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Research Article

EVALUATION OF IN VIVO WOUND HEALING AND IN VITRO ANTIBACTERIAL ACTIVITIES OF THE DIFFERENT EXTRACT OF LEUCAS INDICA LINN.

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ABSTRACT

Objective: The present study was designed to evaluate the *in vivo* wound healing and *in vitro* antibacterial activity of different extract of the aerial parts of *Leucas indica* Linn.

Methods: The preliminary phytochemical screening and acute toxicity studies were performed for both methanolic and aqueous fraction. The excision wound model was employed for wound healing activity on Wistar albino rats. The inflicted wounds were treated by ointment containing methanolic and aqueous fraction (10% and 15% w/w, topically). The antibacterial screening (zone of inhibition) was performed against six pathogenic bacteria using disc diffusion technique for petroleum ether, chloroform, ethyl acetate, methanol and aqueous fraction at different concentration (200, 100 and 50 mg/ml) and compared to reference drug Gentamycin (10 mg/ml). The minimum inhibitory concentration (MIC) was evaluated for both methanolic and aqueous fraction against *Staphylococcus aureus* ML191, *Bacillus subtilis* 6633, *Salmonella typhi* 74 and *Pseudomonas aeruginosa* 25619 in descending order of concentrations (200 to 1.56 mg/ml).

Results: Both the methanolic and aqueous fraction increased percentage of wound contraction when compared with reference drug Povidone iodine (10% w/w). The chloroform and methanolic fraction showed enough sensitivity against *Staphylococcus aureus* ML191, *Bacillus subtilis* 6633, *Salmonella typhi* 74, *Pseudomonas aeruginosa* 25619 and *Escherichia coli* 55/10HD whereas the aqueous fraction inhibited the growth of *Staphylococcus aureus* ML191, *Bacillus subtilis* 6633 *and Salmonella typhi* 74. There was a significant dose dependent zone of inhibition against *Staphylococcus aureus* ML191, *Bacillus subtilis* 6633 and *Salmonella typhi* 74 by all fractions.

Conclusion: From the above experiment it can be concluded that the methanolic fraction showed better wound healing and antibacterial properties than aqueous one.

Keywords: *Leucas indica*, Methanolic extract, Aqueous extract, Excision wound, Antibacterial.

INTRODUCTION

The medicinal plants have a great importance in human being as it shows diverse pharmacological properties. There are numbers of phytochemicals are used for the treatment of microbial infections, cardiovascular disorders, carcinogenic complications, metabolic disorders etc from ancient time to modern medicinal therapy [1]. The wounds may be formed by the several physical and chemical injuries or may be due to various pathogenic microbial infections. The moist wound area is very much susceptible to microbial growth and it delays the healing process remarkably. Although the healing of wound is a natural biological process but according to severity treatment is recommended to increase the rate of healing and minimize the microbial growth around the wounded area. There are numbers of synthetic drugs and antibiotics available for this purpose but it create several unwanted effect. It is already reported that herbal medications are more effective, nontoxic in nature, nonresistant to microorganism, more available, affordable and cheap over the conventional medicine [2]. From this point of view there is a very much growing interest in research field to discover new potential herbal medicine for treatment of infectious diseases along with their diverse medicinal importance as it is safer in concern [3].

The plant *Leucas indica* Linn. (Family: Labiatae) is commonly known as 'Dandokalos' in Bengali. It is distributed almost in every state of India but abundantly present in 'Mahananda Neora Valley' in West Bengal. It is an erect herb with pubescent branching. The leaves of this plant are linear-lanceolate in nature while the flowers are white with four stamens [4]. Traditionally, the leaves of this plant are used as vermifuge, stomachic, sedative and in sores [5]. The phytochemicals like phenylethanoid glycosides were isolated from the aerial parts of *Leucas indica* having antioxidant property [6]. The methanolic fraction of this plant showed significant wound healing activity [7]. The aqueous extract of the aerial parts of *Leucas indica* having significant hypoglycaemic activity [8]. However, based on the literature survey and traditional use the present study was designed to study wound healing and antibacterial activities of different fraction of aerial parts of *Leucas indica* Linn.

MATERIALS AND METHODS

Plant Material

The aerial parts of *Leucas indica* Linn. were collected in August, 2011 from Duars region, Jalpaiguri District, West Bengal, subsequently identified and authenticated from Botanical Survey of India, Central National Herbarium, Botanical Garden, Howrah-711103, West Bengal (Ref No. CNH/32/2012/Tech.II/625 Dated: 06.03.2012). After proper washing, it was dried under shade at a room temperature for seven days and then grinded with a mechanical grinder. Finally, the coarse powders were separated by sieving using 40 mesh and stored in an air tight container for further use.

Preparation of Plant Extract

The fresh coarse powders were subjected to maceration by petroleum ether to remove fatty materials and then successively extracted with chloroform, ethyl acetate, methanol and distilled water according to ascending order of polarity of solvent using a Soxhlet apparatus. The each fraction of the extract was then concentrated to dryness in a rotary vacuum evaporator under reduced pressure and temperature and stored in desiccators. During performing the experiment, the petroleum ether, chloroform and ethyl acetate extract were dissolved in 2% Dimethyl sulfoxide (DMSO) solution but the dried methanloic and aqueous extract were dissolved in distilled water to prepare the subsequent doses for the experiment.

Animals

Wistar albino rats (weighing 150-200 g) of either sex were used to perform the wound healing and acute toxicity study. The animals were randomly grouped (n=6) and housed in polyacrylic cages

(38×23×10 cm) and maintained under standard laboratory conditions (Temp. 25 ± 2º C) with dark and light cycle (14/10 hr). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The rats were acclimatized to laboratory condition for 1 week before commencement of this experiment. Ethical clearance was obtained from Jadavpur University Animal Ethical Committee for using animals in the present study (Vide No. 0367/01/C/CPCSEA, India).

Phytochemical screening

The preliminary phytochemical screening of different extracts of *Leucas indica* was done by the standard procedure described by Trease and Harbone [9, 10].

Acute toxicity study

The acute toxicity study was followed here Litchfield and Hilaly models with some necessary modifications [11, 12]. Wistar albino rats (weighing 150-200 g) of either sex were divided into several groups containing 10 animals of each. Different doses of aqueous fraction (200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000 and 4500 mg/kg) and methanolic fraction (200, 500, 1000, 1500, 2000, 2500, 3000, 3500 mg/kg) were administered orally (single dose) to the treated groups but control groups received only normal saline orally (5 ml/kg) under overnight fasting condition. The sign of toxicity and mortality were recorded within 24-72 h for all groups of animals. The LD_{50} was determined using graphical representation using probit analysis.

Wound healing activity

The protocol for wound healing activity study was followed here Wani *et al* method with little modifications [13]. The animals were divided into six groups of six animals in each. Group-I served as Wound control (apply ointment base only). The animals under Group-II and III were treated with methanolic fraction at the concentration of 10% and 15% w/w ointment respectively. Group-IV and V were designed as aqueous one at the concentration of 10% and 15% w/w ointment treated group respectively. Group-VI was served as reference group (Povidone iodine ointment 10% w/w). An excision wound was inflicted on nape of the neck on dorsal surface in each animal by removing a circular skin piece of full thickness approximately 400 mm^2 by scissor under ether anaesthesia and in aseptic condition. The day of wound inflection was considered as day zero of the experimental period. The wounds were left to open environment without dressing and treated with respective ointment topically in daily until complete healing. The wound diameter was measured by placing mm graph paper upon the wounded area and marking was done accordingly. The gradual reduction of wounded area was measured on 3^{rd}, 6^{th}, 9^{th}, 12^{th} and 15^{th} day after initiation of wound generation. The percentage of wound contraction was calculated by comparing with initial wound area of each animal.

Antibacterial activity

The three Gram positive (*Staphylococcus aureus* ML191, *Bacillus subtilis* 6633 and *Enterococcus faecalis* ATCC 29212) and three Gram negative (*Salmonella typhi* 74, *Pseudomonas aeruginosa* 25619 *and Escherichia coli* 55/10HD) bacteria were selected for the study of antibacterial potency of *Leucas indica* plant. The study was performed using disc diffusion technique on nutrient agar medium. This method was described by Vincent and Vincent with few modifications [14]. The discs were prepared by Whatman filter paper (6 mm in diameter) and after proper sterilization it was soaked in different concentrations (50, 100 and 200 mg/ml) of the petroleum ether, chloroform, ethyl acetate, methanol and aqueous fraction and subsequently dried at 50º C. The 2% DMSO solution was used to dissolve petroleum ether, chloroform and ethyl acetate extract where as methanolic and aqueous fraction were dissolved in distilled water. The isolated bacterial culture were diluted with sterile normal saline to obtained requisite inoculums size (10^6 cfu/ml) and spread on the surface of dried nutrient agar plates with sterile cotton wool swabs. The inoculated plates were subjected to incubation at 37ºC for 48 h. The zone of inhibition by different fraction at different concentration indicated the antimicrobial potency and it was compared with both negative control (without applying any drug on plate) and positive control (reference drug Gentamycin 10 mg/ml).

Minimum inhibitory concentrations (MIC)

The methanolic and aqueous fraction of the plant was selected for the study of MIC on some selected organisms. In case of methanolic fraction four organisms (*Staphylococcus aureus* ML191, *Bacillus subtilis* 6633, *Salmonella typhi* 74 *and Pseudomonas aeruginosa* 25619) and for aqueous fraction three organisms (*Staphylococcus aureus* ML191, *Bacillus subtilis* 6633 *and Salmonella typhi* 74) were selected on the basis of sensitivity test. Both the extract dissolved in sterile water and prepared different concentrations of solution (200, 100, 50, 25, 12.5, 6.25, 3.13, 1.56 mg/ml) by serial dilution technique and transferred about 0.5 ml to each individual sterile test tube. About 0.5 ml of sterile nutrient broth was placed in all test tubes containing extract solution and mixed properly. The selected bacterial cells suspension (0.5 ml) was inoculated in each test tube and incubated at 37ºC for 24 h. The turbidity appeared in those test tubes were considered as bacterial growth and the transparency in test tubes indicated inhibition of microbial growth. The minimum concentration of the extracts that completely inhibited the bacterial growth was considered as MIC of the respective fraction [15, 16]. The test tubes containing only nutrient broth was designed as negative control whereas the nutrient broth along with bacterial culture as positive control for this study.

Statistical analysis

The results were expressed as mean ± SEM. Statistical differences between the treated and control groups were determined by one way ANOVA followed by Dunnet's test using the computer software, Prism graph pad 5 version. The 'P' values less than 0.05 was considered as statistically significant.

RESULTS

Phytochemical screening

The both methanolic and aqueous fraction of aerial parts of the *Leucas indica* contains flavonoids, total phenolic compounds, saponin and tannin. The chloroform, ethyl acetate and methanolic fraction of this plant confirmed the presence of cardiac glycoside. The test results of alkaloids and steroids were negative for all fractions (Table 1).

Acute toxicity study

In case of acute toxicity study the measured LD_{50} values were 1995 and 2630 mg/kg for the methanolic and aqueous extract respectively. However no visual toxicity was found up to 1000 mg/kg for aqueous extract and up to 500 mg/kg for methanolic one (Table 2, 3 and Figure 1, 2).

Wound healing activity

Both the methanolic and aqueous fraction of *Leucas indica* plant showed significant dose dependent (10% and 15% w/w ointment, topically) wound healing potency when compared with reference drug Povidone iodine (10% w/w, topically). On the day 15^{th} the methanolic fraction increased the percentage of wound contraction about 96.32 and 100% whereas the results of aqueous fraction were 92.97 and 94.05 % at lower and higher dose respectively. However the reference drug (Povidone iodine) showed better result regarding percentage of wound contraction (100%) and epithelialization on same day (Table 4, Figure 3A and 3B). On the other hand the methanolic fraction showed comparatively better results than aqueous fraction in this study. The percentage of wound contraction and epithelialization upon the wounded area in different group of animals were clearly visualized on day 15^{th} of the experimental period (Figure 4A to 4F).

Antibacterial activity

In case of sensitivity testing the chloroform and methanolic fraction showed enough positive results against *Staphylococcus aureus* ML191, *Bacillus subtilis* 6633, *Salmonella typhi* 74, *Pseudomonas aeruginosa* 25619 and *Escherichia coli* 55/10HD whereas the aqueous fraction inhibited the growth of

Staphylococcus aureus ML191, *Bacillus subtilis* 6633 *and Salmonella typhi* 74 significantly (Table 5). The measured MIC for both methanloic and aqueous fraction was 12.5 mg/ml against *Staphylococcus aureus* ML191whereas in case of *Salmonella typhi* 74 the results were 6.25 and 200 mg/ml for methanolic and aqueous fraction respectively. The MIC results for both *Bacillus subtilis* 6633 *and Pseudomonas aeruginosa* 25619 were also significant (Table 6 and 7). There was a significant dose dependent (200, 100 and 50 mg/ml) results in zone of inhibition against *Staphylococcus aureus* ML191, *Bacillus subtilis* 6633 and *Salmonella typhi* 74 by the all fraction when compared with reference drug Gentamycin (10 mg/ml). However the petroleum ether fraction was completely resistant to *Staphylococcus aureus* ML191, *Pseudomonas aeruginosa* 25619 and *Escherichia coli* 55/10HD. The ethyl acetate and aqueous fraction also totally resistant to *Pseudomonas aeruginosa* 25619 *and Escherichia coli* 55/10HD where as the methanolic fraction was only resistant to *Escherichia coli* 55/10HD (Table 8 and Figure 5).

Table 1: Phytochemical analysis of different fractions of *Leucas indica*

Phytochemicals	LIPEE	LICE	LIEAE	LIME	LIAE
Flavonoids	-	-	-	+	+
Phenolic compounds	-	-	-	+	+
Alkaloids	-	-	-	-	-
Cardiac glycoside		+	+	+	-
Tannin	-	-	-	+	+
Steroid	-	-	-	-	-
Saponin	-	-	-	+	+

'-' represent not present; '+' represent present; LIPEE: Petroleum ether extract of *Leucas indica*; LICE: Chloroform extract of *Leucas indica*; LIEAE: Ethyl acetate extract of *Leucas indica*; LIME: Methanolic extract of *Leucas indica*; LIAE: Aqueous extract of *Leucas indica.*

Table 2: Determination of LD_{50} of Methanolic Extract of *Leucas indica* (LIME) on rats

Group	Dose mg/kg	Log dose	No of rats	No of dead	Percentage mortality	Corrected mortality	Probit value
I	0 (control)	-	10	0	0	0	0
II	200	2.30	10	0	0	0	0
III	500	2.70	10	0	0	0	0
IV	1000	3.00	10	2	20	20	4.10
V	1500	3.18	10	4	40	40	4.75
VI	2000	3.30	10	5	50	50	5.00
VII	2500	3.40	10	7	70	70	5.52
VIII	3000	3.48	10	8	80	80	5.84
IX	3500	3.54	10	9	90	90	6.28

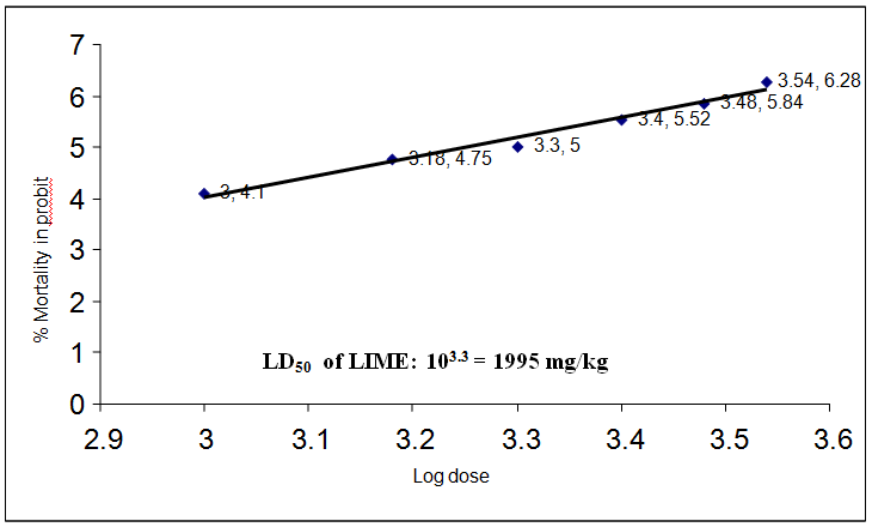

Fig. 1: Determination of LD_{50} of Methanolic Extract of *Leucas indica* (LIME)

Table 3: Determination of LD_{50} of Aqueous Extract of *Leucas indica* (LIAE) on rats

Group	Dose mg/kg	Log dose	No of rats	No of dead	Percentage mortality	Corrected mortality	Probit value
I	0 (control)		10	0	0	0	0
II	200	2.3	10	0	0	0	0
III	500	2.7	10	0	0	0	0
IV	1000	3	10	0	0	0	0
V	1500	3.18	10	1	10	10	3.72
VI	2000	3.3	10	3	30	30	4.48
VII	2500	3.4	10	4	40	40	4.75
VIII	3000	3.48	10	6	60	60	5.25
IX	3500	3.54	10	7	70	70	5.52
X	4000	3.6	10	8	80	80	5.84
XI	4500	3.65	10	9	90	90	6.28

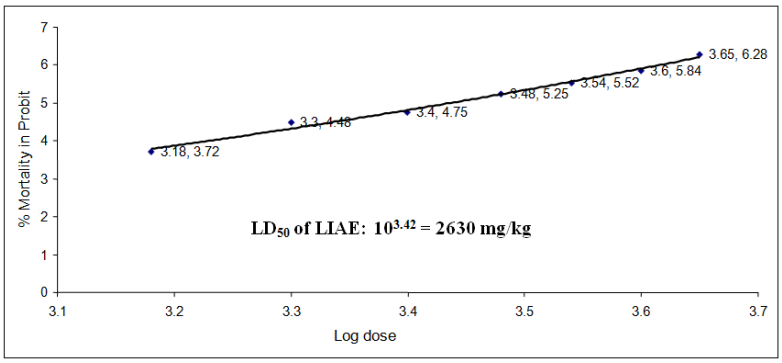

Fig. 2: Determination of LD_{50} of Aqueous Extract of *Leucas indica* (LIAE)

Table 4: Determination of LIME and LIAE on Excision wound area and percentage of wound contraction

Group	Wound area (mm²) and percentage of wound contraction					
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Gr-I: Wound control	399.94 ± 5.24	385.67 ± 10.26 (3.57 %)	363.44 ± 3.43 (9.12 %)	312.65 ± 13.24 (21.82 %)	234.44 ± 4.55 (41.38 %)	123.44 ± 6.34 (69.35 %)
Gr-II: LIME 10% w/w	416.52 ± 4.56	388.56 ± 3.37** (6.71 %)	315.87 ± 2.45** (24.16 %)	217.56 ± 1.38** (47.77 %)	115.43 ± 3.48** (72.29 %)	15.32 ± 2.55** (96.32 %)
Gr-III: LIME 15% w/w	424.76 ± 9.32	348.50 ± 4.21*** (17.95 %)	241.54 ± 5.15*** (43.13 %)	170.25 ± 3.63*** (59.92 %)	62.16 ± 8.65*** (85.37 %)	00 (100 %)
Gr-IV: LIAE 10% w/w	388.45 ± 2.55	359.96 ± 3.54* (7.33 %)	333.36 ± 5.28* (14.18 %)	187.98 ± 8.45* (51.61 %)	122.99 ± 4.66* (68.34 %)	27.29 ± 3.12* (92.97 %)
Gr-V: LIAE 15% w/w	405.35 ± 11.53	355.63 ± 6.76** (12.27 %)	315.80 ± 3.33** (22.09 %)	166.26 ± 2.34** (59.00 %)	111.43 ± 2.35** (72.51 %)	24.12 ± 2.24** (94.05 %
Gr-VI: PI 10% w/w	423.58 ± 14.87	357.25 ± 5.56*** (15.66 %)	302.35 ± 7.23*** (28.62 %)	143.50 ± 6.34*** (66.12 %)	53.68 ± 4.50*** (87.32 %)	00 (100 %)

Values are expressed as Mean ± SEM; n= 6; Where*P< 0.05; **P< 0.01; ***P <0.001;

LIME: Methanolic Extract of *Leucas indica*; LIAE: Aqueous Extract of *Leucas indica*; PI: Povidone iodine

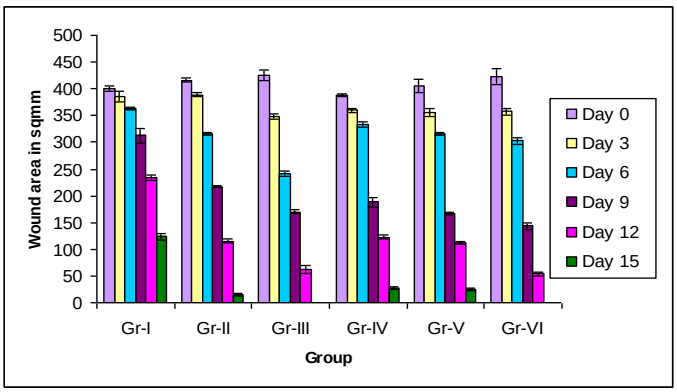

Fig. 3A: Effect of Methanolic and Aqueous extract on wound area (mm²).

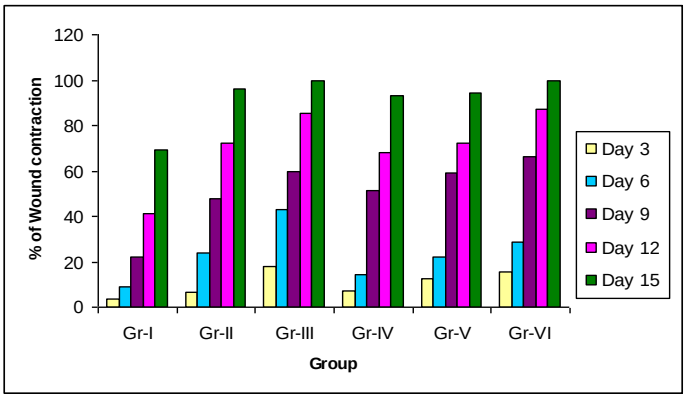

Fig. 3B: Effect of Methanolic and Aqueous extract on percentage wound contraction.

Fig. 4A: Photographical representation of wound control animal treated with ointment base only and it showing much wound (blue arrow) and very less percentage of wound contraction and no epithelialization upon wounded area.

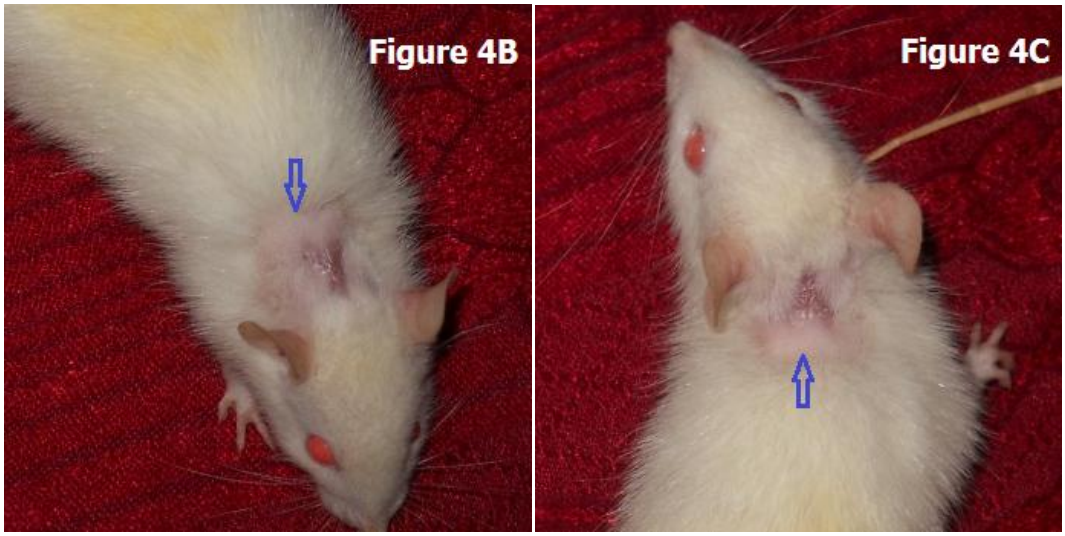

Fig. 4B and 4C: Photographical representation of Aqueous fraction 10 and 15% w/w ointment treated wound (blue arrow) respectively showing decreased in wound area and started to epithelialization.

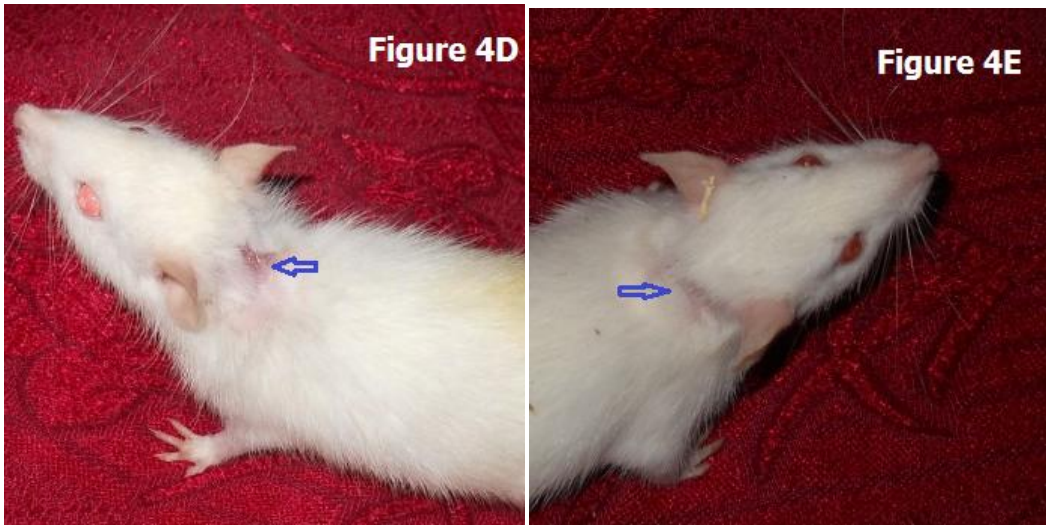

Fig. 4D and 4E: Photographical representation of Methanolic fraction 10 and 15% w/w ointment treated wound (blue arrow) respectively showing very closure of wounded area and Figure 4E showing 100% wound contraction and epithelialization.

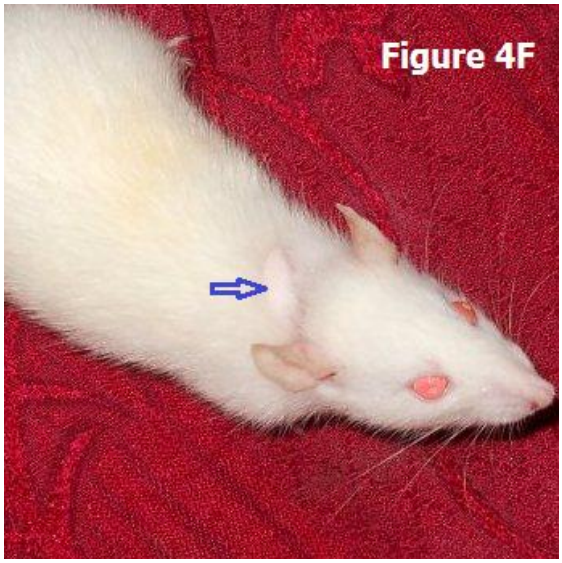

Fig. 4F: Photographical representation of Povidone iodine 10% w/w treated wound (blue arrow) showing complete epithelialization and 100% wound contraction.

Table 5: Sensitivity Test of different fraction of *Leucas indica* on bacterial strain

Bacterial strain	Nature	LIPEE	LICE	LIEAE	LIME	LIAE	GEN
Staphylococcus aureus ML191	Gram positive	-	+	+	+	+	+
Bacillus subtilis 6633	Gram positive	+	+	-	+	+	+
Enterococcus faecalis ATCC 29212	Gram positive	-	-	-	-	-	+
Salmonella typhi 74	Gram negative	+	+	+	+	+	+
Pseudomonas aeruginosa 25619	Gram negative	-	+	-	+	-	+
Escherichia coli 55/10HD	Gram negative	-	+	-	-	-	+

'-' represent not sensitive; '+' represent sensitive; LIPEE: Petroleum ether extract of *Leucas indica*; LICE: Chloroform extract of *Leucas indica*; LIEAE: Ethyl acetate extract of *Leucas indica*; LIME: Methanolic extract of *Leucas indica*; LIAE: Aqueous extract of *Leucas indica*; GEN: Gentamycin

Table 6: Determination of MIC of Methanolic fraction against Bacterial growth

Organism	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.12 mg/ml	1.56 mg/ml
SA	-	-	-	-	-	+	+	+
BS	-	-	+	+	+	+	+	+
ST	-	-	-	-	-	-	+	+
PA	-	-	+	+	+	+	+	+

'+': Detection bacterial growth; '-': No bacterial growth; MIC: Minimum Inhibitory Concentration; LIME: Methanolic extract of *Leucas indica*; SA: *Staphylococcus aureus* ML191; BS*: Bacillus subtilis* 6633*;* ST: *Salmonella typhi* 74; PA: *Pseudomonas aeruginosa* 25619

Table 7: Determination of MIC of Aqueous fraction against Bacterial growth

Organism	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.12 mg/ml	1.56 mg/ml
SA	-	-	-	-	-	+	+	+
BS	-	-	-	+	+	+	+	+
ST	-	+	+	+	+	+	+	+

'+': Detection bacterial growth; '-': No bacterial growth; MIC: Minimum Inhibitory Concentration; LIME: Methanolic extract of *Leucas indica*; SA: *Staphylococcus aureus* ML191;

BS*: Bacillus subtilis* 6633*;* ST: *Salmonella typhi* 74; PA: *Pseudomonas aeruginosa* 25619

Table 8: Determination of Zone of inhibition by Disc Diffusion technique

Group	Zone of inhibition (mm) in disc diffusion technique				
	S. aureus	*B. subtilis*	*S. typhi*	*P. aeruginosa*	*E. coli*
LIPEE 200 mg/ml	R	12	20	R	R
LIPEE 100 mg/ml	R	10	18	R	R
LIPEE 50 mg/ml	R	9	16	R	R
LICE 200 mg/ml	17	16	16	17	17
LICE 100 mg/ml	16	15	14	15	16
LICE 50 mg/ml	13	12	10	13	14
LIEAE 200 mg/ml	13	R	15	R	R
LIEAE 100 mg/ml	12	R	14	R	R
LIEAE 50 mg/ml	8	R	13	R	R
LIME 200 mg/ml	18	19	20	18	R

LIME 100 mg/ml	14	16	18	15	R
LIME 50 mg/ml	10	15	16	14	R
LIAE 200 mg/ml	18	17	16	R	R
LIAE 100 mg/ml	12	15	14	R	R
LIAE 50 mg/ml	11	14	12	R	R
GEN 10 mg/ml	30	29	26	25	26
Negative control	0	0	0	0	0

LIPEE: Petroleum ether extract of *Leucas indica*; LICE: Chloroform extract of *Leucas indica;* LIEAC: Ethyl acetate extract *Leucas indica*; LIME: Methanolic extract of *Leucas indica*; LIAE: Aqueous extract of *Leucas indica*; GEN: Gentamycin; R: Resistant to the organism.

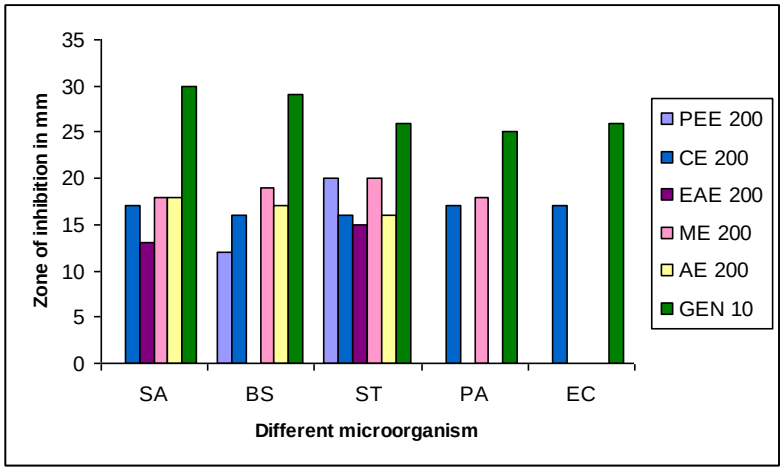

Fig. 5: Determination of Zone of inhibition by different fraction of *Leucas indica* at 200 mg/ml concentration in Disc Diffusion technique.

PEE 200: Petroleum ether extract 200 mg/ml; CE 200: Chloroform extract 200 mg/ml; EAC 200: Ethyl acetate extract 200 mg/ml; ME 200: Methanolic extract 200 mg/ml; AE 200: Aqueous extract 200 mg/ml; GEN 10: Gentamycin 10 mg/ml; SA: *Staphylococcus aureus* ML191; BS: *Bacillus subtilis* 6633; ST: *Salmonella typhi* 74; PA: *Pseudomonas aeruginosa* 25619;

EC: *Escherichia coli* 55/10HD.

DISCUSSION

The methanolic and aqueous fraction was completely safe (no dead of animals) for the animals up to 500 and 1000 mg/kg respectively which was observed in the acute toxicity test of the present study (Table 2, 3 and Figure 1, 2). The wound healing is a complicated biological process in cellular and molecular level for regeneration of the damaged tissue [17]. There are three stages of wound healing process such as inflammation, proliferation and remodeling. The inflammation is the first stage of wound repairing. In this stage the platelets, thrombin, fibrin and other coagulation factors aggregated subendothelially to form haemostatic plug. In case of proliferative phase there is a rapid generations of collagen fibre and it deposited upon the wounded area. The neoangiogenesis in proliferative phase also enhance the wound healing process by proper blood circulation around the wounded area. In final stage of wound healing there is a rapid differentiation of fibroblast cells to myofibroblasts that causes wound contraction [18]. In the present study the methanolic and aqueous fraction of this plant significantly increased the percentage of wound contraction (Table 4, Figure 3A, 3B and Figure 4A to 4F). The mechanism involved in wound healing activity might be rapid cellular proliferation, collagen deposition, epithelialization, myofibroblasts production and angiogenesis around the wounded area. In the present study the chloroform extract of *Leucas indica* showed significant broad spectrum antibacterial activity against the entire selected organisms (Table 8 and Figure 5). The methanolic fraction also showed broad spectrum antibacterial potency except *Escherichia coli* 55/10HD. Others fractions of this plant showed narrow spectrum antibacterial property. It is reported that phenolic compounds are toxic to pathogenic microorganism [19]. The plant *Leucas indica* contain phenolic compound and it is responsible for microbial toxicity. It is also reported that majority of phytochemicals having antimicrobial property in some extent and it is better option to treat microbial infection by plant based phytomedicine due to lesser adverse effect, readily available, cheap and affordable compared to current synthetic medication [20]. Further more the several phytochemicals like flavonoids, saponins, tannins, phenolic compounds promote the wound healing process due to their antimicrobial and astringent property as shown in our present study [21, 22]. However the methanolic fraction of this plant showed better results than aqueous fraction both in wound healing and antimicrobial activity study.

CONCLUSION

From the present studies it can be concluded that the methanolic extract of *Leucas indica* having more potent wound healing property than aqueous one. The chloroform fraction showed comparatively better antibacterial potency than others fraction of this plant. The wound healing and antibacterial properties are due to the significant content of the most important phytochemicals like flavonoids, total phenolic compounds, saponins and tannins. So it can be widely used as a potent and safe medication for various types of wound and pathogenic bacterial infections. Although further studies are highly recommended to elucidate the active compounds and to investigate the specific mechanism of action in cellular and macromolecular levels.

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