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A B S T R A C T

Plumeria acuminata (PA) is known for treating various disorders in folk medicines since a long time. The aim of the study was to explore wound healing activity of PA. The methanol extract of leaves of *Plumeria acuminata* (PAME) was incorporated in simple ointment base B.P. at concentration of 5% (w/w) and 10% (w/w). Wound healing activity was evaluated in excision, wound model, using standard drug 5% (w/w) Betadine to compare the results. The data obtained suggested that PAME has satisfactory wound healing potential in rat models. Both the concentrations 5% (w/w) and 10% (w/w) ointment significantly ($p<0.001$) and ($p<0.001$ reduced wound contraction respectively as well as reduced epithelization period also. The study concluded that *Plumeria acuminata* (PA) has potential to accelerate wound healing process.

***Key words*:** *Plumeria acuminata,* Excision wound, Methanol extract, Epithelization period, Wound contraction

Wound is very common, oldest and unavoidable incidence of daily life. Wounds are produced by physical, chemical, mechanical, microbiological, immunological insults to the tissue and it is one of the important causes for physical disabilities and loss of productive hours of mankind [1]. Human being is born with inbuilt capability of wound healing through continuous repairing and regeneration of tissue. As wounds are of different kinds and every kind of wound is different from others so there is need of individual care to them. Due to this wound healing has been an ancient medical issue. Wound healing is not only the contraction or closure of wound but it should represent quality of scar tissue, restoration of functional and cellular structural for good quality of healing [2]. Wound healing is a major problem in Diabetes mellitus. Most of the diabetic patient face lot of problems when they get an infected wound [3]. Wound care and management include various measures like dressing, use of painkillers, anti-inflammatory agents, corticosteroids, topical and systemic application of antimicrobial agents and other healing agents [4]. Advance development in Pharma sector made availability of various kind of pharmaceutical formulations for promoting wound healing process but due to high cost and undesirable side effects, their use is still a major challenge [5]. From ancient time human being is dependent on plant sources to fulfill health care needs. Through trial-and-error method wound healing potential of various plants has been discovered. Most of the drugs are associated with the isolation of active phytoconstituent present in a particular plant. Since ancient times, plant originated medicines are preferred in the developed world for basic health care due to safety, low cost, easy availability, low undesirable effects and multitasking in nature [6].

Plumeria acuminata belongs to the Apocynaceae family. This plant is an evergreen or partly deciduous, small tree, upto 7 meter in height. It is one of the well-recognized genera of laticiferous trees and shrubs documented in Ayurveda. This pant is widely distributed throughout the southern part of India. They are considered as an ornamental plant and most commonly found in the graveyards although they are popular for fragrance and attractive flowers [7]. *Plumeria acuminata* is very commonly grown species and known as “Temple tree” or “Champa” in common language. Frangipani, Golainchi, Gorur champa, Dalan phul, Kshira champa, Velachampakan, Sonachampa, Radha champa, Kat champa are some other names used in different languages in India. It is recognized as “Kembang kamboja” in Indonesia, “Kalachuchi” in Philipines and Dead man’s fingers in Australia. The plant material is widely used as purgative, remedy for pain, diarrhea and cure for itch. The milky juice is employed for the treatment of inflammation [8]. After thorough literature search *Plumeria acuminata* was selected for the present study on the basis of its traditional use and phytoconstituents present in it to scientifically claimed the wound healing potential of this plant hence there is no any such kind of scientific study is available on *Plumeria acuminate.*

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MATERIALS AND METHODS

Collection and authentication of plant material

The fresh leaves of *Plumeria acuminata* were collected from the surrounding area of C.S.J.M. University, Kanpur. The botanical identification and authentication of plant was done by Scientist S.K. Srivastava, Botanical Survey of India (BSI), Dehradun with authentication No. BSI/NRC Tech/Herb (ident.)/2015-16/301.

Preparation of crude plant extract and phytochemical screening

The leaves of *Plumeria acuminata* was washed, cut into small pieces, air-dried and powered with the mechanical grinder. The powdered material was extracted in a soxhlet extractor, with methanol. The extract was concentrated, in rotary evaporator (Evator Mumbai) under reduced pressure (below 40°C), then concentrated extract was stored in an air tight container and kept in refrigerator for further use. Methanol extract was analyzed for the confirmation of different phytoconstituents through various tests viz alkaloids (Dragendroff's test), flavonoids (Shinoda's test), tannins (5% ferric chloride test), terpenes and steroids (Libermann-Burchard's test), saponins (foam formation), fixed oil (spot test), proteins (ninhydrin test) according to standard methods [9].

Experimental animals

Wistar albino rats (150-210 g) of either sex were taken in the study. The animals were kept under standard laboratory conditions. Animals were caged according to ethical guidelines under standard laboratory conditions, at room temperature 25-30°C with relative humidity 60 to 65% and 12-hours light and dark cycle. Animals were provided free access to rodent diet and water ad libitum. The study was performed according to CPCSEA guidelines, under the Institutional Animal Ethics Committee with approval no IAEC/SHIATS/PA16III/SMTPG02.

Preparation of 5% and 10% ointment

Simple ointment was prepared according to fusion method mentioned in British Pharmacopoeia. To prepare 5% and 10 % w/w ointment, 5 g and 10 g of dried powder of and methanol extract of *Plumeria acuminata* were incorporated into 95 g and 90 g of simple ointment base respectively by crumbling on the surface of ointment slab to get smooth texture and uniform consistency. The preparations were packed in wide-mouth plastic jars with air tight lid [10].

Acute dermal toxicity

The highest concentration of the extract ointment 10% (w/w) was applied thinly and uniformly on shaved back of the rats for a period of 24 hrs. After application of the test substance, cage side observation was performed daily for the next 14 days to notice late development of dermal toxicity [11].

Excision wound model

For the excision wound studies, albino rats were divided into four groups and six animals were placed in each group (n=6). Treatment given to respected groups was as follows:

Group I: Control group given simple ointment base topically

Group II: Standard group treated with 5% w/w Betadine ointment.

Group III: Test group treated with 5% ointment of methanol extract of PA (F1)

Group IV: Test group treated with 10% ointment of methanol extract of PA (F2)

Creation of excision wound

All animals were made unconscious by administering 1ml of ketamine hydrochloride (10 mg/kg b.w. i.p.). With a razor blade their dorsal hairs were shaved. To create an excision wound an area of 500 mm^2 in length and 0.2 cm in depth was created on the shaved region. The wound left exposed to the environment. The animals were kept in separate cages.

Treatment of wounds

The ointments were applied daily on wound of respective groups for 15 days or till the complete epithelization from the initial day of wounding. The percentage wound closures were traced on mm^2 graph paper on the day 0, 3, 6, 9, 12 and 15 for all groups. Epithelization time (days) and size of scar were noted [12].

Wound contraction

The wound contraction is the reduction in wound area with respect to initial wound area. Wound contraction (WC) was calculated as a percentage change in the initial wound size [13].

$$\% \text{ of wound contraction} = \frac{\text{Wound area on day 0} - \text{Wound area on day } n}{\text{Wound area on day 0}} \times 100$$

Histopathological study

After the study period samples of healing skin tissues were taken from each group of rats of excision wound model and burn wound model for the histopathological examination. The sample tissues were kept in 10% neutral buffered formalin embedded in paraffin wax and cut into 5µm thick sections and mounted on slides and stained with hematoxylin and eosin solution and clicked images under 100 or 400x magnification [14].

Statistical analysis

The findings obtained from different activities were analyzed by one-way ANOVA followed by Benferroni post-test. The difference was considered significant when p value <0.05 when compared to control and other groups. All the values were expressed as mean ± standard error mean (S.E.M.).

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Preliminary phytochemical screening confirmed the presence of flavonoids and tannins which possess various pharmacological properties like antioxidant, anti-inflammatory, antimicrobial etc. These properties may have remarkable contribution to accelerate the wound contraction, shorter epithelization duration and reorganization of cellular structure.

Table 1 Preliminary phytochemical screening of the methanol extract of *P. acuminata* leaves

Phytoconstituents	Methanol extract of *P. acuminata*
Alkaloid	--
carbohydrate	+
Protein /amino	--
saponin	--
Flavonoids	+
Tannins	+
Steroids	--

Excision wound model

Wound contraction is the retraction of healing of wounds. Better efficacy of medication depends upon speedy wound

closure. In excision wound model the extract revealed significant wound contraction by enhancing epithelization. During healing process, wound contraction is the reflection of reduction in area of wound. Rate of wound contraction represents the efficacy of drug. The wound healing activity was supported by histopathological study of granuloma tissue of excision wound model. The results of excision wound model are given in (Table 2).

Observations indicated that topical application of ointment at both doses (5% and 10% w/w) had significant **p<0.01 and ***p<0.001 wound healing potential respectively when compared to control group. Wound contraction was expressed in dose dependent manner by plant extract i.e., highest dose 10 % had exhibited 90.11% wound contraction and lowest concentration (5%) showed 84.95% at day 15th post wounding. As per histopathological observations, cellular proliferation, fast epithelization and collegenation were found in dose dependent manner and dose 10% indicated fast wound contraction better cellular reorganization and shorter re-epithelization period.

Table 2 Effect of PAME ointments on reduction in wound area (mm^2) in excision wound model

Group	0 day	3rd day	6th day	9th day	12th day	15th day	Epithilization period (days)
Control	504.11±0.39	484.29±2.78 (3.93%)	396.19±0.63 (21.4%)	361.97±1.16 (28.19%)	274.90±0.68 (45.58%)	186.18±0.56 (63.06%)	25.45±0.27
Standard	506.46±0.44	408.41±0.68^{ns} (19.35%)	321.18±0.97^{ns} (36.58%)	251.47±0.73** (50.34%)	135.19±0.77*** (73.3%)	9.39±0.37*** (98.14%)	19.87±0.23
PAME 5% w/w	508.23±1.08	453.03±1.91^{ns} (10.32%)	386.75±2.03^{ns} (23.56%)	312.21±2.14^{ns} (38.21%)	186.79±1.30* (63.16%)	76.68±1.4** (84.95%)	22.13±0.56
PAME 10%w/w	507.75±1.22	423.17±1.90^{ns} (16.4%)	331.06±1.98^{ns} (34.58%)	272.04±2.43* (46.24%)	153.44±1.75** (69.76%)	50..54±0.43*** (90.117%)	19.45±0.82

Values are expressed ± SEM, ***p<0.001, **p<0.01, *p<0.05, ns=not Significant

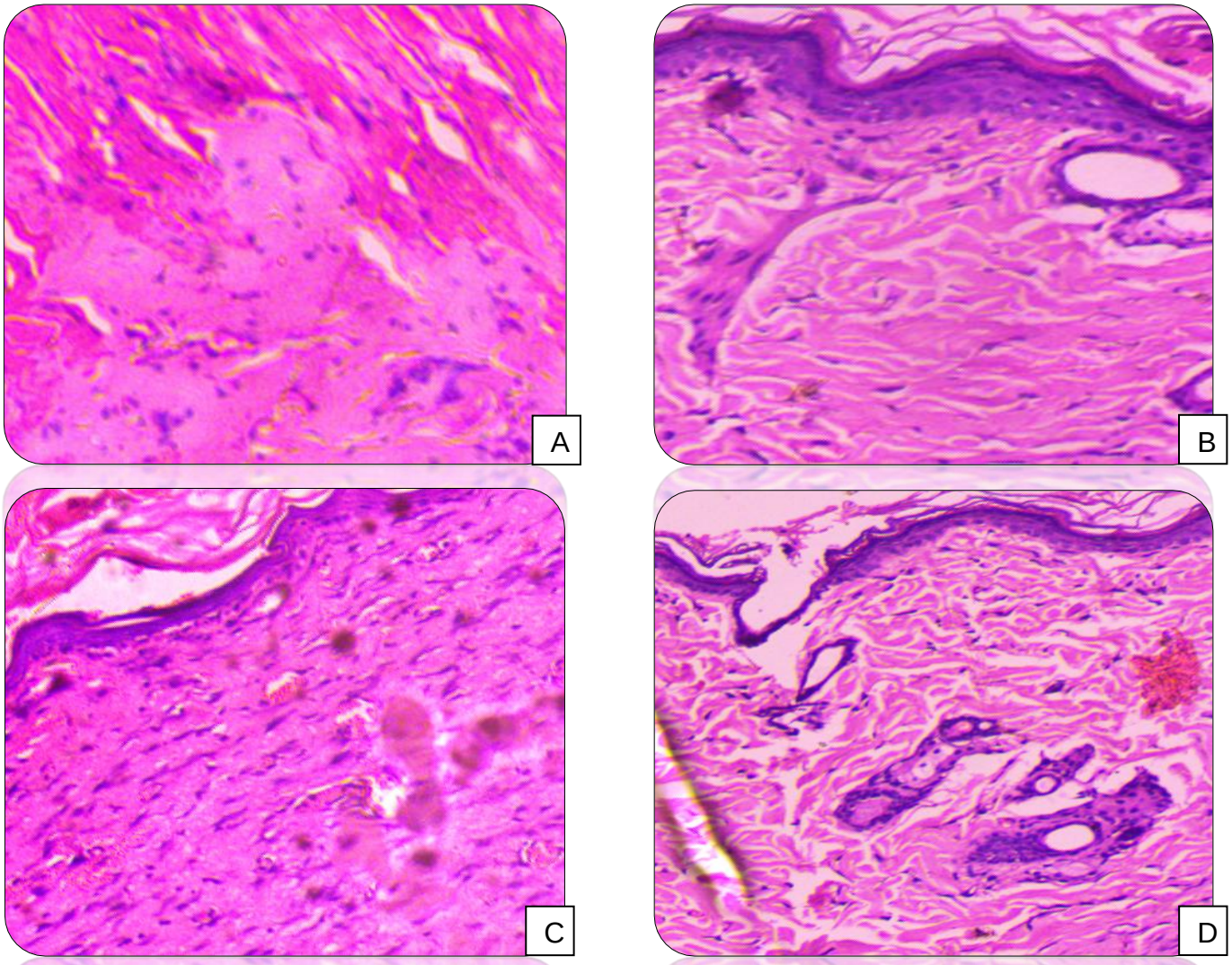
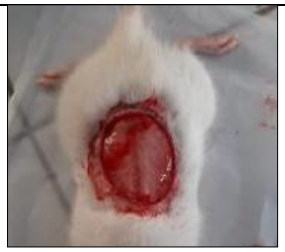
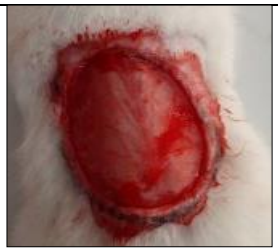
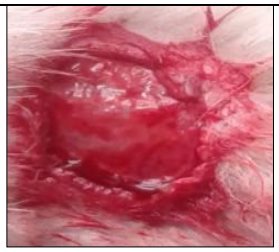
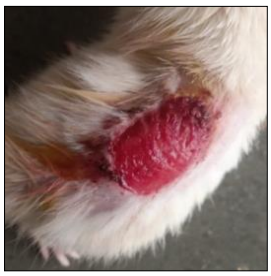
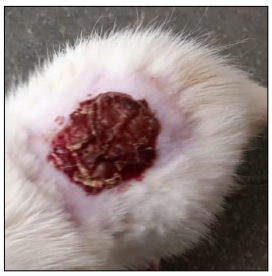
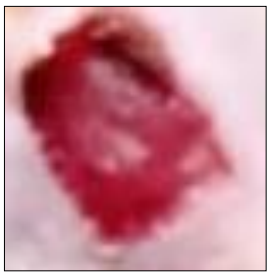
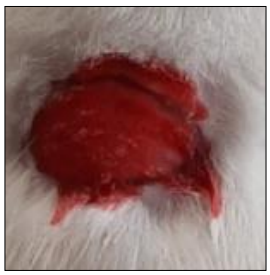
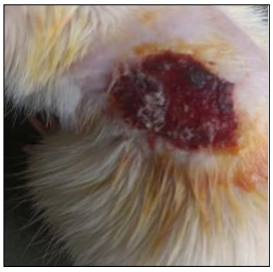
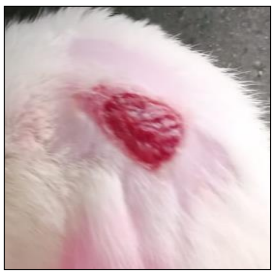
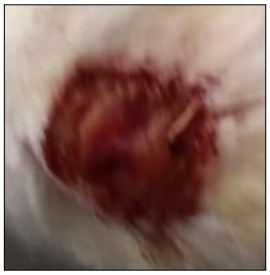
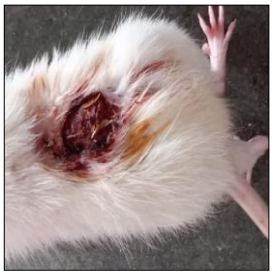
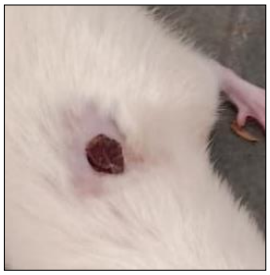
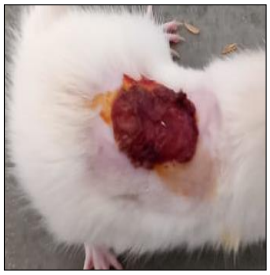
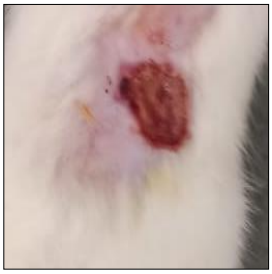
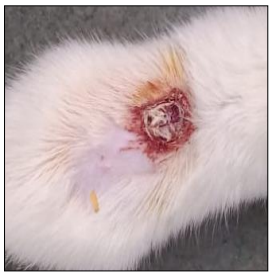
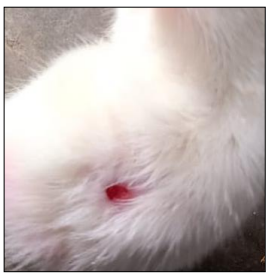
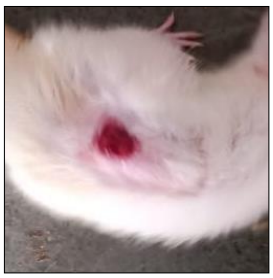
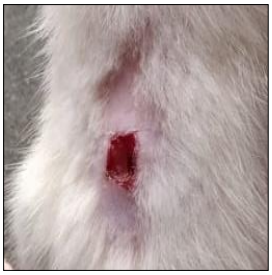
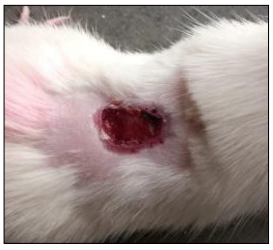
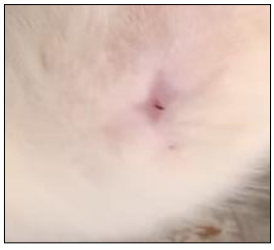
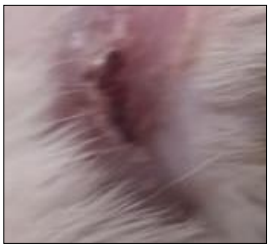
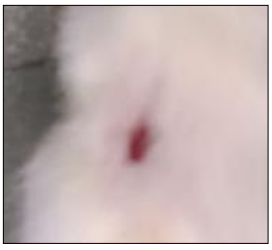


Fig 1 Histopathological features of excision wound model at day 15

Histopathological changes in granulation tissue of excision wound model at day 15 is shown in **(A)** control group showing, no regeneration of epithelial layer, inflammatory cells, mild increase in fibrous issue, loose deposition of collagen. **(B)** Standard treated group showing complete and thick re-epithelization and high number of fibroblasts with minimum inflammatory cells and dense collagen with sebaceous gland and hair follicles and **(C)** F3 PAME 5% treated group showing thick epithelial layer and dense fibrosis and numerous fibroblasts **(D)** F4 PAME 10% treated group showing adnexa with regular organization of collagen with few fibroblasts, blood vessels and fat cells. Some muscles are also observed.

Table 3 Photographic presentation of contraction of wound in excised wound model

Day	Control	Standard	F1(PA 5%)	F2 (PA10%)
0				
3				
6				
9				
12				
15				

CONCLUSION

The present study concluded that methanol extract of leaves of *Plumeria acuminata* have tremendous potential to promote and accelerate the wound healing process. It can be developed scientifically into an ointment which may be available commercially for management of wounds specifically diabetic as well as chronic wound.

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