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Study of Wound healing activity In Dead space and burn woundleaf extract of Aglaia elaeagnoidea

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Abstract

Introduction: The present study provides a scientific evaluation for the wound healing potential of ethanolic (EtOH) extract of Aglaia elaeagnoidea Linn. (AE) plant.

Materials and Methods:dead space and burn 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, in both studies.

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

Conclusion: The ethanolic extract ointment of AE effectively stimulates wound contraction in dead space and burn wound models.

KEY WORDS: dead space and burn wound, *AE* Linn. wound healing

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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 RafflesiaHasseltii. 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:

Aglaia elaeagnoidea, the **droopy leaf** or **priyangu**,belongs to Meliaceae family. It is a 10m tall tree found in American Samoa, Australia, western Australia and queensland),cambodia, india, Indonesia, Malaysia, New caledonia, srilanka, Taiwan, Thailand.

Timber is bright red color is a hard wood Leaf is greyish brown in color. White latex can be exudate. Leaves are compound, imparipinnate, alternate; lamina narrow-elliptic to oblanceolate; apex bluntly acute to subacuminate; base acute to cuneate. Flowers show axillary panicles inflorescence. Fruit is a brown or red, indehiscent globose berry.

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Common names

- English — droopy leaf, priyangu, coastal boodyarra
- Hindi — *priyangu* (प्रियंगू)
- Tamil — *chokkala*, *chokla*
- Malayalam — *nyalei*, *punniyava*, *cheeralam*
- Telugu — *yerraadugu*, *erranduga*, *kondanduga*
- Kannada — *gadagayya*, *kempunola*, *thottilu*, *priyangu*
- Mandarin — *shanluo*
- Sinhala — *puwanga*

Kingdom	:	Plantae
Clade	:	Tracheophytes
Clade	:	Angiosperms
Clade	:	Eudicots
Clade	:	Rosids
Order	:	Sapindales
Family	:	Meliaceae
Genus	:	Aglaia
Species	:	A.elaeagnoidea

Habit: Small tree which grows up to 10 m tall.

Trunk and Leaf: Leaf greyish brown, lenticellate; blaze reddish brown.

Branches and Branchlets: Young branchletsterete, densely lepidote scaly.

Exudates: White latex, not profuse.

Leaves: Leaves compound, imparipinnate, alternate, spiral; rachis 1.5-3.5 cm, slightly canaliculate above, lepidote scaly; leaflets 2-3 opposite or subopposite pairs with one at terminal, 3-7 x 1-3 cm, narrow-elliptic to oblanceolate, apex bluntly acute to subacuminate, sometimes obtuse, base acute to cuneate, chartaceous, young leaves densely lepidote scaly, glabrous when; midrib slightly raised above, secondary nerves 8-13 pairs; tertiary nerves very slender, broadly reticulate.Inflorescence axillary panicles, shorter than leaves, lepidote scaly.

Fruit and seed: Berry, nearly globose, lepidote scaly, to 1.5 cm across, not depressed at apex, 2 loculed; seeds 1 per locule.

Trees, to 15 m high, leaf reddish-brown, smooth; blaze pink; branchlets 3-4 mm thick, covered with brownish scales. Leaves imparipinnate, alternate, estipulate; rachis 60-80 mm long, stout, grooved above, swollen at base, lepidote; leaflets 5-7, opposite, estipellate; petiolule 10-18 mm long, lepidote scales present; leaflets 6-10 x 3-4.5 cm, elliptic, elliptic-obovate, lanceolate or oblanceolate, base oblique, acute or attenuate, apex acute, acuminate or caudate-acuminate, margin entire, chartaceous, foveolate above, lepidote above and beneath; lateral nerves 5-12 pairs, parallel, prominent, secondary laterals present, intercostae obscure. Flowers polygamodioecious, yellow in axillary branching panicles; calyx 5 lobed campanulate, scaly,

margins ciliate; petals 5, free, imbricate; staminal tube entire at apex; anthers 5, included; ovary small, superior, slightly depressed, 1-2-celled, ovules 1-2 in each cell. Fruit a berry, globose, 1-15. cm across, buff coloured; seeds 1-2.

Cultivation Details: A plant of the lowland tropics, preferring non-equatorial areas with seasonal climates. . It is intolerant of frosts Grows well in dappled shade. Prefers a moist, humus-rich soil, which must be well-drained. The flowers are strongly fragrant, both male and female forms need to be grown if fruit and seed are required.

Edible Uses: Fruit – raw, An acid flavour. The seed is covered with a thin, white, gelatinous flesh (aril) that is sweet and tasty.The fruit is up to 20mm long and 15mm wide, containing up to 2 seeds.

Plant Material

The whole plant of *AE* 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 *AE* 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 AE.

Preparation of Formulation and Standard Used

Simple ointment was prepared from the 10% ethanolic extract of AE 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. Povidinewas 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 ***Aglaiaelaeagnoidea*** in a ceramic mortar and pestleto 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, cetostearyl 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 melting[7-9].

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 ***Aglaiaelaeagnoide.***

Experimental design

Table 1: For Experimental Design of *Aglaiaelaeagnoidea*

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%*Ae*extract ointment	Topical	6
Group 4	2 %*Ae*extractointment	Topical	6

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

Dead Space Wound Model[10,11]: 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 Model[12] **:** 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 AE 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 $P< 0.01$.

Results

Preliminary Phytochemical:

The results of the preliminary phytochemical screening of the ethanolicextracts reveals the presence of phytoconstituents such as alkaloids, carbohydrates, glycosides, phytosterol, saponins, tannins, Proteins, flavonoids and diterpenes.

Table No.2. Preliminary Phytochemical screening of *Aglaiaelaeagnoidea*

S.No	Phytocontituents	MEAE
1	Alkaloids	Present
2	Carbohydrates	Present
3	Glycosides	Present
4	Phytosterol	Present
5	Saponins	Present
6	Tannins	Present

7	Proteins and free mino acids	Present
8	Flavonoids	Present
9	Diterpenes	Present

***Acute toxicity* studies**

In the acute toxicity studies the extracts of MEAE did not showed any toxic symptoms or mortality up to the dose level of 5000mg/kg, body weight in rats, and hence the extracts was considered to be safe and non toxic for further pharmacological screening.

Table No.3. Acute toxicity studies of leaves *Aglaiaelaeagnoidea*

S.No	Treatment	Signs of toxicity	Onset of toxicity	Weight variation	Duration of observation
1	MEAE (2000mg/kg)	observed	After 20 hrs	5g	14 days
2	MEAE (3000mg/kg)	observed	After 20 hrs	5g	14 days
3	MEAE (4000mg/kg)	observed	After 20 hrs	5-10g	Till animals are alive

Wound healing activity:

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 ofethanolic leaf extract treated animal groups, moderate collagen deposition, macrophages and fibroblastswere 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 4: 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 No:5: Wound healing percentage in experimental groups by burn wound healing model

Groups	**14th day**
Control	56 ± 0.035
Standard	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 *AE* 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*Aglaiaelaeagnoidea* 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*Aglaiaelaeagnoidea* 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 ointment[13].

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 efficient[14].

In excision wound healing model, the crude extract (80% methanol) of the leaves of*Aglaiaelaeagnoidea* 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*Aglaiaelaeagnoidea* 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[15,16]. 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*Aglaiaelaeagnoidea* 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*Aglaiaelaeagnoidea* leaves which may possess antioxidant, free radical scavenging properties and promote cell proliferating properties. To mention some, a study on the leaf extracts of*Aglaiaelaeagnoidea* 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 AE 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.

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