

Wound healing activity of *lepidagathis pungens* nees

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Abstract

The use of plant based medicine has increased significantly in recent years due to the attraction of people towards to natural therapies. The herbal drugs traditionally used cure a variety of human ailments. The pharmaceutical companies are searching for new bioactive active compounds from angiospermic plants for a novel drug. The present study focused on the wound healing activity of ethanolic extract of *Lepidagathis pungens* Nees. (Acanthaceae). Male albino Wistar rats were used in the present study. Povidone iodine ointment was applied as standard drug. Sample at low dose (5%) and high dose (10%) were used to record the efficiency of plant extract. In excision wound model the potency of wound healing activity of the *L. pungens* ethanolic extract was found to be highly significant.

Key words: Acanthaceae, ethanolic extract, *Lepidagathis pungens*, standard drug, wound

1. Introduction

Angiospermis plants possess a great therapeutic potential and many such a plants are unexplored to-date. *Species Plantarum* by Linnaeus (1753) described 5,940 species of flowering plants, including all identified species then known globally. The exploration of plant identification across the world continues since publication of the *Species Plantarum* has led to describe approximately 374,000 plant species¹. According to the latest data available, currently being 308,312 plants were described, accepted. Among these vascular plant species 295,383 are angiosperms (monocots: 74,273, eudicots: 210,008). It is estimated that there currently India being recorded with 48218 species of plants, of which 18,666 species are angiosperms². In eudicots, family Acanthaceae is one of large families. It consists of herbs, shrubs or twining vines. 4300 species are recorded in 346 genera in the family³. In India, "Flora of British India" by J. D. Hooker (C.B Clarke) reported about 49 genera, 504 species and 127 varieties of Acanthaceae. In "Flowering Plants of India" reported 47 genera, 475 species and 118 varieties⁴. In Acanthaceae, traditionally leaves are used externally to treat wounds. Still, several potent plants of Acanthaceae particularly from the rural areas are unexplored which deserve attention and research. *Lepidagathis pungens* Nees. are such plants which has not been explored extensively by the scientific world so far. *L. pungens* has very little previous record of use in traditional medicine. It is a spiny herb, found in dry lands of South Tamilnadu and endemic to peninsular India^{5,6}. Many species in the Acanthaceae family have antifungal, cytotoxic, anti-inflammatory, antipyretic and antioxidant properties^{7,8}. The most common mechanisms behind phytochemical-mediated wound healing activity are antioxidant, anti-inflammatory and anti-microbial effects⁹ suggesting *Lepidagathis pungens* leaves may have wound healing activity. Hence the present study was carried out to evaluate the wound healing activities of the crude extracts of leaves of *L. pungens*, this experimental analysis provide a basis for isolation and identification of pharmacologically active compounds.

2. Materials and Methods

Leaves from the mature and healthy plants were collected after the rainy and summer seasons. The specimens were authenticated by Dr. M. Padma Sorna Subramanian, Research Officer (Scientist-II) in Botany, (CCRS, Govt. of India), Mettur Dam. Voucher specimens of the collections are deposited at the Herbarium of Medicinal Plants Garden, Mettur Dam, Tamil Nadu, India. The leaves are dried in the shade and ground into coarse powder. In the Soxhlet apparatus 100 g of powder extracted with ethanol. The extracted solution was concentrated.

2.1. Experimental animals

Male albino Wistar rats (150-180 g) used in the present study were procured from the small animals breeding station, Mannuthy, Kerala, India. They were housed in polypropylene cages (38 x 23 x 10 cm) with not more than six animals per cage and maintained under standard environmental conditions (14h dark /10h light cycles; temp $25\pm 2^{\circ}\text{C}$; 35-60% humidity, air ventilation) and were fed with standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai, India) and fresh water *ad libitum*. The animals were acclimatized to the environment for two weeks prior to experiment use. Animals were fasted overnight before the experimental schedule, but have free access for water *ad libitum*. The experiment was carried out according to the guidelines prescribed by Animal Welfare Board and with the prior approval of animal ethic committee.

2.2. Preparation of Ointment

Simple ointment was prepared by using 10 g of petroleum jelly and 0.5 g of the ethanolic extract of the sample was added and stirred to produce the 5% low dose ointment. The 10% high dose ointment was prepared by stirring 10 g of Petroleum jelly with 1.0 g of the sample. This 5% and 10% ointment was used for topical application¹⁰.

2.3. Excision Wound model

Wistar albino male rats (150-180 g) were divided into four groups of six animals each (n=6). The experiment was designed as follows:

- Group I : Excision wound (1.0 sq cm) induced rats
- Group II : Induction + standard drug (Povidone iodine ointment for 21 days)
- Group III : Induction + Sample 1 low dose (5%) for 21 days
- Group IV : Induction + Sample 1 high dose (10%) for 21 days

Group I served as induced and Group II as standard which was topically applied with Povidone iodine ointment for 21 days after the wound excision. Groups III - IV were topically applied with 5% and 10% (w/w) ointments prepared using the sample extracts and petroleum jelly for 21 days. Wounds were created at the back of each animal of Groups I–IV. An area of about 1.0 sq cm is marked out. The marked area is excised with sharp knife and scissors under ether anesthesia¹¹. The length and breadth of the wounds were measured for 21 days using a vernier caliper. On days 0, 6, 12, 18 and 21 the wounds were photographed

3.0. Results

In *L. pungens* a rapid closure of wound in standard and extract treated groups was observed between 6 to 9 days of post surgery as represented by a decrease in the wound area (Table: 1). After day 9 of post surgery, the wound closure was gradual till the closure of the wound.

In excision wound model the potency of wound healing activity of the *L. pungens* ethanolic extract was found to be highly significant. Excision wound showed that there is almost complete (95.89%) healing on the 21st post wounding day with ethanolic extract. The topical application of *L. pungens* ointment increased the percentage of wound contraction and this indicates rapid epithelization. The administration of this 10% concentration extract *L. pungens* accelerated the progression of wound healing by 21st day i.e. (95.89%) compared with control (83.10%) in Table 1. The percent wound contraction by ethanolic extracts of *L. pungens* from day 3 to day 21 has been shown in Fig. 1.

Table: 1 Percentage reduction of samples on Wound size

Day	Induced		Low Dose (5%)		High Dose (10%)		Standard	
	Wound size (mm)	% reduction	Wound size (mm)	% reduction	Wound size (mm)	% reduction	Wound size (mm)	% reduction
0 th day	131.33 ±1.57	-	131.44 ±1.86	-	123.71 ±0.43	-	127.26 ±0.89	-
3 rd day	124.71 ±0.74	5.04	15.31 ±1.44	21.27	110.79 ±0.98	10.44	104.08 ±1.03	18.22
6 th day	113.01 ±1.33	13.94	112.67 ±2.12	14.28	104.04 ±0.54	15.90	89.80 ±1.05	29.44
9 th day	81.49 ±0.92	37.95	86.84 ±1.88	33.93	66.57 ±1.30	46.19	40.90 ±0.97	67.86
12 th day	72.73 ±0.68	44.62	69.01 ±0.98	47.49	55.85 ±1.34	54.85	21.00 ±0.78	83.50
15 th day	60.06 ±1.15	54.26	51.30 ±0.10	60.97	41.58 ±0.60	66.63	5.04 ±0.19	96.04
18 th day	50.24 ±0.58	61.74	34.86 ±0.25	73.48	27.23 ±0.26	77.99	0.04 ±0.09	99.68
21 st day	22.20 ±0.53	83.10	15.43 ±1.61	88.26	5.71 ±0.40	95.89	-	100

Table: 2 Wound healing activity of the ethanolic extract of *Lepidangathis pungens* (Excision model)

Day	Wound measurement (mm)							
	Induced		Standard		Low Dose (5%)		High Dose (10%)	
	Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth
0 th day	11.47 ±0.09	11.44 ±0.07	11.29 ±0.06	11.27 ±0.04	11.43 ±0.10	11.5 ±0.11	11.18 ±0.04	11.07 ±0.03
3 rd day	11.18 ±0.06	11.16 ±0.05	10.28 ±0.04	10.13 ±0.06	10.71 ±0.08	10.76 ±0.07	10.55 ±0.08	10.50 ±0.07
6 th day	10.56 ±0.10	10.7 ±0.06	9.48 ±0.08	9.48 ±0.05	10.57 ±0.10	10.65 ±0.11	10.21 ±0.03	10.20 ±0.04
9 th day	9.12 ±0.09	8.93 ±0.03	6.24 ±0.09	6.55 ±0.09	9.21 ±0.08	9.42 ±0.13	8.34 ±0.12	7.98 ±0.08
12 th day	8.62 ±0.08	8.44 ±0.05	4.46 ±0.12	4.71 ±0.07	8.16 ±0.04	8.45 ±0.08	7.59 ±0.10	7.34 ±0.09
15 th day	7.82 ±0.09	7.68 ±0.11	2.31 ±0.08	2.18 ±0.04	7.10 ±0.04	7.23 ±0.04	6.41 ±0.06	6.44 ±0.04
18 th day	7.03 ±0.06	7.15 ±0.08	0.65 ±0.10	0.6 ±0.09	6.01 ±0.09	5.80 ±0.06	5.21 ±0.05	5.23 ±0.03
21 st day	4.70 ±0.09	4.73 ±0.09	-	-	3.97 ±0.16	3.84 ±0.26	2.39 ±0.12	2.38 ±0.08

In *L. pungens* ethanolic extract at high dose the measurement on 6th and 9th day showed that the percentage closure of the original excision wound area was found to be 15.90 and 46.19, for standard

ointment treated group 29.44 and 67.86. The tested extracts less significantly promoted wound closure compared to controls.

4.0. Discussion

The animals that did not have the *L. pungens* ethanolic extract treatment, the wounds show to be hard and crusty with undermined margins and generally unclean with biofilm glaze on the surface. In contrast the animals treated with plant extract were clean, and showed bright red healthy granulation tissue. The wound treated with standard drug Povidone iodine ointment showed the healthy granulation tissue. *L. pungens* ethanolic extract treated excision wounds showed an increased rate of wound contraction, leading to faster healing as confirmed by the increased healed area when compared to the control group. Tensile strength was measured to confirm the wound healing activity claimed for this plant. An increase in tensile strength of treated wounds may be due to an increase in collagen concentration and stabilization of the fibres facilitating wound healing.

Granulation, collagenation, collagen and scar maturation are the different phases of wound healing¹². Many plant extracts and medicinal herbs have shown potent antioxidant activity. Research into the role of antioxidants from plant extracts in wound healing is also supported by different studies¹³. Tannins the chief components of many plant extracts, act as free radical scavengers^{14,15}. Tannins are also having the capacity as active detoxifying agents and restrain bacterial growth¹⁶. Terpenoids in the plant extracts also significantly enhance the wound healing action mainly due to their astringent and antimicrobial property¹⁷. Flavonoids are also important antioxidants, free radical scavengers^{18,19}. Polyphenols and flavonoids are preventing the synthesis of prostaglandins and possess anti-inflammatory and antimicrobial activities²⁰. Glycosides (iridoid glycosides) isolated from the Acanthaceae possess antioxidant, antimicrobial, analgesic, antitumor, immunomodulatory, and anti-inflammatory effects enhance the antioxidant enzyme level in granuloma tissues²¹. Therefore, the presence of various phytochemicals in the selected crude extract such as terpenoids, flavonoids, glycosides, saponins, tannins, and phenolic compounds may contribute to wound healing activities independently or synergistic effects

5.0. Conclusion

The results agree with the fact that the natural compounds rich in antioxidants involve a considerable improvement in the enzyme activity and reduce oxidative stress, which plays a significant role in wound healing.

Ethical approval

The study was approved by postgraduate research coordinating committee of Dr Manian Research Laboratory, Coimbatore, Tamil Nadu.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgements

The authors would like to acknowledge Head, Department of Botany, Rani Anna Government College for women Tirunelveli for accessing its laboratory facilities and providing chemicals and reagents

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Fig 1: Wound healing activity of the *Lepidangathis pungens* extract in low dose (5%)

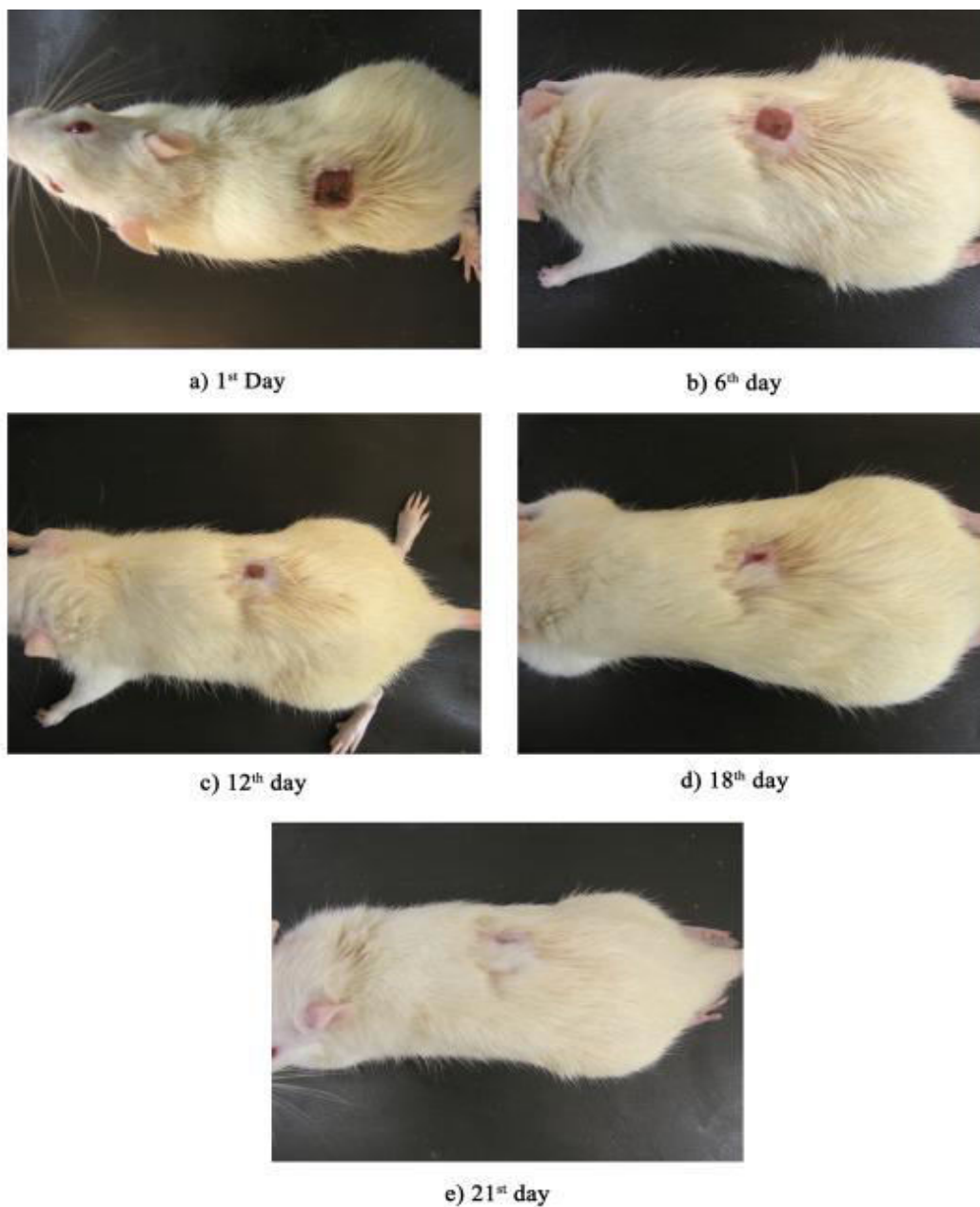


Fig 2: Wound healing activity of the *Lepidangathis pungens* extract in high dose (5%)

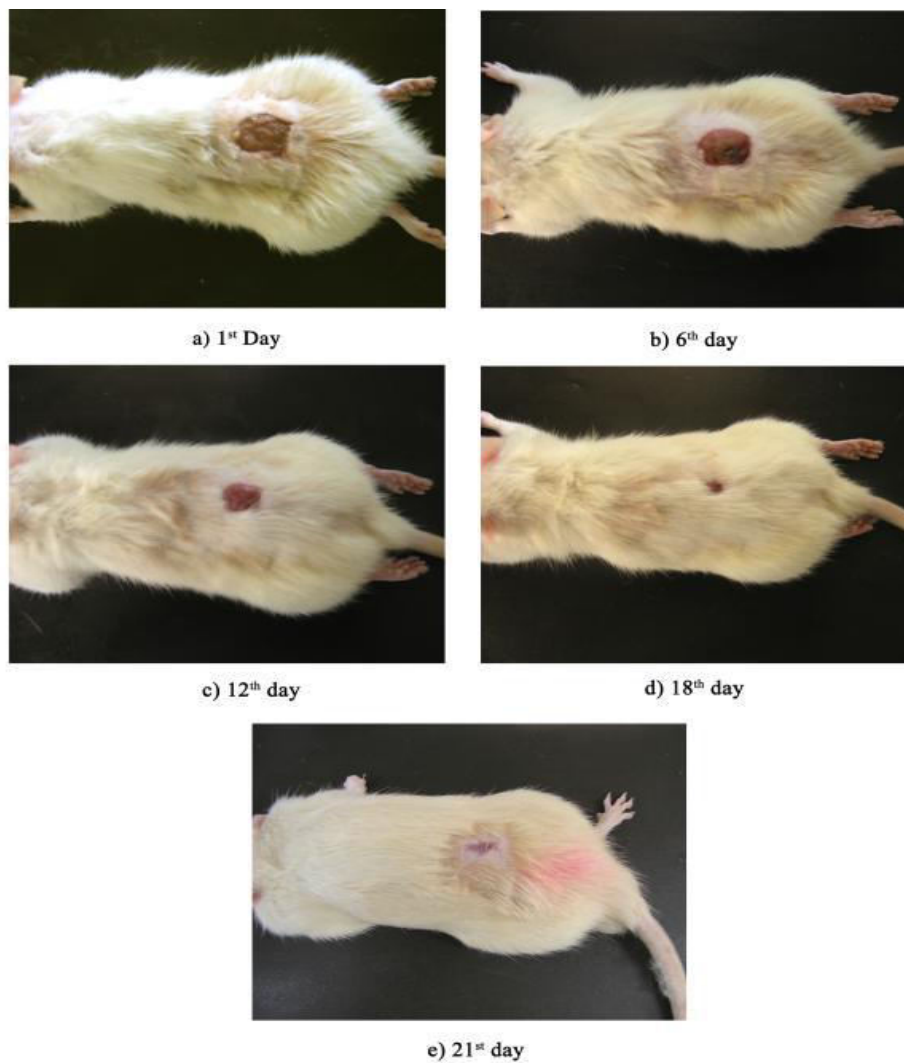


Fig 3: Wound healing activity of the standard drug

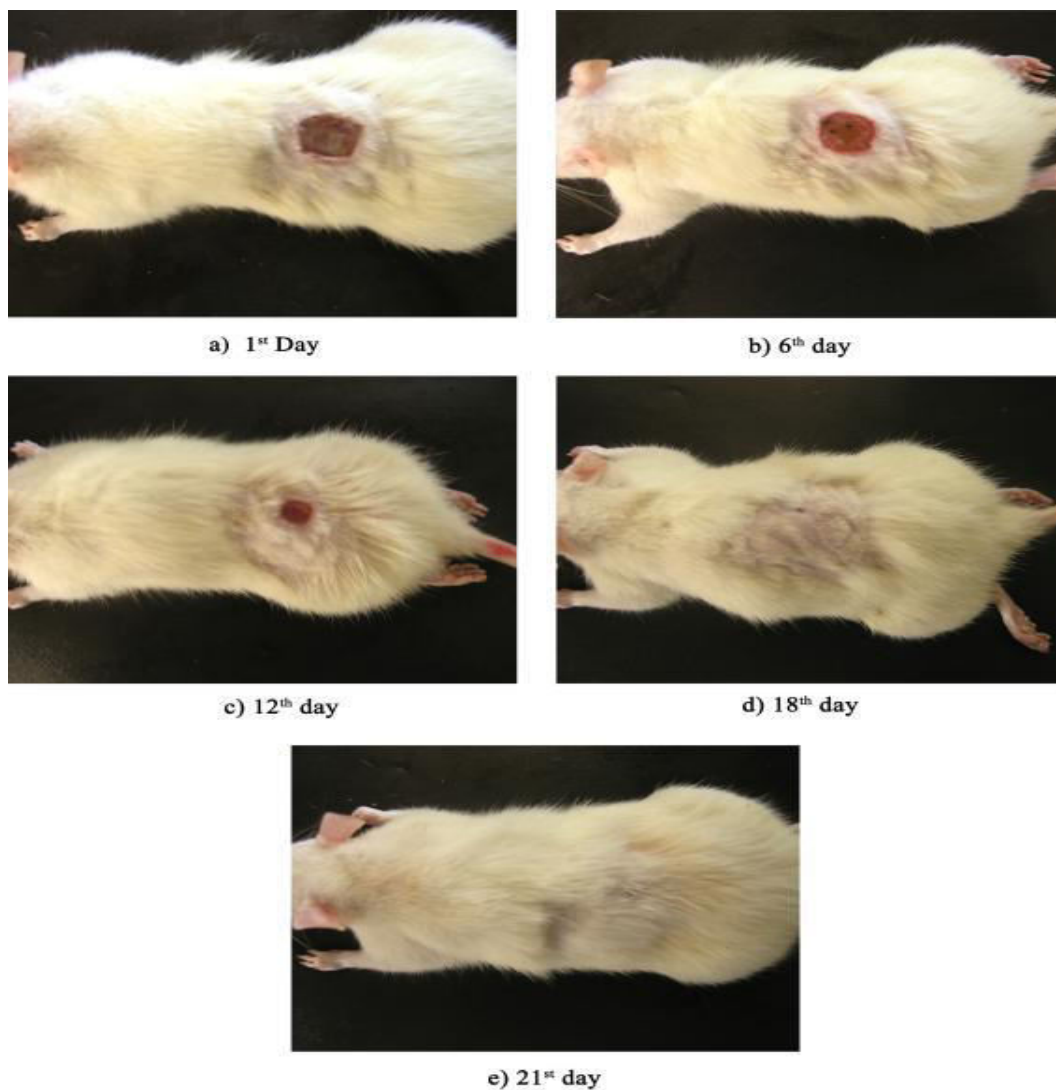


Fig 4: Wound healing activity of the induced drug

