

PHYTOCHEMICAL INVESTIGATION AND EVALUATION OF WOUND HEALING POTENTIAL OF CRUDE LEAVE EXTRACTS OF DALBERGIA SISSOO (ROXB.), IN WISTAR RATS

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ABSTRACT

The present study was an attempt to investigate the phytochemicals and wound healing potential in two different types of wound models in albino rats viz., incision and excision of various extracts of Dalbergia sissoo leave. The different crude extracts such as ethanol, ethyl acetate, n-butanol and petroleum ether are obtained by successive soxhlet extraction. The ethanolic extract showed significant increase in wound contraction and formation of scar in excision wound model. The extract showed significant increase in the breaking strength of resutured incision wound as compared to control group ($p < 0.05$). The result of the present study indicate that ethanolic extract of D. sissoo

has more significant wound healing property than the other three extracts in excision and incision wound model. The literature survey reveals that there are no reports on the wound healing activity of the leaf extracts of Dalbergia sissoo. This prompted the authors to undergo the present study.

KEYWORDS: Dalbergia sissoo; Excision and incision wound model.

INTRODUCTION

In tribal areas different plant drugs used to treat various skin disorders including wound. Some medicinal plants have been employed in folk medicine for wound care. Wound may often possess in problems in clinical practices. Even no synthetic drug has been attributed in market for direct healing of wounds. WHO has been promoting traditional medicine as a source of less expensive, especially in developing countries and also recognized the traditional medicine.^[1] The tribal areas of Baipariguda, Koraput (District) of Eastern Orissa. due to its unique varieties geographical and climatic factors has had a rich variety of

medicinal plant. *Dalbergia sissoo* (family: fabaceae.) also known as sisu (Oriya) is frequently distributed. And extensively used traditionally by the tribal people. The plant species are found generally in many tropical areas of the globe, particularly Africa, Asia, central and southern America where they are used to manage a number of ailments.^[2,3,4] Some *Dalbergia* species have been investigated and found to possess antimicrobial, antioxidant, anti-inflammatory and anti-diarrhoeal activities.^[5,6,7,8] Traditionally Different parts such as roots, bark, wood, leaves and seeds are being used as remedy in many diseases including skin diseases, blood diseases, syphilis, stomach problems, dysentery, nausea, eye and nose disorders, aphrodisiac, expectorant. Leaf extract has been used to treat sore throats, heart problems, dysentery, syphilis, and gonorrhoea. In India and Nepal rural people use *Dalbergia sissoo* leaves to treat animals suffering from non-specific diarrhoea.^[9] Chemically leaves contain sissotrin and an isoflavon-O-glycoside all so reported.^[10]

MATERIALS AND METHODS

Collection of Plant Material

The leaves of *Dalbergia sissoo* were collected from the tribal belts of the local area of Patrapur of Koraput district.(India) in the month of October 2015. The plant was identified, confirmed and authenticated by the Biju Patnaik Medicinal Plants Garden and Research Centre, Dr. M. S. Swami Nathan Research Foundation, Jeypore, Koraput (District), Orissa (Letter No. MJ/SS/P-207/15, dated 10.5.2015). After authentication leaves were collected in bulk and washed under running tap water to remove adhering dirt. Then leaves were shade dried. The dried materials were made into coarse powder and stored in a closed air tight container for further use.

Preparation of Extracts

The coarse powder was taken in Soxhlet apparatus and extracted successively with ethanol, ethyl acetate, n-butanol and petroleum ether as solvent. A total amount of 650 g coarse powder was extracted with 1000 ml of each solvent. For each solvent, 10 cycles were run to obtain thick slurry. Each slurry was then concentrated under reduced pressure to obtain crude extract. All crude extracts were kept in closed air tight containers under cool and dark place for further study.^[11,12,13]

Phytochemical Investigation

The crude ethanol, ethyl acetate, n-butanol and petroleum ether extracts of the leaf of *Dalbergia sissoo* were subjected to preliminary phytochemical analysis in order to detect the presence of various groups of phytoconstituents by carrying out the chemical analysis.^[12,13]

Animals

Healthy adult wistar strain of albino rats weighing approximately 180 to 250gms were used. They were housed in standard conditions of temperature (25 ± 2 °C), 12 hours light per day cycle, relative humidity of 45-55 % in animal house of Jeypore College of Pharmacy. They were fed with standard pellets of food and water. Animals were kept and all operation on animals was done in aseptic condition. All the studies conducted were approved by the Institutional Animal Ethical Committee (1200/ac/08/CPCSEA), Dadhichi college of pharmacy, Vidya vihar, Cuttack, according to prescribed guide-lines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

WOUND HEALING ACTIVITY WOUND MODEL

Excision wound

For the excision wound study, animals were divided into 5 groups of six rats in each group. Group-I served as control and applied with Vaseline, Group-II, Group-III, Group-IV and Group-V were treated with ethanol, ethyl acetate, n-butanol and petroleum ether extracts respectively. An impression was made on the dorsal thoracic central region 5mm away from the ears, by using a round seal of 2.5 cm diameter as described by Morton and Malone [14]. The skin of the impressed area was excised to the full thickness to obtain an area of about 500 mm² under light ether anaesthesia in aseptic condition. The animals were housed individually. The methanol extracts in simple ointment base (5% w/w) were applied on the wound once a day for 18 days starting from the day of wounding. The percentage wound closure was observed on 4th, 8th, 12th, 16th, 18th post wounding day. Epithelization time (in days) and size of the scar area was noted.

Incision Wound

Incision wound model was performed according to Ehrlich and Hunt.^[15] The animals were divided into 5 groups of six rats in each group, and kept in separate cage. Group-I served as control, received only 2% gum acacia suspension (1 ml/kg, p.o), ethanol, ethyl acetate, n-butanol and petroleum ether extracts (250 mg/kg) were given orally once a day to group-II,

III, IV and V respectively for 10 days. Under light ether anesthesia, the animals were secured to operation table in its natural position. Two paravertebral straight incisions of 6 cm each were made through the entire thickness of the skin, on either side of the vertebral column with help of sharp blade. Removal of the sutures was done on 8th post wounding day. Tensile strength was determined on both wounds by continuous constant water flow technique of Lee.^[16]

STATISTICAL ANALYSIS

The results are reported as Mean±SE. Statistical analysis was done using ANOVA (Tukey-Multiple Comparison Test). When probability (p) was less than 0.05 was considered as significant.^[17]

RESULT AND DISCUSSION

The preliminary phytochemical screening showed that the different solvent extracts of *D. sissoo* contain the alkaloids, flavonoids, terpenoids, saponins, phenols, steroids and tannins were present in all the solvent extract & carbohydrates absent. In the study using excision wound model, animals treated with ethanol extract of *D. sissoo* leaf showed significant decrease in epithelization period as evidenced by shorter period for fall of eschar as compared to control group ($p < 0.05$) Fig-1. The extract also facilitated the increase in rate of wound contraction than control group. The petroleum ether extract treated animal (Group-II) showed wound contraction by 71.18%. The ethyl acetate extract treated animals (Group-III) showed wound contraction by 77.33%. The n-butanol extract treated animal (Group-IV) showed wound contraction by 82.43%, The ethanol extract treated animal (Group-V) showed wound contraction by 86.23% as compared with the control (Group-I) by 61.26%. In all the extract. Fig-2 the result of present study reveals that ethanolic leaf extracts of *Dalbergia sissoo* possess a prominent prohealing activity in incision wound model. This was demonstrated by significant increase in the skin tensile strength in methanol extract treated groups ($p < 0.05$) on 10th post wounding day are presented in Table-1.

Table 1: Phytochemical screening for the different solvent extracts of *Dalbergia sissoo* leaves.

Extracts	Phytochemicals						
	glycoside	Flavonoids	Steroids	Terpinoids	Tannins	Saponins	Phenols
Cadiac							
Ethanol	+++	--	++	++	+++	-	++
Ethyl-acetate	+	--	-	+	+	-	+

n-butanol	++	--	++	+	++	-	++
Petroleum ether	++	--	++	+	+	--	+

 +++, strong; ++, moderately; +, poor presence, --, absence

Table-1: Effect of extracts of Dalbergia sissoo leave on the breaking strength in incision wound

Sl no.	Group	Breaking strength
1	Control	276.17 ± 21.06
2	petroleum ether	327.14 ± 12.26
3	ethyl acetate	337.22 ± 13.72
4	n-butanol	357.17 ± 16.32
5	ethanol	438.83 ± 17.32*

Values are mean ± SE (n=6)* p<0.05 vs control

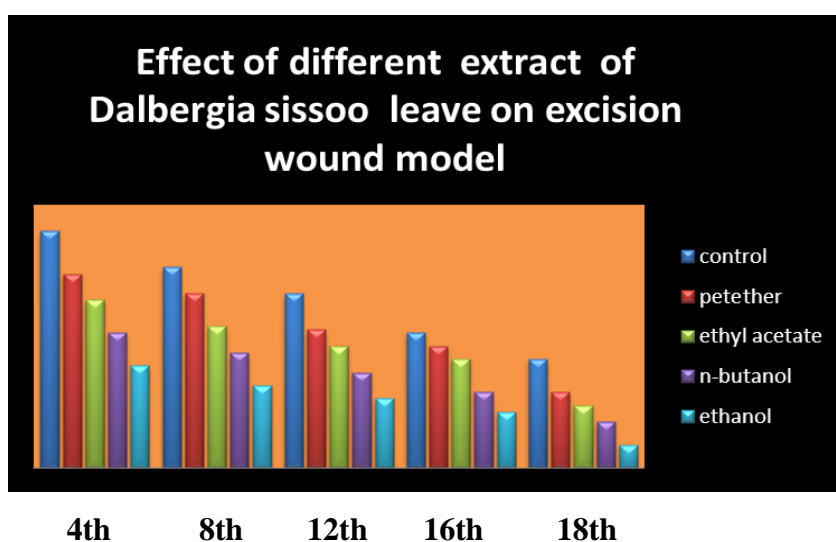


Figure-1 (wound size mm²)

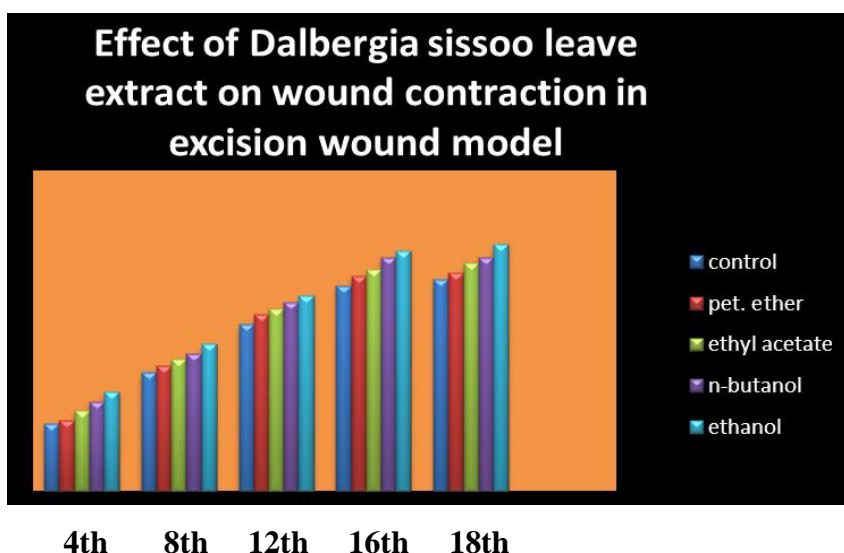


Figure-2 (% of wound closure)

CONCLUSION

In the present study, wound healing activity of *Dalbergia sissoo* was studied and the results of the present study suggest that local application and systemic administration of ethanol extract of the leaf has shown more significant wound healing activity in excision and incision wound models and support the popular use of plant to open wound in folk medicine. The wound healing property of *D.sissoo* has been attributed to its antimicrobial effects. The presence of phytoconstituents like cardiac glycoside, terpenoids, phenols, steroids and tannins either individually or combined together may exhibit the synergistic effect towards healing of wounds. However, further investigation employing isolation of constituents and screening models are needed for further confirmation of wound healing potential of *Dalbergia sissoo* leave.

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