PRELIMINARY PHYTOCHEMICAL ANALYSIS AND WOUND HEALING ACTIVITY OF VARIOUS EXTRACTS OF THE FRONTAL LEAVES OF *TECTONA GRANDIS*.

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Summary

Wound healing activity of different extracts of *Tectona grandis* on excision wound, incision and burn wound models was evaluated using Sprague dwaly rats. The activity was assessed by the rate of wound contraction and the period of epithelization in excision and burn wound model while tensile strength was the parameter in incision wound model. The extracts were formulated using different hydrophilic and hydrophobic bases. The results revealed that the polar extracts i.e. methanolic and aqueous extracts in hydrophobic bases showed significant activity when compared to the control and the other nonpolar extracts i.e. petroleum; ethyl acetate and chloroform extracts did not show significant activity.However the rate of contraction was slightly faster in the aqueous extract when compared to the methanolic extract. It was also noted that the emulsifying ointment showed a better activity than the paraffin ointment.

Key words: *Tectona grandis*, wound healing, excision wound.

Introduction

Tectona grandis. (F.Verbinaceae) is a deciduous tree widely distributed in India, Burma, and Myanmar. It is a tree with an erect trunk. It is commonly called as teak. The wood has been reported to be used as a cooling agent, laxative and sedative, flowers are used in bronchitis, as diuretic and in the treatment of urinary discharge, juice is used in the treatment of the common cold and headache , oil is used in the treatment of hair problems and in scabies [1,2], Juglone isolated from the methanolic extract has been reported to posses anti-microbial activity[3], Betulin aldehyde

isolated from the bark has been reported to posses anti tumor activity[4], Lapchol a nitroquinone derivative was reported to posses anti ulcerogenic activity[5]. We had earlier demonstrated that the hydroalcoholic extract of *Tectona grandis* leaves posseses wound healing activity in different wound model[6].The present work was carried out to evaluate the effect of different extracts of *Tectona grandis* on healing of excision wounds, incision and burn wound models in rats and also to find the most potent extract and the best base for the formulation of the extract.

Materials and methods

Plant material

The frontal leaves of *Tectona grandis* were collected from the rural areas of Bangalore in the month of October 2006. The plant was identified and authenticated by the Regional Research Institute, Bangalore where the specimen voucher (RRCBI Acc no 12474) has been deposited for future reference. The material was shade dried, pulverized and preserved in air tight containers until further use.

Preparation of the extracts

The dried powder (1 kg) was extracted successively with various solvents .i.e. Petroleum ether, Chloroform, Ethyl acetate and Methanol in a Soxhlet apparatus. The aqueous extract was prepared by macerating the residue of successive extraction in distilled water. The extracts were vacuum dried and the percentage yields calculated.

The extracts were subjected to preliminary phytochemical analysis [7] and majority of the constituents were found to be polar in nature.

Selection of animals

Healthy Sprague dwaly rats of either sex weighing 200-250 gm were selected. The animals were maintained under standard conditions and were fed with commercial diet and water ad libitum during the experiment. The Institutional Animal Ethical Committee (No Krp/IAEC-27/2006) approved the experimental protocol and the guidelines for the animal care were strictly adhered to during the experimentation as recommended by committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt of India.

Drug formulation

The extracts were formulated using different bases. The non polar extracts i.e. petroleum ether(PE), chloroform(CH), and ethyl acetate(EA) were formulated as gels using hydrophilic bases i.e. hydroxy propyl methyl cellulose (HPMC),carboxy methyl cellulose (CMC) and sodium alginate (SA) and the polar extracts i.e. methanol (ME) and aqueous extracts (AQ) were formulated in paraffin ointment (PO) and emulsifying ointments (EO)[8]

Wound healing activity

Excision wound, incision wound and burn wound models[9] were used for the assessment of wound healing activity

Excision wound model

The animals were anesthetized using ether and an impression was made on the dorsal thoracic region 1cm away from the vertebral column and 5cm away from the ear. The skin was excised to the full thickness to obtain a wound area of about 500mm^2. Haemostasis was achieved by blotting the wound with a cotton swab soaked in normal saline. The animals were grouped and the formulations were applied topically once a day. Groups 1,2,3,4 and 5 were used as controls for HPMC, CMC, SA, PO base and EO base respectively. Group 6 was treated with standard i.e. nitrofurazone(0.2% w/w ointment) , groups 7,8, and 9 were treated with formulations of petroleum extract (i.e. PE+HPMC ,PE+CMC,PE+SA), groups 10,11,and 12 were used for chloroform extract (i.e.CH+HPMC ,CH+CMC , CH+SA) , groups 13,14,and15 were treated with ethyl acetate extract formulations (i.e. EA+HPMC ,EA+CMC , EA+SA), groups16 and 17 were used for methanolic extract formulations (ME+PO and ME+EO) and groups 18 and 19 were used for formulations prepared with aqueous extract (AE+EO and AE+PO).

The wound area was measured by tracing the wound on a millimeter scale graph paper. The % of wound was calculated of the original wound size (500mm^2) for each of the animal groups on predetermined days i.e. 2,4,6,8,10,12,14,16,18,20, and 22 days post wounding for final analysis of the results. Complete healing i.e. no leaving of the wound was considered as the end point of complete epithelization and the days required for this was taken as the period of epithelization.The parameters measured were wound area, wound contraction and period of epithelization.

Incision wound model

In the incision wound model, the rats were anesthetized using ketamine.Para vertebral incisions of 6 cm length were made through the entire thickness of the skin. The wound was closed by means of interrupted sutures placed 1 cm apart. The animals were divided into 6 groups of 6 animals each.ie ME+PO,ME+EO,AE+EO,AE+PO,PO control and EO control. The sutures were removed on the 7th post wounding day. Animals were treated daily with ointments from 0 day to 10th post wounding day. The wound strength was estimated on 10th day by continuous, constant water flow technique.

Burn wound model

The animals were anesthetized as mentioned earlier and grouped as in the incision model. Partial thickness burn wounds were inflected by pouring hot molten wax at 80^0 C. The wax was poured on the shaven back of the animal through a cylinder of

300 mm^2 circular opening. The wax was allowed to remain on the skin till it solidified.Immediatly after injury and on subsequent days, the ointments were applied as mentioned above in the incision wound model.

Skin irritation study

The skin irritation study was evaluated using rabbits[10].Healthy rabbit was selected and was shaved in three different areas of the dorsal side, each about 500mm^2.The rabbit was kept in rabbit holder and the 1st area was kept as a control, to which paraffin ointment base was applied.2 nd area was applied with the ME in PO base and the 3rd area was treated with the AQ in PO base. After 4 hrs the skin was observed and compared with the control .The same was repeated using ME in EO and AQ in EO

Statistical analysis

Results were expressed as mean ±SEM.The difference between experimental groups were compared by one way analysis of variance (ANOVA) followed by Dunnet test $P<0.05$ was considered significant

Results

The wound healing activity of the non polar and polar extracts was evaluated using excision, incision and burn wound models. The activity was assessed by the rate of wound contraction, the period of epithelization and tensile strength.

Effect on excision wound

The extracts obtained with non polar solvents - PE, CH and EA did not show any significant effect on wound contraction – 50 % and the falling of scar. The wound contraction-50 % and days required for falling of scar were significantly reduced by both ME and AE in paraffin and emulsifying ointment bases when compared to the controls i.e. paraffin and emulsifying ointment bases.100 % wound closure was obtained in the methanolic and the aqueous extracts by the 12th day and 10th day respectively. Since the non polar extracts did not show any significant effect on the wound healing they were not used for the incision and the burn wound models.

Table 1: Effect on wound contraction-50 % and falling of scar in excision wound model.

Treatment	Wound Contraction-50%	Falling of scar
HPMC control	8.00 ± 0.89	17.50 ± 1.87
HPMC PE	7.17 ± 0.75	16.50 ± 1.05
HPMC CH	7.83 ± 0.75	17.66 ± 1.75
HPMC EA	7.83 ± 0.75	17.83 ± 1.94
CMC Control	11.00 ± 0.58	19.83 ± 0.48
CMC PE	10.50 ± 0.43	18.60 ± 0.67
CMC CH	10.16 ± 0.48	18.16 ± 0.48
CMC EA	11.16 ± 0.48	17.16 ± 0.60
SA Control	7.67 ± 0.49	18.66 ± 0.49
SA PE	8.33 ± 0.49	18.33 ± 0.49
SA CH	8.50 ± 0.56	18.50 ± 0.56
SA EA	8.83 ± 0.60	18.33 ± 0.99
Nitrofurazone	5.60 ± 0.40	15.20 ± 0.49
EO Control	7.60 ± 6.00	18.80 ± 4.90
EO ME	4.60 ± 0.25**	12.00 ± 0.00**
EO AQ	4.40 ± 0.25**	10.40 ± 0.40**
PO Control	6.40 ± 0.60	17.20 ± 0.49
PO ME	4.40 ± 0.51*	12.40 ± 0.40**
PO AQ	4.20 ± 0.49*	10.40± 0.40**

All values are mean ± SEM, n=5-6, *P<0.05 indicates significant and **P<0.001 indicates extremely significant when compared with respective control.

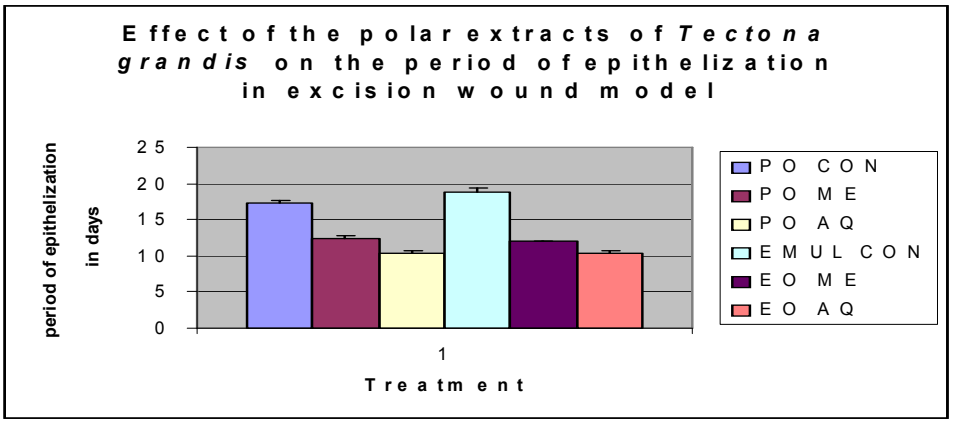

Fig 1: Effect of the polar extract of *Tectona grandis* on period of epithelization in excision wound model

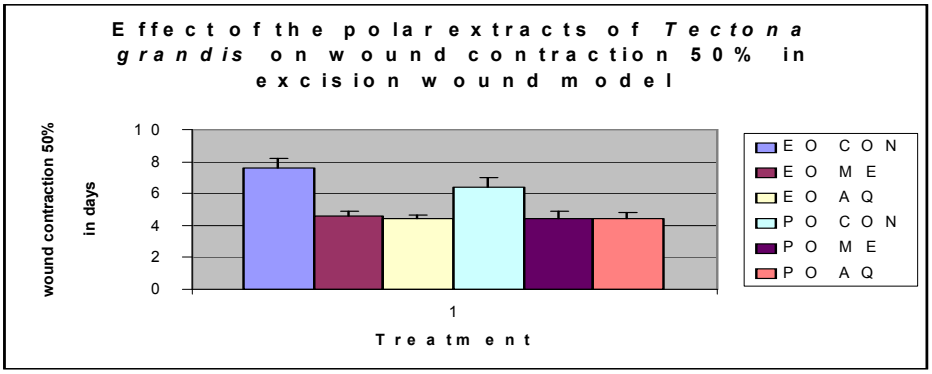

Fig 2: Effect of the polar extract of *Tectona grandis* on wound contraction 50% in excision wound model

Effect on incision wound model

In the incision wound model, all the treatments produced a significant increase ($P<0.001$) in the breaking strength i.e. tensile strength of the wound when compared to the controls. The aqueous and the methanolic extracts showed better activity in the emulsifying base when compared to the paraffin base.

Table 2:.Effect of polar extracts of *Tectona grandis* on tensile strength in Incision wound model

Treatment	Tensile strength
EO Control	423.33±15.84
EO ME	733.33±13.33**
EO AQ	758.33±21.90**
PO Control	488.33±11.37
PO ME	693.50±30.29**
PO AQ	698.00±24.22**

All values are mean ± SEM, n=5-6, **p<0.001 indicates extremely significant.

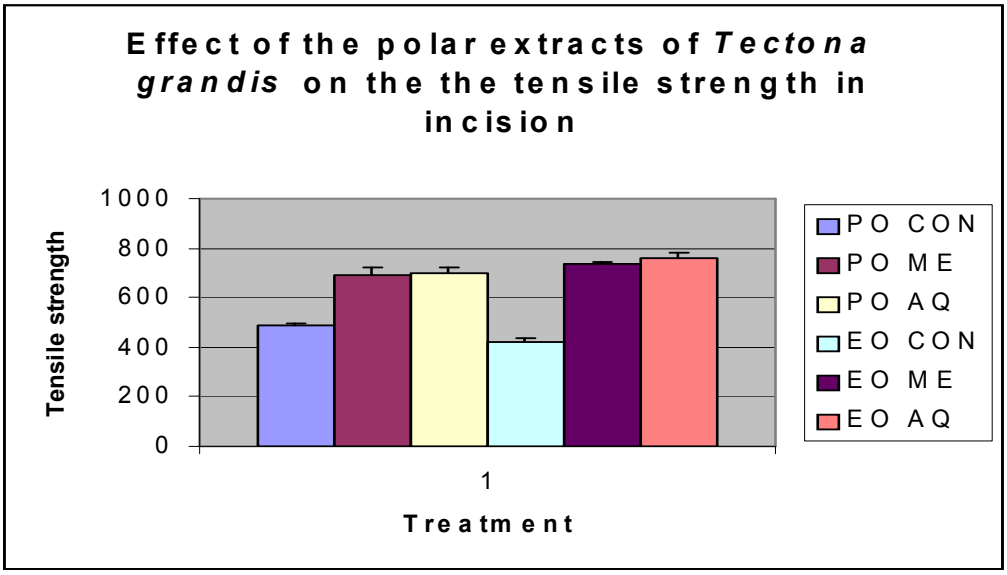

Fig 3: Effect of the polar extracts of *Tectona grandis* on the tensile strength in the incision wound model

Effect on burn wound model

Topical application of the aqueous and the methanolic extracts of *Tectona grandis* in both the emulsifying base as well as paraffin ointment base shortened the period of epithelization significantly($P<0.001$) and also produced significant decrease in the wound contraction($P<0.001$) when compared to the controls i.e. paraffin and emulsifying bases respectively. However similar to the results of the incision wound model, the extracts in the emulsifying base showed better activity when compared to the extracts in the paraffin bases.

Table 4: Effect of *Tectona grandis* on period of epithelization and wound contraction in burn wound model

Treatment	wound contraction 50% (days)	period of epithelization (days)
EO Control	9.00±0.36	19.00±0.44
EO ME	5.30±0.21	11.33 ±0.42**
EO AQ	5.50±0.22	10.66±0.42**
PO Control	8.50±0.34	17.33±0.33
PO ME	5.80±0.30	12.66 ±0.42**
PO AQ	5.50±0.22	11.00±0.44**

All values are mean ± SEM, n=5-6, ** $p<0.001$ indicates extremely significant when compared with respective control.

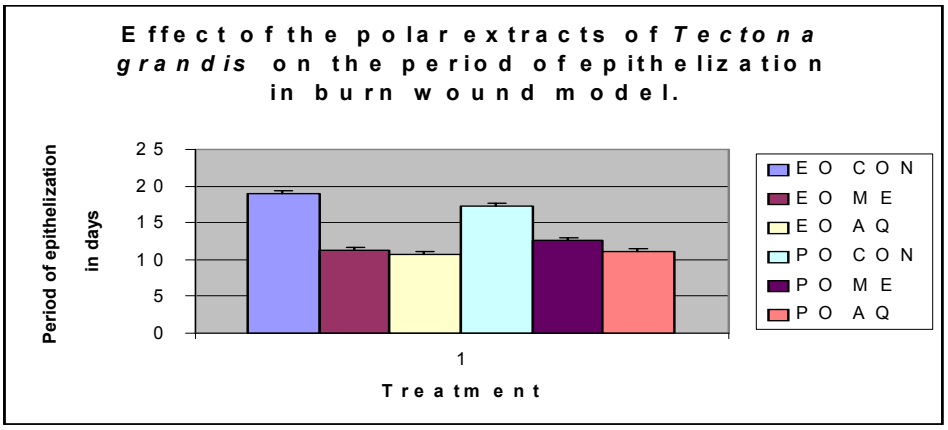

Fig 4: Effect of the polar extracts of *Tectona grandis* on the period of epithelization in the burn wound model.

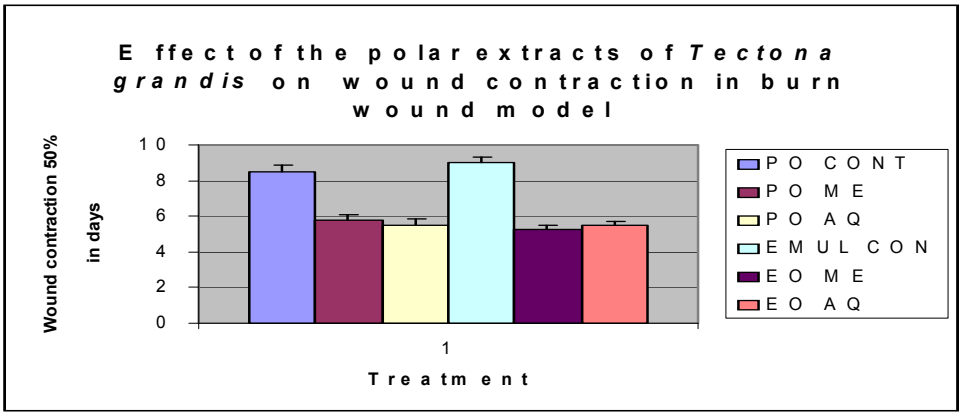

Fig 5: Effect of the polar extracts of *Tectona grandis* on the wound contraction 50 % in the burn wound model.

Skin irritation study

Both the ointment bases containing the ME and AQ extracts did not show any severe type of irritation and there was no evidence of any noticeable inflammation .However a slight redness was observed in the case of the ointments prepared with the paraffin bases. This result further substantiates the results obtained in the incision and the burn wound model, indicating that the emulsifying base is a better base when compared to the paraffin base for both the AQ and the ME extracts.

Table 4: Effect of the polar extracts of *Tectona grandis* on the skin irritation study

Group	Sign	Score
PO control	No noticeable inflammation, but slight redness	0.5
PO ME	No noticeable inflammation, but slight redness	0.5
PO AQ	No noticeable inflammation, but slight redness	0.5
EO control	No change	0.0
EO ME	No change	0.0
EO AQ	No change	0.0

Discussion

Tectona grandis is commonly referred to as teak and is mainly used as timber. There are very few reports available on the chemical constituents and pharmacological effects of this plant. Traditionally, it is used for the treatment of wounds in Mangalore district of Karnataka state in India. As mentioned earlier, the plant is reported to possess antimicrobial, antitumor, antiulcer activity and is also reported to be beneficial in treatment of bronchitis and common cold.

Wound healing is a complex process by which damaged tissue is restored as close as possible to its normal state. It comprises of different phases such as contraction, epithelization, granulation and collagenation[11]. The contraction of the wound depends on the reparative abilities of the tissue, type and the extent of damage. Collagen is a protein of the extra cellular matrix that provides support as it is the main component of the fibrous tissues and contributes to the wound strength.

The wound healing activity of the various extracts of the frontal leaves of *Tectona grandis* was assessed by the rate of wound contraction, the period of epithelization and tensile strength. The extracts were formulated using different hydrophilic bases for nonpolar extract and hydrophobic bases for polar extracts. These bases do not retain the constituents of the extracts[8]. The results revealed that the ME and AE in hydrophobic bases showed significant activity when compared to the control and the other extracts did not show significant activity.However the rate of contraction was faster in the AE when compared to the ME. It was also noted that the extracts in the EO showed slightly better activity than the PO.

The results indicate that the wound healing activity is due to the polar constituent(s) as none of the nonpolar extracts showed any significant wound healing effect and EO is the best base for the release of active constituent(s) from polar extract. The skin irritation study further confirmed that the emulsifying base was a better base for formulation of the extracts than the paraffin base as slight redness was noticed with the extracts in paraffin base. The present study was carried out to identify the best extract and the best base for formulation of the extracts. Further studies are being undertaken to identify the active constituent(s) that may be responsible for the wound healing activity, which may lead to be a promising agent for wound healing.
The preliminary phytochemical analysis revealed the presence of tannins, anthraquinones, saponins, sterols, carbohydrates and proteins in both the aqueous and the methanolic extracts. It has been reported that tannins promote wound healing activity through several mechanisms that includes chelation of free radicals, scavenging of reactive oxygen species, astringent property and antimicrobial activity[12], which seem to be one of the factors responsible for the wound contraction and increased rate of epithelization.

Conclusion

The results of this work give scientific support to the folkloric accounts to the use of the frontal leaves of *Tectona grandis* in the treatment of wounds. It has also been concluded that the aqueous and the methanolic extracts have significant wound healing activity and that the best base is emulsifying base. Isolation of the phytoconstituents is being done identify the active constituent(s) responsible for wound healing activity. This supports the results of our previous work which reports the wound healing activity of the hydro alcoholic extract of the leaves of *Tectona grandis* which was comparable to the marketed *Aloe vera* gel formulation.

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