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EVALUATION OF ANTI-MICROBIAL AND WOUND HEALING ACTIVITY OF AQUEOUS EXTRACT OF MEDICINAL PLANT ON WISTER ALBINO RATS

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ABSTRACT

Wounds are major case of physical disabilities. A wound which is disturbed state of tissue caused by physical, chemical, microbial (or) immunological insults (or) typically associated with loss function. From the results it was clear that the formulated herbal ointment have antimicrobial activity, that the plant extract have potent antimicrobial activity against E.coli at different concentrations Excision wounds were created in rats to study the epithelization period, scar area and rate of wound contract, the measurement of progress of wound healing induced by reference drug, (5% povidone iodine ointment), two herbal test formulations viz., formulation (5% lagerstroemia microcarpa extract ointment). Day of fall of Escher indicated the Epithelization period and scar area was noted on the same day. Wound area was noted from the day of wounding and subsequently at regular time intervals, i.e. 3rd , 6th, 9th, day and then up to 18th day or till complete Epithelization period of study. On the 12th day wound areas were measured to be 224.3 ± 2.23 mm2, 261.3±1.36 mm2 in animals respectively. From 15th day onwards, we have observed the complete closure of wound in ointment treated and reference ointment (povidone iodine ointment) treated groups. Complete closure of wound was observed in Group IV, on the 18th day. On the 18th day, the average wound areas measured animals were found to be 111.5±1.73 mm2 and 11.3±1.36 mm2 respectively. From the above research work it was clear that, the selected plant lagerstroemia microcarpa has potent antimicrobial and wound healing activity. Phytochemical test showed the presence of Carbohydrates, Flavonoids, Proteins & Amino acids, Phenols, Diterpenes and Saponins, Alkaloid was found to absent in extract lagerstroemia microcarpa which indicates the presence of phenols and flavonoids in higher percentage .The Isolation and characterization and various instrumental analysis were required for further studies.

Keywords: Lagerstroemia Microcarpa, Antimicrobial activity, Wound Healing.

INTRODUCTION

Wounds are major case of physical disabilities [1]. A wound which is disturbed state of tissue caused by physical, chemical, microbial (or) immunological insults (or) typically associated with loss function. According to the wound healing society wounds are physical injuries that results in an opening (or) break of the skin that cause disturbance in the normal skin anatomy and function [2].

Wound healing is an interaction of complex cascade of cellular and bio chemical actions healing to the restoration of structural and functional integrity with regain of strength of injured tissues. Involves continuous cell – cell interaction and cell matrix interactions that allow the process to proceed in different overlapping phases and process including inflammation , wound contraction , epithelialization tissue, re modeling , & formation of granulation tissue with angiogenesis [3].

Several factors delay (or) reduce the wound healing process including bacterial infection, necrotic tissue, & interference with blood supply, lymphatic blockage & diabetes mellitus, generally if the above factors could be altered by any agent, an increased healing rate could be achieved [4]. Many Ayurvedic plants have a very important role in the process of wound healing. Plants are more potent healers because they promote the repair mechanisms in the natural way [5].

Plant based therapy not only accelerate healing process and also maintains the aesthetics . More than 70% of wound healing Pharma products are plant based, 20% are mineral based and remaining containing animal products as their base material. The plant base materials are used first aid – antiseptic coagulants and wound wash [6] Lagerstroemia microcarpa wight deciduous trees, to 30 m high, bark 6-8 mm thick, greyish or greyish-white, smooth, peeling off in thin long and broad flakes blaze creamy yellow, outer parts brittle, inner layers fibrous; branches knotted. Leaves simple, opposite,

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distichous, stipulate; stipules 2, intrapetiolar, deciduous; petiole 6-15 mm long, slender, glabrous; lamina 4.5- 10 x 3.7-6.5 cm, elliptic, ovate, elliptic-lanceolate or ovate-lanceolate, base attenuate or acute, apex acute or acuminate, margin entire, glabrous and shining above, velvety Lagerstroemia microcarpa leaf extracts showed the presence of phytochemical constituents such as terpenoids, steroids, phytosterols, flavonoids, carbohydrates, proteins and saponin. There are no previous preliminary phytochemical and other reports in this plant [7].

MATERIAL AND METHOD

Collection of plant material

The plants used in this study consisted of *Lagerstroemia microcarpa* leaves were collected from local area and purchased commercially from recognized Ayurvedic shop from Bhopal (Madhya Pradesh Province).

Authentication of plant material

The plant material was collected from local area of Bhopal in Jan 2023 and was authenticated at the Department of Botany, SRK University Bhopal. A voucher specimen number or the herbarium number is SRKU/BPL/2023/10 has been deposited.

Extraction of Plant material:-

The preparation of extract was carried out according to the method of (Oktay, et al, 2003)[8]. Briefly, the leaves of *lagerstroemia microcarpa* were shade dried after collection for 5 days and was powdered. Approximately 0.95 kg of powdered drug material was extracted using 99% pure ethanol in the ratio of 1:2 (w/v) in a air tight container. The extract obtained was dried in a steam bath and the dried mass was weighed and recorded. The percentage of yield was calculated.

Method (Maceration)

The process consist of keeping the crude drug in intimate contact with whole of the menstruum in a closed vessel with occasionally shaking for 7 days, straining, pressing the marc. Mixing the liquid & finally clarifying by subsidence or filtration. The drug should be properly communicated. The cellular structure gets penetrated & the soluble portion is softening & dissolved. Occasionally shaking brings about a rapid equilibrium between the intra and extracellular fluid. A closed vessel is recommended so as to prevent loss of menstruum. As the degree of pressing the marc may vary the final product in not adjusted to any complete extraction. The drug menstruum ratio is 1:10. a sediment may form on standing for a few day before use. Maceration process is very simple & does not require a skilled operator [9].

Determination of preliminary phytochemical

Qualitative Phytochemical Analysis :

95% Ethanolic extract of selected plants were subjected to qualitative phytochemical investigation for the various phytoconstituents & phenolic compound like, saponins, tannins, flavonoids, alkaloids and glycosides[10].

Determination of total phenolic content

The total phenolic content of the crude extract was estimated using Folin-ciocalteu's reagent (FCR) based assay (Swain and Hills, 1959). To 200 µl of the aliquot taken from a stock solution (1 mg/ml) of the extract, 2.45 ml of water and 150 µl of FCR were added. The mixture was kept at room temperature for 5 min and then 300 µl of 1 N sodium carbonate solution was added. The mixture was kept at room temperature for 30 min and the colour developed was recorded at 765 nm. Total phenols (mg/g) in the crude extract was expressed as gallic acid equivalent (GAE), using a standard curve prepared from gallic acid (0.1 mg/ml) solution [11].

Preparation of ointment

Simple ointment containing the Ethanolic extract of *lagerstroemia microcarpa* was prepared by trituration method in a ceramic mortar and pestle using white soft paraffin ointment base. The batches of the ointment containing 5% w/w of methanol and aqueous extract were prepared for the study. Povidone iodine ointment (5% w/w) was used as the standard drug for comparing the wound healing potential of the extract in different animal models (Sunilson et al 2004)[12].

Herbal Formulations:

Formulation-

10% Polyherbal extract ointment 10 gm 95% ethanolic extract of selected herb was mixed in specific proportion according to their antimicrobial effect in 100gm simple ointment base as given below:

Antimicrobial studies of the formulation

The solutions of the ointment was prepared using 100 gm of gel in 80%w/w of Whit Soft Paraffin and 0.05%w/w . The antibacterial activity was tested by well diffusion method. *P. acne* was incubated in Nutrient agar medium for 48 hrs under anaerobic conditions. The solidified agar plates were swabbed with inoculums on the surface. The equidistance wells were cut in the plates with the help of 8 mm borer. In each of these wells the ointment solutions in dimethyl sulfoxide were placed and the plates were left at ambient temperature for 30 minutes to allow pre-diffusion prior to incubation at 37°C for 72 hrs under anaerobic conditions in an anaerobic bag (Hi-Media) with gas pack and indicator tablets and the bag was kept in an incubator for 72 hrs at 37±1°C. Gas packs containing citric acid, sodium carbonate and sodium borohydride were used to maintain and check the anaerobiosis. The indicator tablet of methylene blue changed from dark pink-blue-light pink finally, which indicated the achievement of anaerobic condition [13].

The culture of *p.acne* was prepared in nutrient agar medium at 24 hrs under aerobic conditions. Test samples of this aerobic bacterium were incubated at 37°C for 24 hrs under aerobic conditions. The antibacterial activity was estimated by measuring the diameter of the zone of inhibition. All well diffusion tests were performed experiments and antibacterial activity was expressed as the mean ± standard deviation.

Wound healing activity

Screening for wound healing activity was performed by excision wound model and incision wound model without infection. All the test sample and standard drug were applied topically once a day after cleansing with surgical cotton wool [14].

Evaluation of wound healing activity

Treatment Protocol

The Animals were depilated and wounded under light ether anesthesia, semi-aseptically. Then they were divided into 5 groups of 6 animals each & treated as follows:

Evaluation of wound healing activity of ethanol extract of lagerstroemia microcarpa.

Treatment Protocol

The Animals were depilated and wounded under light ether anesthesia, semi-aseptically. Then they were divided into 4 groups of 6 animals each & treated as follows:

Group I: Animals of this group were treated by plain ointment base.

Group II: Animals of this group were treated with Povidone iodine ointment (5%w/w).

Group III: Animals of this group were treated with Herbal ointment 5 % w/w topical .

Group IV: Animals of this group were treated with Herbal ointment 10 % w/w topical .

MODEL FOR WOUND HEALING

Incision wound model

Mid-dorsal part of the paravertebral region of rats was prepared before the experiment by cleaning and shaving the part. Incision wound was produced both in NR and DM anesthetized (ketamine 50 mg/kg, ip) rats by two parallel paravertebral incisions, 1.5cm long, made through the full thickness of the skin, 1 cm lateral to the midline of vertebral column (Ehrlich and Hunt, 1969). Wounds were closed with interrupted sutures, 1 cm apart, with surgical suture (1.0 Silk). The sutures were removed on the 7th post-wounding day[15].

Parameters used to assess Incision wound healing activity

(a) Wound breaking strength (WBS)

It was measured on the 10th post-wounding day in anaesthetized rats secured on to the operation table. A line was drawn on either side of the incision line 3 mm away from the wound. Two Allis forceps were firmly applied on to the line facing each other. One of the forceps was fixed, while the other was connected to a freely suspended lightweight polypropylene graduated container through a string run over to a pulley. Standard weights were put slowly and steadily into the container. A gradual increase in weight was transmitted to the wound site pulling apart the wound edges. As and when the wound just opened up, the weight was stopped and noted. Three readings were recorded for a given incision wound at three different sites and the procedure was repeated on the wound on the contra lateral side. The average reading of the group was taken as an individual value of WBS. Mean value gives the breaking strength for a given group.

b) Determination of tensile strength

The tensile strength of wound represents the effectiveness of wound healing. Usually wound healing agents promote the gaining of tensile strength. Tensile strength (the force required to open the healing skin) is used to measure the completeness of the healing. The tensile strength was measured by using tensiometer on 18 post wounding day [15].

Excision wound model

Each animal was anesthetized by open mask method with mild anaesthetic ether. The rats were depilated on the back and a predetermined area of 500mm^2 full thickness skin was excised in the dorsal inter scapular region. The areas of the wounds were measured (sq.mm) immediately placing a transparent polythene graph paper over the wound and then tracing the area of wound on it. This was taken as the initial wound area reading. All the test samples were applied once daily. The wound area of each animal was measured on days 0, 3, 6, 9, 12, 15, 18 after inflicting the wound [16].

Parameters used to assess Excision wound healing activity

a) Measurement of surface area of wound

To assess the area of the healing wound, the surface area was measured by tracing the boundary on semitransparent paper and calculation was done using graph paper [16] .

b) Percentage of wound contraction

Wound contraction (WC) was calculated as a percentage change in the initial wound size.

% of wound contraction (WC) = [(Initial wound size-specific day wound size)/Initial wound size] X 100.

(c) Period of Epithelisation and Scar Area

Changes in wound area were measured regularly and the rate of wound contraction calculated as given in the formula below. Significance in wound healing activity of the test groups were derived by comparing treat wound area on respective days with healed wound area of control group. The period of epithelization, *i.e.* day of fall of eschar and the scar area were also noted down.

$$\%\ \text{wound contraction} = \frac{\text{Healed Area}}{\text{Total Wound Area}} \text{x} 100$$

(Healed Area = original wound area – present wound area)

EXTRACTION OF PLANT DRUGS

The plant drug (100g) was subjected to extraction by (maceration) using ethanol as solvent for about 24 hrs. The liquid extracts were collected in a tarred conical flask. The solvent removed from the extract by evaporation method using hot plate. The extracts obtained with each solvent were weighed to a constant weight and percentage

w/w basis was calculated. The weighed extract of plant drug was stored in desiccators for further use.

The yields were found to be (11.2% w/w of crude drug) of ethanol extract ***lagerstroemia microcarpa***.

OBSERVATIONS

The percentage yield of ethanolic extract of ***lagerstroemia microcarpa*** was found to 11.2% by using maceration method. The percentage yield was found to be slight higher due to polar nature of solvent, methanol and water.

The percentage yield of ethanolic extract of ***lagerstroemia microcarpa*** was found to 11.2% by using maceration method. The percentage yield was found to be slight higher due to polar nature of solvent, methanol and water.

Results of Phytochemical test showed the presence of Carbohydrates, Flavonoids, Proteins & Amino acids, Phenols, Diterpenes and Saponins, Alkaloid was found to absent in extract ***lagerstroemia microcarpa***.

Estimation of total phenolic content

Total phenolic content was estimated by gallic acid and expressed as milligrams of gallic acid equivalent (GAE). The extract contained a considerable amount of phenolic contents of GAE/g of extract. The results were presented in Fig 3.

Estimation of total flavonoids content

Flavonoid content was calculated from the regression equation of the standard plot (y=0.020x, R^2 =0.992) and is expressed as quercetin equivalents (QE) (fig.). Total Flavonoid content was 0.165mg/100mg quercetin equivalent in HELM. Flavonoids are the most common and widely distributed group of plant's phenolic compounds.

Model for Wound Healing

Excision Wound Model: -

Excision wounds were created in rats to study the epithelization period, scar area and rate of wound contract, the measurement of progress of wound healing induced by reference drug, (5% povidone iodine ointment), two herbal test formulations viz., formulation (5% ***lagerstroemia microcarpa*** extract ointment). Day of fall of Escher indicated the Epithelization period and scar area was noted on the same day. Wound area was noted from the day of wounding and subsequently at regular time intervals, i.e. 3rd , 6th, 9th, day and then up to 18th day or till complete Epithelization period of study.

Effect of *lagerstroemia microcarpa* extract on Epithelization period and scar area

The mean epithelization period observed with Group II and IV was 14.0 and 13.6 days and scar area was 70.2 and 65.0 mm^2 respectively.

It was observed that the epithelialization period of Group II treated by reference drug were found to be almost similar i.e test formulation Group IV was 12.2 ± 0.53 and 12.9 ± 0.40 days respectively. The epithelialization times were found to be 14.5 ± 0.43 and 13.5 ± 0.45 days in experimental group I and III respectively. Average number of days that took for the shedding of scar without leaving any residual raw wound in these rats was 13.7 days and mean of scar area was 51.1 mm^2. The mean, scar area was 50.8, 57.4 and 52.6 mm^2 respectively.

Results are mean ± SEM of 6 animals in each group. P values: ^{a}<0.05, ^{b}<0.01, ^{c}<0.001 compared to respective rats (Statistical analysis was done by one way ANOVA followed by Dunnett's test for multiple comparisons).

Results are mean ± SEM of 6 animals in each group. P values: a<0.05, b<0.01, c<0.001 compared to respective NR rats and x<0.05, y<0.01 compared to respective DM rats (Statistical analysis was done by one way ANOVA followed by Dunnett's test for multiple comparisons).

TABLE 1: Preparation of Formulations

Ingredients	Quantity in100 gm
lagerstroemia microcarpa (Extract)	10%w/w
Whit Soft Paraffin	80%w/w
Methyl Paraban	0.05%w/w

Table: 2 Extractive values obtained from Lagerstroemia microcarpa leaves

S.N.	Solvent	% Yield
1.	ethanol	11.2%

Table: 3 Absorbance of standard and sample at 760nm.

Standard	Concentration (µg/ml)	Mean Absorbance
Gallic acid	5	0.127
	10	0.241
	15	0.338
	20	0.421
	25	0.578

Table: 4 Total Phenolic Content of Hydroalcoholic extract of lagerstroemia microcarpa.

Sample	Total phenolic content GAE mg/100mg
Hydroalcoholic extract 100µg/ml	0.314

Table: 5 Absorbance of standard and ethanolic extract of lagerstroemia microcarpa.

S. No	Concentration of Quercetin (µg/ml)	Mean absorbance
1	10	0.214
2	20	0.464
3	30	0.624
4	40	0.814
5	50	1.024

Table: 6 Total Flavonoid content of Hydroalcoholic extract of lagerstroemia microcarpa.

S. N.	Extracts 100µg/ml	Flavonoid content Quercetin equivalent mg/100mg
1	Hydroalcoholic extract (100µg/ml)	0.178

n=3, values are given in SEM.

Table: 7 Preparation of Formulations.

Ingredients	Quantity in100 gm
lagerstroemia microcarpa (Extract)	10%w/w
Whit Soft Paraffin	80%w/w
Methyl Paraban	0.05%w/w

Table: 8 Physical evaluation of formulation at the time of ointment formulation.

Formulation	***lagerstroemia microcarpa (Extract) Ointment***
Colour	Brown
Homogeneity	Homogenous
Ph	6.7
consistency	good
Spreadibility (gm.cm/sec)	33
Extrudability	514
Viscosity (CPS)	37.1

Table: 9 Results of sensitivity of ointment formulation.

S. No.	Microbes Codes	Microbes	Aqueous extract
1.	Bact-1	*E. coli*	Yes

Table: 10 Antimicrobial activity of standard drug against selected microbe..

S. No.	Name of drug	Microbes	Zone of Inhibition		
			10 µg/ml	20 µg/ml	30 µg/ml
1.	Ciprofloxacin	*E. coli*	14±2.21	20±3.10	28±5.12

Table: 11 Antimicrobial activity of ointment against selected microbes.

S. No.	Name of microbes	Zone of inhibition		
		25mg/ml	50 mg/ml	100mg/ml
1.	*E. coli*			
	Herbal ointment	6±0.5	7±0.3	10±0.2

Table: 12 Effect of lagerstroemia microcarpa on epithelization period and scar area in rats

Treatment Group (mg/kg, od)	Epithelization period (Days)	Scar area (mm^2)
	Rats	Rats
(I) Normal Control (Plain ointment)	14.5 ± 0.43	60.1 ± 2.3
(II) Povidone Iodine (5% w/w)	12.2 ± 0.53	50.8 ± 4.5
(III) Herbal ointment 5 % w/w topical	13.5 ± 0.45	57.4 ± 4.1
(IV) Herbal ointment 10 % w/w topical	12.9 ± 0.40	52.6 ± 4.5

Table: 13 Evaluation of wound healing activity of test formulations

Group	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
(I) Normal Control (Plain ointment)	386.2	324.3	319.1	281.4	224.3	111.5	81.5
(II) Povidone Iodine (5% w/w)	389.4	355.2	341.3	289.5	261.3	206.7	111.5
(III) Herbal ointment 5 % w/w topical	386.6	344.6	305.3	209.3	87.6	33.3	00
(IV) Herbal ointment 10 % w/w topical	383.3	342.0	292.7	187.6	187	21.3	11.3

Table: 14 % Wound Area Contraction.

Group No.	Post Wounding Days					
	Day3	Day 6	Day 9	Day12	Day15	Day18
	%Wound Area Contraction / Closure					
(I) NC (1% CMC)	9.35%	15.78%	30.67%	45.05%	60.54%	72.34%
(II) PI (5% w/w)	15.56%	30.56%	50.45%	75.90%	100%	100%
(III) HERBAL OINTMENT (5% w/w)	13.34%	26.73%	45.42%	69.38%	93.50%	100%
(IV) TFEET (10% w/w)	14.67%	28.59%	50.67%	75.98%	98.56%	100%

Figure: 1 Standard (Gallic acid) Calibration curves.

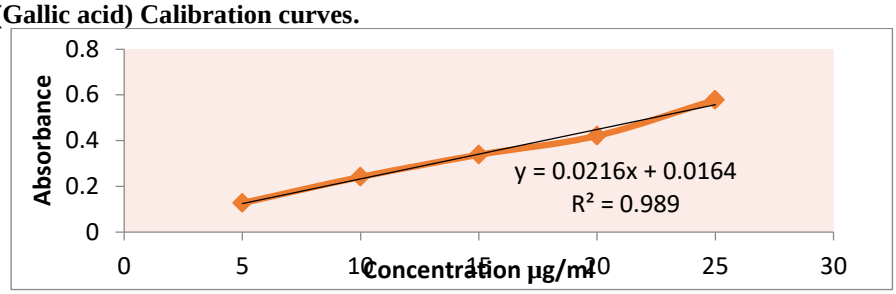


Figure: 2 Standard (Quercetin) Calibration curves.

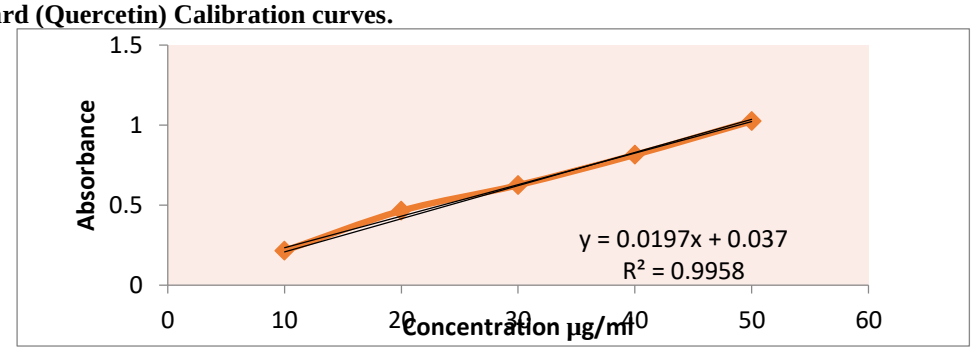


Figure: 3 Effect of lagerstroemia microcarpa on scar area in rats.

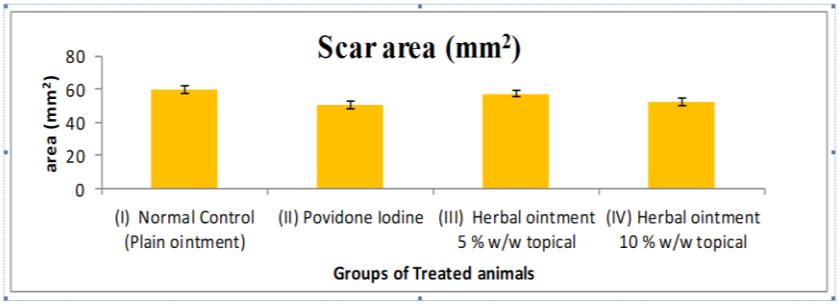


Figure: 4 Effect of Lagerstroemia microcarpa on epithelization in rats.

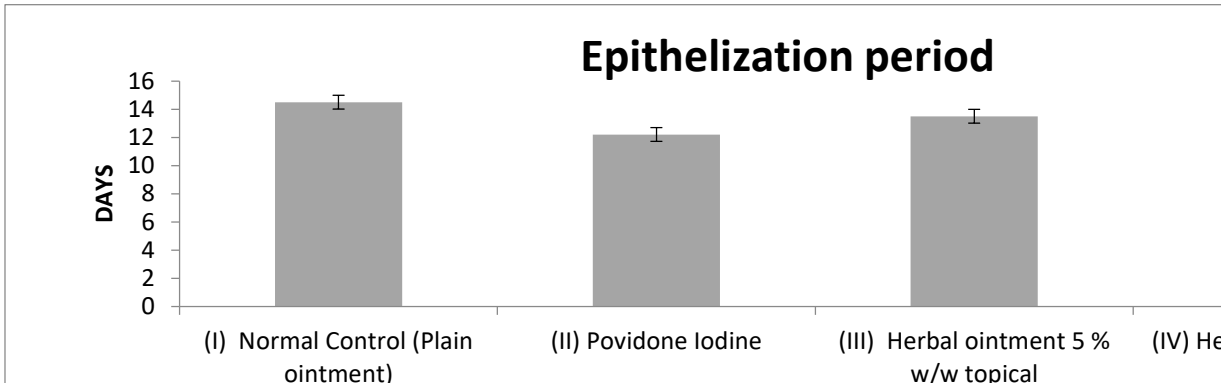


Figure: 5 Wound area contraction in mm2 in Group- I Normal Control animals treated with simple ointment base from day 0 to day 18th.

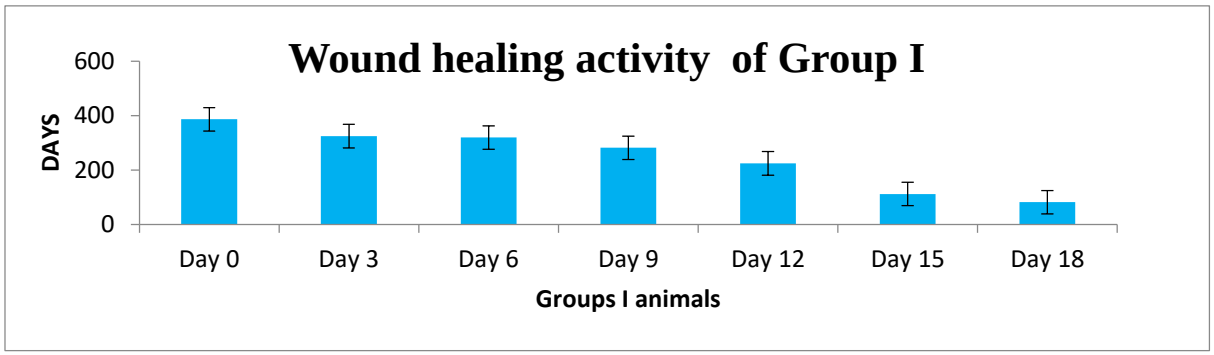


Figure: 6 Wound area contraction in mm2 in Group-II animals treated with simple ointment base from day 0 to day 18th.

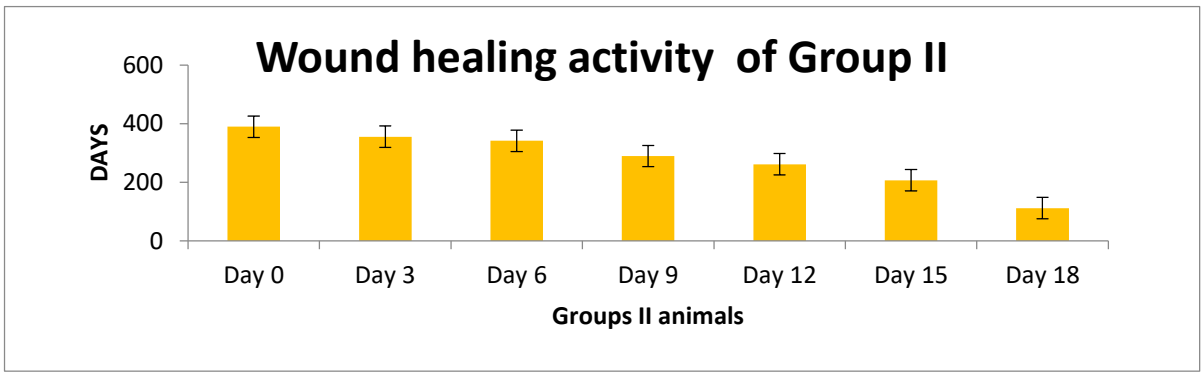


Figure: 7 Wound area contraction in mm2 in Group III reference ointment (5% povidone iodine) treated group from day 0 to days 18th.

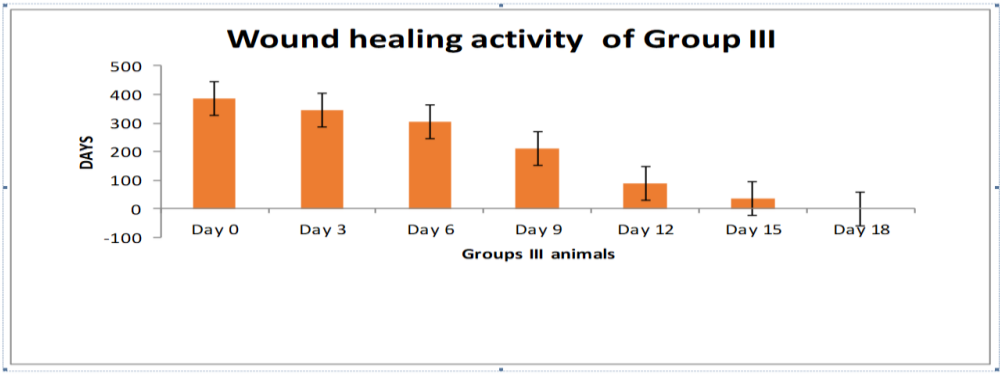


Figure: 8 Wound area contractions in mm2 in Group-IV Diabetic experimental animals treated with formulation I from day 0 to 18th day.

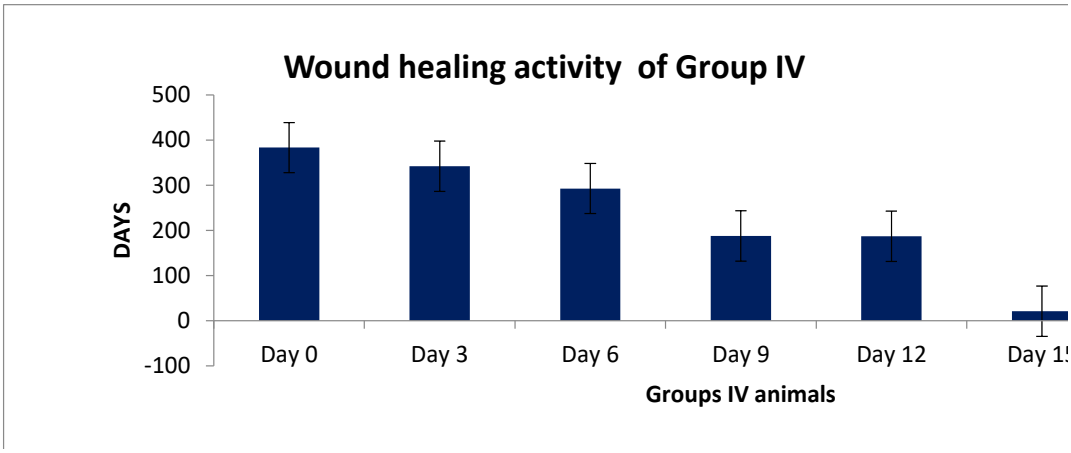
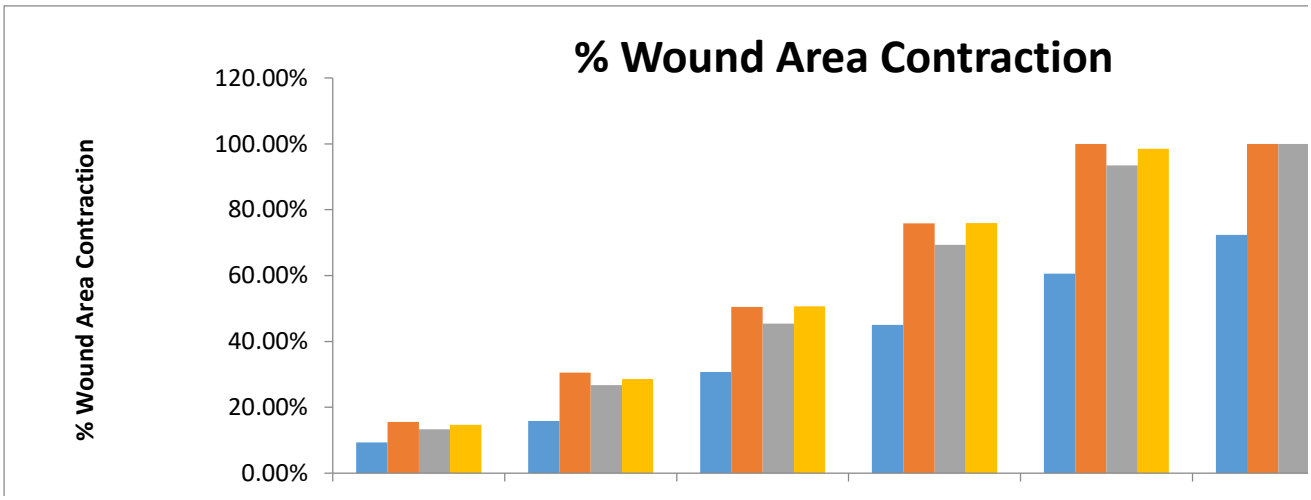


Figure: 9 Percent wound contraction from day 0 to day 18th in Group I Normal control animals treated with simple ointment base.



Average mean wound area closure: -

Wound area was traced manually and photographed in each 3 days interval and healed area was calculated by subtracting from the original wound area.

The results of the mean excision wound area of different groups on the day of wound infliction (day 0) were found to be 386.2± 0.82, 389.4 ± 3.57 , 386.6 ± 5.27, 383.3 ± 0.81 mm^2 in Group I, Group II, Group III, Group IV respectively.

Group II as reference ointment (5% povidone iodine ointment) treated animals group. On the day 0 till day 6^{th} there were no significant wound contractions observed in experimental groups. On the 9^{th} day, significant wound contraction process started in experimental treatment groups. On the day 2, wound area was measured.

On the 12^{th} day wound areas were measured to be 224.3 ± 2.23 mm^2, 261.3±1.36 mm^2 in animals respectively. From 15^{th} day onwards, we have observed the complete closure of wound in ointment treated and reference ointment (povidone iodine ointment) treated groups. Complete closure of wound was observed in Group IV, on the 18^{th} day. On the 18^{th} day, the average wound areas measured animals were found to be 111.5±1.73 mm^2 and 11.3±1.36 mm^2 respectively.

The analysis of values obtained in this particular parameter reveals that all three different herbal test formulations topically applied, have shown significant effect on wound closure.

Percent Wound Contraction

The values calculated for percentage reduction of wound area for excision wound model in different treatment groups have been given .According to it, 100% wound closure was observed for test formulation (5% Polyherbal extract ointment treated group) and reference drug (povidone iodine ointment treated group) on the15th day while 98.56%, and 93.59%% wound closures were observed with test formulation

Whereas, 100% wound closure was observed on 17th & 18th day. However, animals of the test group were observed for the complete wound closure for more than 18 days. It was observed that both the above mentioned groups exhibited 72.34% and 60.56% wound closure respectively on the 18^{th} day.

CONCLUSION

From the above research work it was clear that, the selected plant lagerstroemia microcarpa has potent antimicrobial and wound healing activity. Phytochemical test showed the presence of Carbohydrates, Flavonoids, Proteins & Amino acids, Phenols, Diterpenes and Saponins, Alkaloid was found to absent in extract ***lagerstroemia microcarpa*** which indicates the presence of phenols and flavonoids in higher percentage .The Isolation and characterization and various instrumental analysis were required for further studies.

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