties such as soil binders, sand stabilizers, as well as its ability to grow in the poorest soils. It is a shrub or tree having 8-12 metres long. Growing to a height of up to 12 metres (39 ft), *P. juliflora* has a trunk diameter of up to 1.2 metres (3.9 ft). Its leaves are

deciduous, geminate-pinnate, light green, with 12 to 20 leaflets. Flowers appear shortly after leaf development.

The various chemical agents that are present in it show the medicinal value that may alters certain physiological actions in the human body. The several biochemicals present in the plant are terpenes, alkaloids, flavonoids and phenolic compounds. Terpenes are used as insecticides and their pharmacological properties include antibacterial, antifungal, anthelmintic, antimalarial and molluscicidal. Extracts of *P. juliflora* seeds and leaves have several in vitro pharmacological effects such as anti-bacterial, anti-fungal and anti-inflammatory properties. Since it is a main source of fuel this plant provides more than 90% of the fuel wood in some Indian villages because *P. juliflora* wood has excellent burning qualities. Thus, it is called wooden anthracite. It also has high calorific value.

It has been used as a folk remedy for catarrh, cold, diarrhea, dysentery, excrescences, flu, hoarseness, inflammation, measles, sore throat and in healing of wounds. Decoction prepared from leaf and seed extracts are used in wound healing, as disinfectant and also to treat scury. *P. juliflora* syrup prepared from ground pods is given to children showing weight deficiency or retardation in motor development, the syrup is believed to increase lactation. Tea made from *P. juliflora* is thought to be good for digestive disturbances and skin lesions. It has soothing, astringent, antiseptic, antibacterial and antifungal properties. It has been used to treat eye problems, open wounds, dermatological ailments and digestive problems by the native tribes of many countries. The flavonoid, patulitrin isolated from its flowers and fruits showed significant activity against lung carcinoma in vivo.. Leaves and pods are to be the richest source of plant metabolite, followed by flower, root and stem. Very high flavonoids content (16%) of *Proposip juliflora* makes it a potential candidate bearing antioxidant and anticancer properties. Tannins and Phenols although found in low concentrations, (0.33 and 0.66% respectively) can synergize the antioxidant and anticancer potential of flavonoids. Phenols are reported to prevent the platelets from clumping and have the ability to block specific enzymes that cause inflammation. *P. juliflora* pods are characterized by elevated sugar content, about 300 g/kg of dry matter. With 120 g/kg of crude protein on a DM basis. Once concentrated, the methanolic extract obtained from these beans becomes dark and dense and can be used in beverages and jellies. Roasted and ground, the beans can be used to make a coffee-like beverage

Plant Material collection: The whole plant of *PJ* were identified, and collected in the month of October from the forest of Telangana. The plants were washed, shade dried, pulverized into moderately coarse powder, passed through a 40 mesh sieve and stored in an air tight container for further use.

Plant Drug Extraction

The powdered plant (60 g) of *PJ* was extracted with ethanol using the Soxhlet apparatus for 24 h until the extraction was completed. The solvent was removed under reduced pressure. The dried extract was weighed and percentage yield was obtained with respect to dry powdered material.

Preliminary Phytochemical Screening

The different identification tests were performed to detect the presence of metabolites in ethanolic extract of PJ.

Preparation of Formulation and Standard Used

Simple ointment was prepared from the 10% ethanolic extract of PJ by trituration method in a ceramic pestle and mortar using White soft paraffin obtained from S.D. Fine Chemical, India. About 10 g of semisolid extract was incorporated into the 100 g of simple ointment base B.P. Simple ointment base was used as the control group and was applied twice per day. Extract ointment was used twice per day to treat different groups of animals. Povidine was used as standard drug for comparing the wound healing potential of extract in different animal models and was applied twice per day.

Animals

Wistar albino rats (150-180 g) of either sex were selected for the experiment. They were housed individually in standard laboratory environment for 7 days of period, fed with commercial pellets and water *ad libitum*. Animal study was performed in Division of Pharmacology,

Preparation of Ointment

The ointment was prepared by using white liquid paraffin wax as a base. Ointment was formulated by grinding base and ethanol extract of **PJ** in a ceramic mortar and pestle to get different concentrations on w/w basis. Viz. 0.5% and 2%. The prepared fresh ointment was stored in the plastic airtight container, labelled and maintained at room temperature.

Ointment Formulation

Simple ointment B.P. was prepared using hard paraffin, ceto stearyl alcohol, white soft paraffin, and wool fat. The master formula used for the preparation of ointment was taken from British Pharmacopoeia.

Ingredients	M.F	R.F
Wool fat.....	50 g	10g
Hard paraffin.....	50 g	10g
White soft paraffin.....	850g	170g
Cetostearyl alcohol.....	50g	10g
	1000g	200g

M.F is Master Formula; R.F is Reduced Formula. The 200 g of simple ointment base was prepared by placing hard paraffin (10 g) in a beaker and melted over water bath. The other ingredients such as cetostearyl alcohol (10 g), white soft paraffin (170 g), and wool fat (10 g) were added in descending order of melting point, respectively, after removing from melting⁷⁻⁹.

All the ingredients were melted over a water bath with constant stirring until they became homogeneous. The mixture was removed from the heat and stirred until cold. To prepare hydroalcoholic extract ointment, 0.5 g and 2 g of the powdered extract were incorporated into portion of simple ointment base to prepare 0.5% and 2% (w/w) ointment, respectively, by levigation. The remainder of simple ointment base was gradually added and mixed thoroughly. Finally, the extract ointment was transferred to a clean container for topical application during the experiment.

Wound healing Studies

Excision wound experimental paradigm was used to assess the wound healing activity of ethanol extract of leaves of *PJ*.

Excision Wound Model

Animals were anesthetized prior to and during creation of the wounds. The rats were inflicted with excision wounds as described by Morton and Malone. The dorsal fur of the animals was shaved with an electric clipper and the anticipated area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of circular area 500 mm² and 0.2 cm depth was created along the markings using toothed forceps, a surgical blade, and pointed scissors. The entire wound was left open⁹⁻¹¹. The animals were divided into 4 groups of six each. Group 1 and 2 animals were topically treated with the simple ointment base I.P. as a placebo control and positive control. The animals of group 3 and 4 were topically treated with the 0.5 and 2 % ointment of the ethanolic extract of *PJ* formulated in simple ointment base I.P. till complete epithelialization. The wound closure rate was assessed by tracing the wound on days 0, 2, 4, 8, 12, 16, 18, and 20 post-wounding using transparency sheets and a permanent marker. The wound areas recorded were measured using a graph paper. Number of days required for falling of eschar without any residual raw wound gave the period of epithelialization.

(a) Measuring the contraction of the wound.

The tracing of the wound was transferred to a one mm² graph paper to determine the wound area. The wound contraction was calculated as a percentage of the original wound size taken as 100% of each animal in the group by using the equation. The progressive reduction in the wound area is monitored plan metrically by tracing the raw wound boundaries initially on a sterilized

transparency paper sheet in mm² without causing any damage to the wound area, and then, the wound area recorded is measured using a graph paper on every 2–4 d interval. The period of epithelialization is expressed as the number of days required for falling of the eschar (dead-tissue remnants) without any residual raw wound is considered as the end point of complete epithelialization. Percentage wound contraction is calculated as:

Percentage wound contraction on

$$N\text{th day} - 100 = \frac{\text{wound area on } N\text{th day}}{\text{wound area on 1st day}} \times 100.$$

Final statistical analysis was done by taking the mean of individual groups after inducing the wound at 5th, 9th, 13th and 17th day. Epithelialization period was monitored by noting the number of days required for the eschar (Eschar is dead tissue found in a full-thickness wound) to fall off leaving no raw wound area behind.

Experimental design

Table 1: For Experimental Design of PJ

Group	Drug	Route	No.of animals used
Group 1	Normal saline (Control)	Topical	6
Group 2	Povidone-iodine (2%) (Standard)	Topical	6
Group 3	0.5% <i>Ae</i> extract ointment	Topical	6
Group 4	2 % <i>Ae</i> extract ointment	Topical	6

PJ. For the excision model a total of 24 rats were used and divided into 4 groups (n=6 rats).

Incision Wound Model

As the above model, rats were anesthetized prior to and during creation of the wound. The dorsal fur of the animals was shaved with an electric clipper. A longitudinal paravertebral incision, 6 cm in length, was made through the skin and cutaneous muscle on the back, as described by Ehrlich and Hunt. After the incision, surgical sutures were applied to the parted

skin at intervals of 1 cm^{12,13}. The wounds were left undressed. The rats were given *PJ* extract (dissolved in tween-80, 0.5%) orally at a dose of 200 mg kg⁻¹ day⁻¹. The controls were given with tween-80 0.5% only. The sutures were removed on the eight post-wound day and the treatment was continued. The skin-breaking strength was measured on the 10th day by the method described by Lee. The anesthetized animal was secured to the table, and a line was drawn on either side of the wound 3 mm away from the line. This line was gripped using forceps one at each end opposed to each other. One of the forceps was supported firmly, whereas the other was connected to a freely suspended light-weight metal plate. Weight was added slowly and the gradual increase in weight, pulling apart the wound edges. As the wound just opened up, addition of weight was stopped and the weights added was noted as a measure of breaking strength in grams. Three readings were recorded for a given incision wound. The mean reading for the group was taken as an individual value of breaking strength. The mean value gives the breaking strength for a given group.

Dead Space Wound Model^{14,15}: In this model, the physical and mechanical changes in the granuloma tissue are studied. The subcutaneous dead space wounds are inflicted one on either side of axilla and groin on the ventral surface of each animal, by making a pouch through a small nick in the skin. The cylindrical grass piths (2.5 × 0.3 cm) or sterile cotton pellets (5–10 mg each) are introduced into the pouch. Each animal received 2 grass piths/cotton pellets in different locations. The dead space wound is created by subcutaneous implantation of a sterilized, shallow, metallic ring (2.5 × 0.3 cm) known as the cylindrical pith or polypropylene tube (2.5 × 0.5 cm) on each side beneath the dorsal paravertebral lumbar skin surface, and wounds are sutured. The respective therapeutic treatment is administered either orally or topically to the animals of respective groups for 10 consecutive days. The physical changes in the granuloma tissue are studied in this model. In dead space wound model also significantly increase in weight of the granulation tissue and breaking strength was observed in the animals treated with deoxyelephantopin.

Burn Wound Model¹⁶ : Rats were divided into 5 groups including; SSD cream 1% as the reference standard, eucerin as the control, and 5 %, 10 % and 20 % ointments of *PJ* flowers extract as the treatment groups, starting right after burn wound induction. Ointments were used topically over the wounds every day for 14 days. The wounds areas were cleaned, photographed with a digital camera and calculated using Adobe Photoshop CS5. The wound contraction rate was measured according to this formula: Wound contraction % = 100 × [(first day wound area - specific wound area)/ first day wound area] On the day 14 (the end of experiment), animals were sacrificed, the granulated tissues were collected, and preserved in buffered formalin 10% to evaluate the histological changes. Series of 3-4 μm thickness sections were prepared for each sample, stained with hematoxylin/eosin, and microscopic photographs were captured under × 400 magnification.

Statistical Analysis

All treated groups were compared with the control groups. The results were analyzed statistically using one-way analysis of variance (ANOVA). The result were found to be significantly at $P < 0.01$.

Wound healing activity:

The percentage of wound contraction was measured on 5th, 9th, 13th, and 17th days of drug treatment to the respective group of rats. On 5th and 9th days; rats treated with povidone-iodine (58.00±0.22 and 80.97±0.39), 0.5% PJ extract (49.45±0.28), 2% PJ (54.48±0.32 and 81.13±0.47) showed significant p (<0.05) increase in the percentage of wound contraction vs. control. However, povidone-iodine and 2% PJ showed a maximum % of wound contraction on the 5th and 9th days, respectively. Rats treated with the (2% PJ + dexamethasone) combination exhibited the least % of wound contraction i.e. 29.86±0.83 and 43.86±0.46 is on 5th and 9th day respectively. The 2% PJ extract treated rats produced relatively a better wound contraction as compared to the 0.5% lower dose. On the 9th day, the percentage of wound contraction showed by the 2% PJ treated rats were as comparable to the povidone-iodine group of rats. On the 13th and 17th days, none of the rats administered with the test drugs produced significant wound contraction vs. control. The mean period of epithelialization shown by control, standard, 0.5% and 2% test drug-treated rats were 18.30 ± 0.17, 12.33 ± 0.25, 18.19 ± 0.17, 14.14 ± 0.09 days respectively. The mean period of epithelialization with povidone-iodine (12.33 ± 0.25), 2% PJ extract (14.14 ± 0.09). However, the mean period of epithelialization was least in rats treated with povidone-iodine compared to control.

Table 2: Effect of ethanolic extract of leaves of PJ on the rate of wound contraction in an excision wound.

Group	Drug (n=6)	Wound contraction % Mean ± SEM			
		5 th day	9 th day	13 th day	17 th day
Group 1	Normal saline (p. o.)	47.16±0.15	73.26±0.84	90.93±0.40	99.08±0.12
Group 2	Povidone-iodine (Topical)	58.00±0.22 ^a	80.97±0.39 ^a	91.91±1.02	99.35±0.17
Group 3	PJ Ointment (0.5%)	49.45±0.28 ^{a,b}	74.62±0.36 ^b	91.62±0.49	98.20±0.21
Group 4	PJ Ointment (2%)	54.48±0.32 ^{a,b}	81.13±0.47 ^a	90.45±0.42	99.24±0.09

Values were expressed as Mean \pm SEM. Statistical analysis was done using one way ANOVA followed by Tukey post hoc test; ^a p<0.05 Vs control, ^b p <0.05 Vs standard (povidone iodine), n= No. of rats/gr.

Table 3: Effect of ethanolic extract of leaves of *PJ* on the period of epithelialization in an excision wound.

Group (n=6)	Drug	Period of epithelialization
Group1	Normal saline(PO)	18.30 \pm 0.17
Group 2	Povidone iodine(Topical)	12.33 \pm 0.25 ^a
Group 3	Ointment of ethanolic extract of leaves of <i>PJ</i> 0.5%	18.19 \pm 0.17 ^b
Group 4	Ointment of ethanolic extract of leaves of <i>PJ</i> 2%	14.14 \pm 0.09 ^{a,b}

Values were expressed as Mean \pm SEM. Statistical analysis was done using one way ANOVA followed by Tukey post hoc test; ^a p <0.05 Vs control, ^b p <0.05 Vs standard (povidone iodine), n= No. of rats/gr.

In incision wound model, methanol leaf extract treated animals showed increase in breaking strength (383.3 \pm 7.43). when compared to the control (297.4 \pm 7.45). The mean breaking strength was also significant in animals treated with standard drug FSC (422.0 \pm 6.35).

Table 4: Effect of topical application of ethanol extracts of on incision wound model

S.No	Group (n)	Breaking strength (g)
1	Control	297.5 \pm 7.45
2	Nitrofurazone (0.2% W/W)	422.0 \pm 6.35

3	Ethanol extract (0.5% w/w)	383.3±7.43
4	Ethanol extract (2% w/w)	422.0± 10.23

n=6, albino rats per groups, values are represents mean± SEM *P<0.01. (comparison of I, II and III). The results were analyzed statistically using one-way analysis of variance (ANOVA)

In dead space wound model, histological studies of the granulation tissue of the control group of animals showed more aggregation of macrophages with few collagen fibres. In the case of ethanolic leaf extract treated animal groups, moderate collagen deposition, macrophages and fibroblasts were noticed, whereas the methanol leaf extract treated animal group evidenced significant increase in collagen deposition showing lesser macrophages and fibroblasts. Compared to the control group of animals, methanol leaf extract treated animals showed significant increase in dry weight of granulation tissue (75.00±1.29) and breaking strength (436.0±4.30) followed by aqueous leaf extract treated group of animals.

Table 5: Effect of ethanolic extract on dead space wound model

Treatment	Granulation tissue dry weight (mg/100g)	Breaking strength (g)
Control	42.33±2.28	318.0±9.22
Ethanol	61.54±1.89	397.0±5.98
povidone	75.00±1.29	436.0±4.30

n=6, albino rats per groups, values are represents mean± SEM *P<0.01. (comparison of I, II and III). The results were analyzed statistically using one-way analysis of variance (ANOVA)

Table 6: Wound healing percentage in experimental groups by burn wound healing model

Groups	14th day
Control	56 ± 0.035
SSD	73.5 ± 0.018
0.5% Extract	80.6 ± 0.027
2% Extract	89 ± 0.066

n=6, albino rats per groups, values are represents mean± SEM *P<0.01. (comparison of I, II and III). The results were analyzed statistically using one-way analysis of variance (ANOVA)

Discussion

Preliminary phytochemical

Preliminary phytochemical screening revealed that ethanolic extract of *PJ* showed positive response to Alkaloids, Tannins, Flavonoids, Carbohydrates, Lignin's, Proteins, some active principles which are responsible for the different pharmacological activities, they are Tannins and triterpenes are responsible for antidiarrheal activity, antiulcer activity, and wound healing, Hepatoprotective activity, Flavonoids are responsible for neuropharmacological and CNS depressants, anticancer activity Hepatoprotective activity, antidiabetic activity, wound healing, Antiulcer activity and Triterpenoids which are responsible for CNS depressants activity.

Wound healing activity:

Traditionally, the leaves of *PJ* are used for wound healing activity. Applying the extract directly on the affected wound cannot bring the desired effect as it does not stay longer on the wounded skin of the experimental animals. Ointment is necessary to achieve a sustained drug release at the application sites. Hence, a hydrophobic base was selected based on traditional claim and active metabolites of leaves of *PJ* predominate polar components, which would be released better from the nonpolar base and vice versa. The ointment base has additional roles like formation of occlusive barrier for moisture by hard and white soft paraffin. Wool fat and cetostearyl alcohol helps to thicken and used for stabilization of ointment¹⁷.

The results of this study on wound healing activity revealed that the crude extract significantly increases wound healing effects with both 0.5% (w/w) and 2% (w/w) extract ointment treated groups in the excision and incision wound models. This can be supported by the fact that greater the reduction in the rate of wound contraction shows the better efficacy of medication which the wound will close at faster rate if the medication is more efficient¹⁸.

In excision wound healing model, the crude extract (80% methanol) of the leaves of *PJ* showed statistically significant wound area contraction compared to the negative control. The 2% (w/w) extract ointment treated group revealed faster wound area contraction from day 6 to day 14, whereas the 0.5% (w/w) extract ointment treated group showed statistically significant wound area contraction starting from the 8th day onwards. The higher wound contraction rate of the extract ointment may be due to either its dose-dependent antibacterial effect or induction of macrophage cell proliferation.

Furthermore, the period of epithelialization was significantly reduced from 20 days (negative control) to 15, 15, and 13 days for 5% extract, nitrofurazone, and 2% extract ointment treated groups, respectively. The shorter period of epithelialization and faster wound area contraction

could be due to the ability of *PJ* leaf extract to enhance collagen synthesis, induction of cell proliferation, and antimicrobial activities of bioactive constituents.

In the case of infected wound model, the ointments of crude extract revealed statistically significant wound healing effect in mice infected with *S. aureus*. The infiltration, blister formation, edema, and exudates exhibited on the wounds of mice before treatment vanished in all treated groups except the negative control. Groups treated with 2% extract ointment showed faster rate of wound contraction than nitrofurazone and 0.5% extract ointment treated groups. Additionally, the period of epithelialization was shorter in 2% extract followed by nitrofurazone and 0.5% extracts. This finding indicated that the wound healing activity of the extract in infected wound model was presumed to be dose-dependent. In this study, the antibacterial activity of the extract was confirmed against common wound infecting pathogens, which might contribute remarkably to the faster wound healing rate^{19,20}. Supporting evidence explained that the eradication of the colonizing organisms from infected wounds creates a suitable environment for wound healing to take place. As a result, the antimicrobial activity reported in infected wound model shows the promising potential of *PJ* towards wound management.

In incision wound model, significant increase in skin breaking strength was observed. Groups treated with 2% and 0.5% (w/w) extracts and standard ointments showed statistically significant increase in tensile strength as compared to simple ointment base treated group. However, the difference in tensile strength was not statistically significant among standard drug and 2% and 0.5% (w/w) ointment treated groups. The increase in tensile strength in the incision model may be due to the antioxidant activity of the extract, increase in collagen synthesis and maturation, formation of stable intra- and intermolecular cross-link, matrix deposition, and cell migration. Flavonoids are known in promoting wound healing via inhibition of collagen synthesis.

Another possible reason for enhanced wound healing effect could be due to the crude extracts of *PJ* leaves which may possess antioxidant, free radical scavenging properties and promote cell proliferating properties. To mention some, a study on the leaf extracts of *PJ* showed antioxidant activity and scavenging free radicals (superoxide and hydroxyl radicals), due to the presence of flavonoids..

The role of phytochemicals in wound healing is also supported by different studies. For instance, tannins are seen to be active detoxifying agents and inhibit bacterial growth; terpenoids promote the wound healing process mainly due to their astringent and antimicrobial property. flavonoids are potent antioxidants, free radical scavengers. Polyphenols and flavonoids (prevent the synthesis of prostaglandins) possess anti-inflammatory properties and have antimicrobial activities.. Glycosides (iridoid glycosides) isolated from the same family (Acanthaceae) possess antioxidant, antimicrobial, analgesic, antitumor, immunomodulatory, and anti-inflammatory effects. Therefore, the presence of phytochemicals in the crude extract such as terpenoids,

flavonoids, glycosides, saponins, tannins, and phenolic compounds may contribute to wound healing activities independently or synergistic effects.

Conclusion:

The results of the study showed that the ethanolic extract ointment of PJ effectively stimulates wound contraction; increases tensile strength of excision, incision and burn wound as compared with the control group. These finding could justify the inclusion of this plant in the management of wound healing.

References:

1. Yogesh Sharma, G. Jeyabalan and Ramandeep Singh, Current Aspect of Wound Healing Agents from medicinal plants: A review, *Journal of Medicinal plants studies*, Year: 2013, Volume: 1, Issue: 3, First page: (1) Last page: (11), ISSN: 2320-3862.
2. Schultz G.S, Molecular regulation of Wound healing in acute, chronic wounds, nursing management, brgant, R.A,(Ed).2nd Edn, 1999:413-429.
3. Kerstein, M.D, Factors affecting wound healing. *Adv. Wound care*, 2007; 10:30-36.
4. Li J, Chen J and Kirsener R, Pathophysiology of Acute Wound healing, *Clin.Dermatol*.2007; 25:9-18.
5. Stadelmalmann W.K, Digenis A.G and Tobin G.R, Physiology and healing dynamics of chronic cutaneous wounds , *Am. J.Surg*.1998; 176: 26S-38S.
6. Tamara, Book of Pathophysiology, basis for phase of wound healing.2008:12.
7. DE. S., Dey. Y.N., Ghosh. A.K., “Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of *Amorphaphalluspaeoniifolius* (Aracea)”, *Int.J.pharma bio. Res.* 1(5), 2010, 150-157.
8. G. Ayoola, H. Coker, S. Adesegun et al., “Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria,” *Tropical Journal of Pharmaceutical Research*, vol. 7, no. 3, pp. 1019–1024, 2008.
9. K. Nagarajan, P. Saxena, A. Mazumder, L. Ghosh, and G. U. Devi, “Effect of various chromatographic terpenoid fractions of *Luffa cylindrica* seeds on in-vitro antimicrobial studies,” *Oriental Pharmacy and Experimental Medicine*, vol. 10, no. 1, pp. 21–28, 2010.
10. OECD, “Guideline for testing of chemicals: Draft updated Test Guideline 434 on Acute Dermal Toxicity,” *Draft Guideline*, pp. 1–12, 2015.
11. Morton JJP, Malone MH. Evaluation of vulnerary activity by an open wound procedure in rats. *Arch Int Pharmacodyn.* 1972;196:117–26.
12. Diwan PV, Tilloo LD, Kulkarni DR. Influence of *Tridax procumbens* on wound healing. *Indian J Med Res.* 1982;75:460–4.
13. The Indian Pharmacopoeia. 2nd ed. Delhi: Ministry of health, Government of India; 1966.

14. Ehrlich HP, Hunt TK. Effect of cortisone and vitamin A on wound healing. *Ann Surg.* 1968;167:324–8.
15. 16. Lee KH. Studies on mechanism of action of salicylates II Retardation of wound healing by aspirin. *J Pharm Sci.* 1968;57:1042–3.
16. D. Odimegwu, E. Ibezim, C. Esimone, C. Nworu, and F. Okoye, “Wound healing and antibacterial activities of the extract of *Dissotis theifolia* (Melastomataceae) stem formulated in a simple ointment base,” *Journal of Medicinal Plants Research*, vol. 2, no. 1, pp. 11–16, 2008.
17. A. Maria Lysete, R. L. S. H. Bastos, M. Lucia et al., “Antimicrobial and wound healing activities of *Piper hayneanum*,” *Journal of Chemical and Pharmaceutical Research*, vol. 3, no. 4, pp. 213–222, 2011.
18. Wound healing activity ... M. Dibaj, et al *Journal of Medicinal Plants* 112 March 2020, Vol. 19, No. 73: 109-118
19. Küpeli. E., Harput. U.S., Varel. M., Yesilada. E., and Saracoglu. I., “Bioassay-guided isolation of iridoid glucosides with antinociceptive and anti-inflammatory activities from *Veronica anagallis-aquatica* L”. **J. Ethnopharmacol.** 102, 2005, 170–176.
20. Ghosh. M.N., “Fundamental of experimental pharmacology”. 2nd ed. Calcutta, Scientific Book Agency, 1984, 153.
21. Leafwill .F., Charles. K.A., and Mantovani. A., “Smoldering and polarized inflammation in the initiation and promotion of malignant disease cancer cell”, 7, 3, 2005, 211-217.
22. Gene. R.M., Segura. L., Adzet. T., “*Heterotheca inuloides*. Anti-inflammatory and analgesic effects”. **J. Ethnopharmacol.** 60, 1998, 157-162.