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Phytochemical study and Evaluation of wound healing potential of *Oroxylum indicum* leaves

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ABSTRACT: Backgrounds: The traditional information revealed that the *Oroxylum indicum* possessed potential values of stomachic, wound healing, anthelminthic, antiulcer, expectorant and anti-rheumatic. **Aim:** The present study was carried out to evaluate the wound healing potential of different extracts of *Oroxylum indicum* leaves. **Methods:** The crude extract of leaves of O. indicum was extracted by Soxhlation using petroleum ether, chloroform and ethanol as solvents. The various extracts were evaluated for wound healing activity incision and excision method, using rat as animal model. The wound healing activity of extracts was compared with the povidone iodine ointment, which was used as standard drug. **Result:** All the ethanolic leave extract of *O. indicum* showed wound healing activity, which is well comparable with standard drug (Iodine ointment). Among all the extract, the ethanolic extract at a dose of 250 mg/kg, possesses 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 that is saline water. **Conclusion:** It could be concluded that the ethanolic extract of *O. indicum* leaves showed significant wound healing property.

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INTRODUCTIONS:

Herbal medicines are acting as tremendous role of standard healthcare. About 80 % of people are depending on herbal medicines for primary health care aspect, as per the data given by the World Health Organization [1]. Wound healing is a complex process dynamic in nature, which results in the restoration of anatomic continuity and function. Pathologically wound is the sequential events of hemostasis, inflammation, proliferation, maturation and remodeling [2,3]. Literature revealed that various Ayurvedic herbal plants have a very important role in the treatment of wound. Plants are more potent healers because they promote the repair

mechanisms in the natural way. The tribal areas of Baipariguda, Koraput (District) of Eastern Odisha, due to its unique varieties geographical and climatic factors has had a rich variety of medicinal plant. *O. indicum* belonging to family *Bignoniaceae*. It is locally called as Phana Phena (Odia). *O. indicum* is a small sized deciduous tree. It grows up to a height of 12 m. The stem contains light brown bark with corky lenticels [4]. The leaves are very large, 90 to 180 cm long, 2 to 3 pinnate with 5 or more pairs of primary pinnae, rachis very stout, cylindrical, swollen at the junction of branches, leaflets 2 to 4 pairs ovate or elliptic, acuminate, glabrous. The large leaf stalks wither and fall off the tree and collect near the base of the trunk, appearing to look like a pile of broken limb bones [5]. Fruits are flat capsules, 0.33 to 1 m long and 5 to 10 cm broad and sword shaped. Seeds are numerous, 6 cm long, winged all round except at the base. In India, it is distributed in the Himalayan foothills, Eastern and Western Ghats and North East India [6,7]. Many other compounds namely oroxyloside methyl ester and chrysin-7-O-methyl glucoside in leaves and stem bark and an anthraquinone, aloe-emodin in leaves were also reported from this plant [8]. Analysis of phyto-constituents on various extracts of different parts of the plant revealed the presence of flavonoids, alkaloids, saponins, tannins, glycosides, streols, fats and oils in high, moderate and low concentrations [9,10]. Leaves are used as stomachic, carminative and flatulent. Leaf decoction is given in treating rheumatic pain, enlarged spleen, ulcer, cough and bronchitis [11,12]. The fruits are acrid, sweet, antihelminthic, and effective in diseases of the throat and heart, piles, bronchitis, used as expectorant, improves appetite and is used in leucoderma [13]. Thus the objective of the study to evaluate the *O. indicum* leave extracts for wound healing activity.

Fig 1. Leaves of *Oroxylum indicum*.

MATERIALS AND METHODS:

The ethanol, chloroform and petroleum ether were of analytical grade and were procured from Himedia, Mumbai. The Povidone Iodine ointment was procured from market.

Collection and Authentication of Plant Material:

The leaves of *O. indicum* were collected from the tribal belts of the local area of Baipariguda of Koraput District (India) in the month of January 2018. 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), Odisha (Letter No. MJ/SS/P-605/18, dated, 07-02-2018). After authentication, leaves were collected in bulk and washed under running tap water to remove adhering dirt. Then the leaves were shade dried. The dried materials were made into coarse powder by grinding in mechanical grinder 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, chloroform and petroleum ether as solvent. A total amount of 250 g coarse powder was extracted with 500 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 [14,15].

Phytochemical investigation:

The crude petroleum ether, chloroform and ethanol extracts of the leaf of *Oroxylum indicum* were subjected to preliminary phytochemical analysis in order to detect the presence of various groups of phytoconstituents present in the leaves by carrying out the chemical analysis [15,16].

Evaluation of Wound healing activity:

Animals:

Healthy adult Wister strain of albino rats weighing approximately 180 to 250 g were used. They were housed in standard conditions of temperature (25±2 °C) in 12 h light per day cycle with relative humidity of 45 to 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.

Experimental protocol:

Animals were selected, weighed (25 to 30 g) and divided in to five groups (n=6), namely control, standard drug and three groups belonging to three different extract of *O. indicum*. All the studies conducted were approved by the Institutional Ethical Committee (1906/PO/Re/S/16/CPCSEA), Jeypore College of Pharmacy, Jeypore, Odisha, according to prescribed guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Table 1. Phytochemical screening for the different solvent extracts of *O. indicum* leaves.

Phyto-constituents	Leaves extracts		
	PE	CE	EE
Alkaloids	+++	++	+
Flavonoids	+++	++	+
Glycosides	++	+	+++
Saponins	++	--	+
Tannins	+	--	++
Phenols	++	+	++
Carbohydrate	--	--	--

+++ = Strongly, ++ is moderately, + is poorly present and – is absent. PE, CE and EE are petroleum ether, chloroform and ethanol extracts.

Excision wound method:

For the excision wound study, animals were divided into five groups of six rats in each group. Group I served as control, received normal saline water of 2 ml/Kg of body weight, orally, Group II served as standard drug that is povidone iodine ointment, applied topically. The Group III, IV and V were administered with petroleum ether, chloroform and ethanol extracts (250 mg/Kg of body weight) by orally. An impression was made on the dorsal thoracic central region 5 mm away from the ears, by using a round seal of 2.5 cm diameter. The skin of the impressed area was excised to the full thickness to obtained area of about 500 mm^2 under light ether anesthesia in aseptic condition the animals were housed individually.

The animals were treated daily with drugs as mentioned above under experimental design from 0^{th} to 18^{th} days starting from the day of wounding. The percentage wound closure was observed on 4^{th}, 8^{th}, 12^{th}, 16^{th} and 18^{th} post wounding day. Epithelization time (in days) and size of the scar area was noted [17].

Incision wound method:

In the Incision wound model, the animals were divided into five groups of six rats in each group, and kept in separate cage. Group I served as control which received normal saline water at 2 ml/Kg of body administered orally. Group II served as standard drug (Povidone iodine ointment) applied topically. The Group III, IV and V were administered with petroleum ether, chloroform and ethanol extracts (250 mg/Kg) orally. 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. After complete homeostasis, the wound was closed by means of interrupted sutures placed at equidistance points about 1 cm apart. Animals were treated daily with drugs as mentioned above under experimental design from 0^{th} day to 10^{th} post-wounding day. The wound breaking strength was determined on 10^{th} day by continuous constant water flow technique [18,19].

Statistical analysis:

The results are analyzed for statistical significant by applying mean, standard deviation. Statistical analysis was done using ANOVA (Tukey-MultipleCopmarision Test). When probability (p) was less than 0.05 was considered as significant [19].

Table 2. Effect of extracts of *O. indicum* leave on the breaking strength in incision wound.

Groups	Treatments	Breaking Strength (X±S.D)
1	Control	247.11 ± 27.03
2	Pet. ether	263.16 ± 9.33
3	Chloroform	237.21 ± 8.41
4	Ethanol	337.17 ±14. 32*

Values are expressed as mean ± Standard deviation (n=6), *p<0.05.

RESULTS AND DISCUSSIONS:

The preliminary phytochemical screening showed that the different solvent extracts of *O. indicum* contains alkaloids, flavonoids, glycoside, phenolic compounds, saponins and tannins in all the solvent extracts, where as carbohydrates was absent in all the extracts (Table 1). In the study using excision wound model, animals treated with ethanol extract of *O. indicum* left showed significant decrease in epithelization period as evidenced

by shorter period for fall of eschar as compared to control and standard groups ($p<0.05$), as shown in Fig 2. The extract also facilitated the increase in rate of wound contraction than the standard. The petroleum ether, chloroform and ethanol extract (Group III, IV and V) showed wound contraction by 43.22, 51.29 and 81.33 % (Fig 3). The result of present study revealed that ethanolic leave extracts of *O. indicum* possessed a prominent pro healing activity in incision wound model. This was demonstrated by significant increase in the skin tensile strength in ethanol extract treated groups ($p<0.05$) on 10th post wounding day, as presented in Table 2.

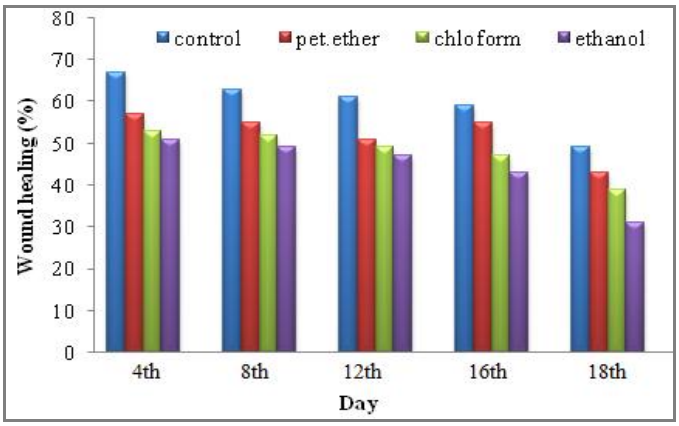

Fig 2. Effect of different extracts of *Oroxylum indicum* on excision wound model.

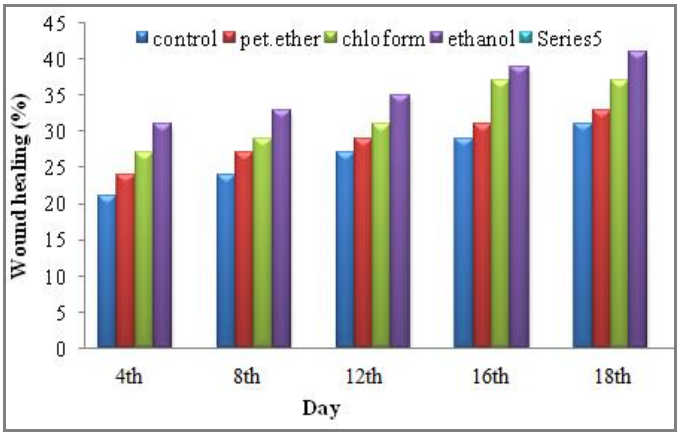

Fig 3. Effect of different extracts of *Oroxylum indicum* on incision wound model.

CONCLUSION:

In the present study, wound healing activity of *O. indicum* was studied and the results of the present study suggested that the local application and systemic administration of ethanol extract of the leaf has shown more significant wound healing activity in excision and incision wound models, which supported the popular use of plant to open wound in folk medicine. The wound healing property of *O. indicum* has been attributed to its antimicrobial effects. The presence of phytoconstituents like flavonoids, saponins, phenols 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 *O. indicum* leave. Thus the traditional use has been pharmacologically validated.

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