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Wound healing activity of the leaves of *Lawsonia alba* Lam.

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Abstract

Objective : To screen the wound healing activity of different extracts of leaves of *Lawsonia alba* on excision, incision wound models in albino rats. Materials and methods : Crude extracts of the leaves of *Lawsonia alba* obtained by successive solvent extraction with petroleum ether (40-60°C), chloroform, ethanol 95% and finally maceration with chloroform-water, were subjected for phytochemical investigation and were screened for wound healing properties in the excision and incision wound models in albino rats. The flavonoid, luteolin present in the leaves was isolated from ethanol extract by column chromatography. Results : Flavonoids, steroids, tannins and glycosides were found to be present in ethanol extract and other extracts of the leaves of *Lawsonia alba.* In excision wound parameters the ethanol extract promotes better wound healing (97.28%) compared to control and other extracts. In resutured incision wound models, ethanol extract and petroleum ether extract showed significant Breaking strength ($p<0.001$) and ($p<0.01$) respectively compared to control. Conclusion : From the results obtained, it can be observed that ethanol extract of leaves of *Lawsonia alba* have significant wound healing property. Also it can be concluded that flavonoid may be responsible for wound healing activity.

Key Words: *Lawsonia alba*, flavonoids, wound healing, successive solvent extraction.

1. Introduction

In the traditional systems of medicine, the leaves of *Lawsonia alba* (Fam. Lythraceae), commonly called as Henna are used in the form of decoction against burns, skin inflammations. An ointment prepared from leaves is used to cure wounds and ulcers. [1] The leaves have also shown to possess anti-fungal and anti-bacterial activities. [2] One of the main constituents of the leaves of *Lawsonia alba* is flavonoids.[3] The free flavonoids present in *Tridax procumbens* have been reported to have pro-healing activity. [4,5] In view of these, has been designed the present

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work to study the possible effect of different extracts of the leaves of *Lawsonia alba* on wound healing process. A survey of literature revealed that the leaves of *Lawsonia alba* has not been scientifically investigated for its wound healing activity.

Present study attempts to investigate comprehensive phyto-pharmacological aspects of the drug on wound healing in albino rats.

2. Materials and methods

The leaves of *Lawsonia alba* were collected from the local areas of Belgaum during November 2001 and were authenticated at Shri.B.M.K. Ayurved Mahavidyalaya, Shahapur, Belgaum.

2.1 Successive solvent extraction

In the present study, air dried leaves around 500g were reduced to fine powder and was subjected to hot continuous extraction in soxhlet extractor, successively with petroleum ether 40-60°C, chloroform and ethanol (95%).

Finally the drug was macerated with chloroform-water. Each time before extracting with next solvent, the powdered material was dried in hot air oven below 50°C. Each extract was then concentrated by distilling off the solvent and then evaporated to dryness on water bath. All the extracts were kept in a desiccator and stored in a refrigerator for chemical and pharmacological studies.

2.2 Preliminary phytochemical studies

The individual extracts were subjected to qualitative chemical investigation for the identification of phytoconstituents; sterols, glycosides, saponins, carbohydrates, alkaloids, flavonoids, tannins, proteins.

2.3 Column chromatography

The preliminary qualitative chemical investigation has revealed the presence of flavonoids, tannins in the ethanol extract. This extract was further subjected to chromatographic studies for separation and identification of flavonoids.

The flavonoid, luteolin was isolated and identified by fractionation of the chloroform layer on Silica gel column, using chloroform with increasing proportions of methanol. The fraction chloroform : methanol (70:30) gave luteolin (m.p. 237°C), yield 0.95%, which was identified by thin layer chromatography, using adsorbant Silica gel G, solvent system chloroform : methanol (96 : 4), spray reagent Ferric chloride : potassium ferricyanide (1% aqueous solution, 1 : 1). [6]

The development of thin layer chromatography has revealed the presence of single spot $R_f = 0.60$.

2.4 Pharmacological screening

2.4.1 Acute toxicity study

Albino mice of either sex weighing 20-25 gm and of 90 days age were used to determine the LD_{50} of various extracts. The animals were fasted over night prior to the acute experimental procedure.

The method of Miller & Tainter (1944) [7] was adopted for determination of LD_{50}. Gum acacia 1%, was used as a vehicle to suspend the various extracts and were administered orally.

LD_{50} was extrapolated by making use of graphical methods [8] to rats, $1/10^{th}$ of Lethal dose was taken as the screening dose. [9]

2.5 Wound Healing Studies

Albino rats of either sex weighing 150-200g were selected, and divided into five groups of six each. Animals were depilated at the desired site before wounding. They were housed individually with free access to food and water, the basal food intake and body weights to the nearest gram were noted.

The animals were starved for 12 h prior to wounding. Under light-ether anesthesia, sterilizing the area with ethanol performed wounding. The first group served as control and given the vehicle (Gum acacia 1%) orally. Second, third, fourth and fifth groups received petroleum ether 40-60°C extract, chloroform, ethanol, aqueous extracts by oral route at a dose of 153.98 ± 7.03mg/kg B.W., 139.66 ± 4.58mg/kg B.W., 153.98 ± 7.03mg/kg B.W., 181.96 ± 9.70mg/kg B.W. respectively. The suspensions of extracts of desired concentrations were prepared in Gum acacia (1%) solution.

2.6. Wound models

2.6.1 Excision wound

It was inflicted on rats as described by Morton and Malone.[10] For the excision wound study, groups containing six animals were selected. A circular wound of about 2.5 cm diameter was made on depilated ethanol sterilized dorsal thoracic region of rats under light-ether anaesthesia and observed throughout the study. To the animals were housed individually, the oral dose was given once a day. The observations of percentage wound closure were made on 4th, 8th, 12th post wounding days and also for epithelization and size of scar area.

2.6.2 Resutured Incision

The method of Ehrlich and Hunt [11] was adopted. Under light-ether anaesthesia, two paravertebral incisions of 6 cm were made through the entire thickness of the skin, on either side of the vertebral column with the help of a sharp blade. The incisions were sutured using 4-0 silk threads with the help of straight round-bodied needle. To the animals were housed individually, the oral dose was given once a day.

On eighth post wounding day, sutures were removed and the Breaking strength was determined on 10th post wounding day by continuous constant water flow technique of Lee. [12]

2.7. Statistical analysis

All the results were analyzed by Student's *t* -test and the level of significance was set at $p<0.05$.

3. Results and discussion

The average percentage yield of petroleum ether (40-60°C), chloroform, ethanol and aqueous extracts were 3.93%, 1.47%, 30.96% and 5.31% w/w respectively. Flavonoids, tannins, steroids were found to be present in ethanol extract and various other extracts as observed by the qualitative tests.

Table 1.
Effect of the extracts of *Lawsonia alba* on the excision wound parameters.

Sl. No.	Group	% Wound contraction on			Period of Epithelization (days)	Mean size of scar area (mm^2)
		4th day	8th day	12th day		
1.	Control	46.58 ± 2.01	83.41 ± 1.46	87.35 ± 1.81	23.50 ± 0.43	31.15 ± 1.57
2.	Petroleum ether (40-60°C)	56.30* ± 1.07	91.19* ± 1.58	96.51* ± 1.82	19.33** ± 0.66	22.40* ± 1.79
3.	Chloroform	58.35 ± 2.54	85.42 ± 2.80	92.02 ± 1.43	20.83 ± 0.94	26.30 ± 1.62
4.	Ethanol	60.96** ± 2.39	96.34** ± 2.08	97.28** ± 0.55	19.00** ± 0.51	10.26** ± 1.21
5.	Aqueous	56.44* ± 1.31	88.40 ± 1.99	94.39* ± 1.26	20.66* ± 0.71	24.02 ± 1.81

All values are in Mean ± SEM; * $p<0.01$ = Significant, ** $p<0.001$ = Highly significant *vs.* Control; n=6.

Table 2.
Effect of the extracts of *Lawsonia alba* on the Breaking strength of incision wounds.

Sl. No.	Group	Breaking strength (g)
1.	Control	124.07±13.92
2.	Petroleum ether (40-60°C)	197.85*±10.54
3.	Chloroform	143.11±7.04
4.	Ethanol	213.77**±12.90
5.	Aqueous	179.06*±7.89

All values are in Mean ± SEM; *p<0.01 = Significant, **p<0.001 = Highly significant *vs.* Control; n=6.

The excision wound heals by contraction and epithelization. The study of parameters includes percentage wound closure, time of epithelization (days) and size of scar area (mm2). The percentage wound closure for different extracts were made at the different intervals of days of post wounding. The results specify that the ethanol extract promote better wound healing (97.28%) compared to control and extracts.

The results also indicate that petroleum ether, chloroform, ethanol and aqueous extracts have shown the complete epithelization on an average 19.33 ± 0.66, 20.83 ± 0.94, 19.00 ± 0.51 and 20.66 ± 0.71 days respectively when compared to control (23.50 ± 0.43 days).

This very well signifies better wound healing activity with ethanol extract. The results also indicated least scar for ethanol extract (10.26 ± 1.21mm^2) followed by other extracts, when compared with control (31.15 ± 1.57mm^2). (Table 1.)

In resutured incision wound models, all the extracts except chloroform extract showed increased mean Breaking strength compared to control. The maximum activity was seen with ethanol extract 213.77 ± 12.90g, petroleum ether extract 197.85 ± 10.54 g and aqueous extract 179.06 ± 7.89 g, which were also found to be significant. (Table 2)

The present investigation reveals that ethanol extract of leaves of *Lawsonia alba* has shown significant pharmacological activity towards wound healing in albino rats. This plant finds mention in Ayurvedic literature and has been used in folk medicine.

From the results obtained in the study, it can be stated that the flavonoid, luteolin in ethanol extract was responsible for wound healing activity. [13] The other solvent extracts have been also shown to possess wound-healing activity as indicated in the tables but to lesser extent.

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