ZITA MEDICAL MANAGEMENT

www.hsmc.gr/en/pharmakeftiki-journal/

ΦΑΡΜΑΚΕΥΤΙΚΗ, 36, I, 2024 | 53-63 ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

PHARMAKEFTIKI, 36, I, 2024 | 53-63 RESEARCH ARTICLE

Pharmacological Exploration of *in Vitro* Antibacterial and *in Vivo* Wound Healing Using *Basella Alba* Ethanolic Stem Extract Ointment On Rats

Narayanaswamy Harikrishnan, Venkat Naga Garlanka Kiranmayi*, Selvadurai Ranjith, Periyasamy Sharan, Mohamed Mujibur Rahman Thalal, Nagappan Tharunkumar

Faculty of Pharmacy, Dr. M. G. R.Educational and Research Institute,
Deemed to be University, Chennai -95, Tamil Nadu, India

KEYWORDS:
Basella alba; Antimicrobial activity; Agar well diffusion method;Epithelialisationperiod;burn wound model.

ARTICLE INFO:
Received: April 3, 2023
1rst revision: June 10, 2023
2nd revision: August 31, 2023
Accepted: September, 9, 2023
Available on line April 4, 2024:

CORRESPONDING AUTHOR:
G.V.N.Kiranmayi*
E-mail: kiranmayi54@gmail.com
7286863529

ABSTRACT

The present study was undertaken to determine the in vivo wound healing activity and in vitro antibacterial activity of Basella alba stem. Wound healing activity was evaluated using burn wound model in rats. Wound healing is a complex phenomenon which consists of three stages that includes inflammatory,proliferative, and remodelling.Traditionally Basella alba is used for wound healing. There is no specific scientific data available for the wound healing activity of Basella alba stem. The wound healing activity of Ethanolic Stem Extract of Basella alba was evaluated by burn wound models. The parameters studied includes the epithelialisation period and rate of wound contraction. It also possess antibacterial activity. Ethanolic Stem Extractof Basella alba was tested against both gram positive and gram negative bacteria using agar well diffusion method. From the results, it was concluded that Ethanolic Stem Extract of Basella alba has significant wound activity and antibacterial activity .

INTRODUCTION

After injury, tissue healing process is initiated for the restoration of integrity and function of damaged tissue in response to injury[1].The inflammatory response starts immediately after injury and the cells beneath the dermis begin to raise collagen production to generate epithelial tissue. The process of wound healing involves three phases which includeinflammation, proliferation and re-

modelling.The inflammatory phase is associated with pain, swelling, heat and redness at the wound site. During proliferative phase,the wound is reconstituted with collagen and granular tissue and is followed by angiogenesis, epithelialisation, and wound contraction. Angiogenesis is characterised by new blood vessels formation from endothelial cells. In the formation of extracellular matrix, collagen and fibronectin are excreted from fibroblast. There upon griping the wound edges by myofibroblasts would undergoes contraction using a mechanism similar to that observed in smooth muscle cells[2].

According to literature, many plants possess potent wound healing activity[3], since they contain many bioactive compounds, such as polyphenols, flavonoids and alkaloids

Phenolic compounds, possess potent antifungal, antiviral and antibacterial activity[4]. Polyphenols have special significance in the case of strains resistant to antibiotics, such as *Staphylococcus aureus* resistant to methicillin, *Enterococci* resistant to glycopeptide antibiotics, vancomycin and pneumococci resistant to β-lactams and macrolides, and *Pseudomonas aeruginosa* with its defense mechanism against phagocytic activity of polymorphonuclear leucocytes[5]. Phenolic compounds as antioxidants plays an important role in skin tissue repair mechanisms and may be used in the treatment of various skin damage, such as wounds and burns. In acute and chronic wounds, they may accelerate the healing process and promote proliferation of normal skin cells[6].

Among phenolic compounds, flavonoids are isolated from a wide range of vascular plants, with over 8000 individual compounds known[7] . They possess wide variety of activities such as antimicrobial[8] , anti-oxidant[9] , anti-cancer[10], anti-inflammatory[11] and wound healing[12]. Flavonoids are essential bioactive compounds found in our daily life in the form of fruits and vegetables.There is a lot of resaerach that shows the importance of flavonoids as a wound healing agent. A representative example are the flowers of *Ipomoea carnea* which belong to the family Convolvulaceae. The bioactive compounds, isolated from the flowers, such as Kaempferol, Kaempferol-3-O-β-D-glucoside confirm the wound healing activities on all animal models[13]. The role of flavonoids in wound healing is recently reviwedby Zulkefliet al[14] .

Many alkaloids, an important class of bioactive compounds present in plants, also cause an increased rate of formation of epithelial cells thus speeding up the re-epithelialization process which is critical in wound healing. There is also the possibility that they accelerate angiogenesis.This will in turn increase blood supply to the newly formed epithelial cells and thus in effect cause an overall increase in the rate of wound contraction[15].

Basella alba is a medicinal plant which belongs to the family of Basellaceae.It is also known as Malabar spinach, Indian spinach, Ceylon spinach and vine spinach. The stem is succulent with tender leaves. Both stem and leaves are used in Culinary practice in Southern parts of India. It is found to be a good source of calcium, iron, vitamin A and Vitamin C[16-17]. So far much pharmacological work has not been carried out on *Basella alba stem*. In folkloric medicine it is used for treating various injuries and wounds. Since it grows as a weed and found abundantly in wastelands, we made an attempt to explore its wound healing efficacy and highlight itsimportance.

In this aspect the aim of present research is to preliminarily determine the phytochemical constituents and the wound healing activity of Ethanolic Stem Extract of *Basella alba stem*. As a part of this study, effort was made to determine also, the antimicrobial activity of *Basella alba* against gram positive organism like *Staphylococcus aureus,Bacillussubtilis* and gram negative organism like *Pseudomonas aeruginosa* and *Escherichia coli*.

MATERIALS AND METODS

Collection of Plant

The *Basella alba stems* were collected near Peddapuram,Andhra Pradesh, India. The plant was authenticated by Dr.T.Raghuram, Taxonomist, Maharani College, Peddapuram.

Preparation of Ethanolic *Basella alba stem* extract

The freshly collected stems of *Basella alba* were

washed with water to remove dirt and sand particles and dried under shade for 40 days.They were grounded into powder using a mechanical grinder. The powder was extracted with 95% ethanol for 3 days, followed by hot percolation for 3 hours. Then it was filtered and distilled at 80°C. Subsequently it was transferred into the empty china dish and evaporated to get an ethanolic extract and kept in anhydrous calcium chloride containing desiccators.

Preparation of Ointment

The ethanolic extract Ointment of *Basella alba stem* was prepared in two concentrations. One is 10% and the other one is 20%.The 10% ointment of *Basella alba stem* consists of 2 grams of stem extract in 20grams of ointment base.The 20% ointment of *Basella alba stem* consists of 4 grams of stem extract in 20 grams of ointment base. This ointment was stored in air tight container.

Phytochemical Testing

Qualitative screening

The qualitative phytochemical screening of the extract was performed to identify the main groups of chemical constituents (glycosides, alkaloids, tannins, saponins, terpenoids, carbohydrates, anthraquinones glycosides, flavonoids, and phenols) present in the extracts using the color reactions[18].

Test for tannins

About 0.1 g of the extract boiled in 2 ml of water/ dimethyl sulfoxide (DMSO) was filtered and mixed with a few drops of 0.1% of ferric chloride. Then, it was examined for brownish green or a blue black coloration.

Test for alkaloids

10 ml of acidified alcohol was added to 0.1 g of the extract and was boiled and filtered. Then, 0.4 ml of dilute ammonia and 1ml of chloroform was added to 1 ml of filtrate and shaken gently. 2 ml of acetic acid was used to extract chloroform layer. This was then divided into two portions, and Mayer's reagent was added to one portion while Dragendroff's reagent to another. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Dragendroff's reagent) was taken as positive for a test for alkaloids.

Test for glycoside

0.2 g of the test material was extracted with 5 ml of each dilute sulfuric acid and water by boiling/ warming on a water bath. Then, the acid extract was filtered and neutralized with 5% solution of sodium hydroxide. Similarly, in the case of water extract an equal amount of water instead of sodium hydroxide was added. Fehling's solution A and B were added until both solutions became alkaline and heated for 2 min using a water bath. If the quantity of red precipitate from acid extract is higher than that obtained from water extract, the presence of glycoside is identified.

Test for carbohydrates (Molisch's test)

The development of purple ring at the interface between the test material and the acid on the addition of Molisch's reagent (a-naphthol dissolved in ethanol) to the extracts followed by addition of a few drops of concentrated sulfuric acid indicates the presence of carbohydrates.

Test for saponins

Development of emulsion on vigorous shaking after addition of 3 drops of olive oil to froth extracted by adding 0.1 g of extract to 1 ml of distilled water indicates the presence of saponins.

Test for flavonoids

Zinc hydrochloride test: Development of red color after a few minutes of addition of a mixture of zinc dust and conc. hydrochloric acid to the test solution was taken as positive for flavonoids.

Test for terpenoids (Salkowski's test)

Development of a reddish brown coloration at the interface after addition of 0.4 ml of chloroform followed by concentrated sulfuric acid to 0.1 g of the extract indicates the presence of terpenoids.

Test for proteins

Development of violet color on addition of biuret reagent (2 ml) to the test solution (2 ml), indicates the presence of proteins.

Test for phenol

50 mg of the extract was dissolved in 5 ml of distilled water and a development of a dark green color after addition of a few drops of neutral 5% ferric chloride solution was regarded as positive for phenolic compounds.

Quantitative phytochemical testing

10 mg of extract is dissolved in 10 mL of methanol to make 1000 g/ml aliquots of extract.

Phenolic contents estimation

Using the Folin-Ciocalteu method[19], the phenolic content of ethanolic stem extract (1 mg/ml, aliquots) was determined. 0.5 ml extract was combined with 3 ml Folin-Ciocalteu reagent (1:10 v/v) and allowed to stand for 5 min. In the mixture tube, 4 ml of sodium carbonate solution (20% w/v) was applied. For color growth, the tubes were held at 30°C for 15 min. A spectrophotometer was used to calculate the absorbance at 765 nm. The Gallic acid equivalent (mg-GAE/g) dry weight of extract was measured using a calibration curve . The results were expressed as Gallic acid equivalent mg/100mg dry weight of the extract. Fig 1 shows the mean absorbance of various concentrations of gallic acid and regression equation used to calculate total phenolic content of the extract.

Flavanoids content Estimation:

The total flavonoid content ethanolic stem extract (1 mg/ml, aliquots) was determined using the aluminum chloride method[20]. 0.6 ml extract, 1.8 ml methanol, 0.1 ml 10 percent aluminum chloride, 0.1 ml 1 M sodium acetate, and 3 ml distilled water were added to 0.6 ml extract, 1.8 ml methanol, 0.1 ml 10 percent aluminum chloride, 0.1 ml 1M sodium acetate, and 3 ml distilled water were added and left at 30°C. After 30 min at 415 nm, the absorbance was assessed individually. Total flavonoid was calculated using standard quercetin calibration curve and expressed as quercetin equivalent mgQE/g dry weight of the extract. Fig 2 shows the mean absorbance of various concentrations of Quercetin and regression equation used to calculate flavonoid content of the extract.

Alkaloid content estimation

The alkaloid content of the extract was calculated using the Fazel et al. process, in which an ethanolic stem extract (1 mg/ml, aliquots) was dissolved in 2N Hydrochloric acid and filtered. 0.1 N NaoH was applied to the filtrate, 1 ml was transferred to a separating funnel, and 5 ml of bromo cresol green solution and 5 ml of phosphate buffer were added. Chloroform was used to remove the mixture after shaking it. At 470 nm, the absorbance was measured. The concentration of alkaloid content in atropine equivalents was determined using the unit's mg/100mg dry weight of extract[21], and the alkaloid content was calculated by using calibration curve. Fig 3 shows the mean absorbance of various concentrations of Atropine and regression equation used to calculate alkaloid content of the extract.

Experimental Animals

Rats of either sex weighing about 150-200 grams were used for the study. Three animals were used in each groupand totally 12 animals were used. All the animals are properly caged and maintained under standard pellet diet and water ad libitum, placed in a properly air conditioned room with 12hrs light and dark cycles. The animal experiments were performed based on the Institutional Animal Ethical Committee (IAEC) approval no.V/IAEC /DR.MGR/2053/PoReb1/S/19/ CPCSEA/18.06.22/01.

Table 1: Preliminary Phytochemical Screening of *Basella alba* stem Ethanolic Extract

Compounds	EE
Alkaloids	+ve
Phenolics	+ve
Glycosides	+ve
Flavonoids	+ve
Saponins	+ve
Triterpenoids	+ve
Tannins	+ve
Carbohydrates	+ve
Proteins	+ve

+ve -Presence

Table 2 : Quantitative screening of phytochemicals of of *Basella alba* stem Ethanolic Extract

Content	*Basella alba* stem Ethanolic Extract (mg/g)
Alkaloid content	3.82 ± 0.39
Flavanoid content	4.42 ± 0.43
Phenolic content	1.16 ± 0.26

All the values were expressed in mean ± SEM, n = 3

Wound Healing Activity

Model taken for the evaluation of wound healing activity, was burn wound model[22].The ethanolic stem extract of *Basella alba stem* was used for the study. For Burn wound model, animals were divided into four groups each consisting of three animals as follows: Group I, left untreated and considered as Control, Group II, which served as standard and was treated with 5% (w/w) povidone iodine ointment USP (Betadine), Groups III and IV which were treated with 10% and 20% (w/w) ointments of ethanolic *Basella alba stem extract*, respectively. All the treatments were given topicallythricea day. In wound healing model simple oitment base was used as an control.

Burn Wound Model

Partial thickness of burn wounds were inflicted on overnight in starved animals under lignocaine anesthesia by pouring the hot molten wax (2 g) at 80 °C. The wax was poured on the animal shaven area through a cylinder of 300 mm2 circular opening. The wax gets to remain on the skin till it gets solidified. Immediately after the injury and on subsequent days, the drug or base was applied topically as mentioned [22].

Percentage wound healing = 100-[final diameter(cm) × 100/ initial diameter (cm)]

Microbial Cultures

Microbial cultures were obtained from Microbes Speciality Lab.They are aseptically maintained in our laboratory. The gram positive bacteria are*Staphylococcus aureus (ATCCBAA 1026), Bacillus subtilis (ATCC 11774) . The* gram negative bacteria used in the study were *Escherichia coli (ATCC 10536)*and *Pseudomonasaurgenosa(ATCC 10662).*

Antimicrobial Activity

The antimicrobial screening is based on measuring the diameters of the zones of inhibition around the cylindrical cups incubated with different concentrations of ethanolic *Basella alba stem extract. For this,sabouraud dextrose agar plates (SDA) seeded with microbial cultures were used. The cups of diameter 6mm wereprepared by using sterile borer in the microorganisms diffused agar medium. The 50µL ethanolicBasella alba stem* extract inconcentration of 100,200mg/ml dissolved in DMSO were filled in the wells. For antibacterial activity, gentamycin (25µg/ml) was used as standard drug. All the prepared

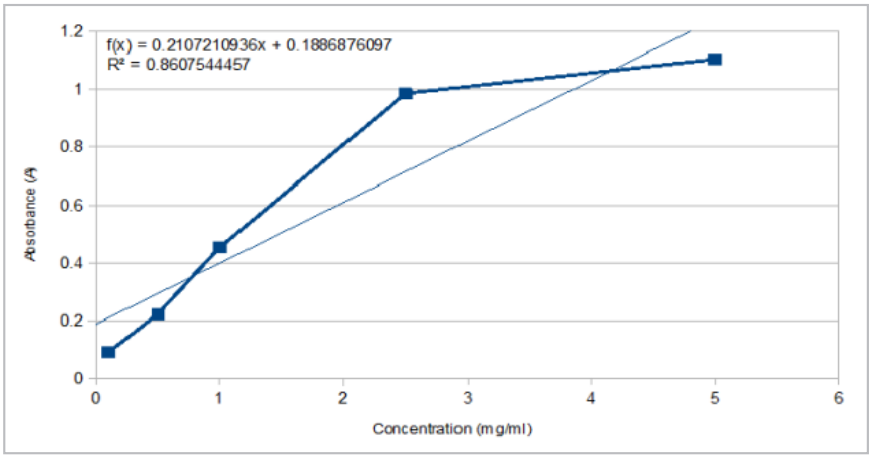


Figure 1 : *Calibration curve of Gallic acid*

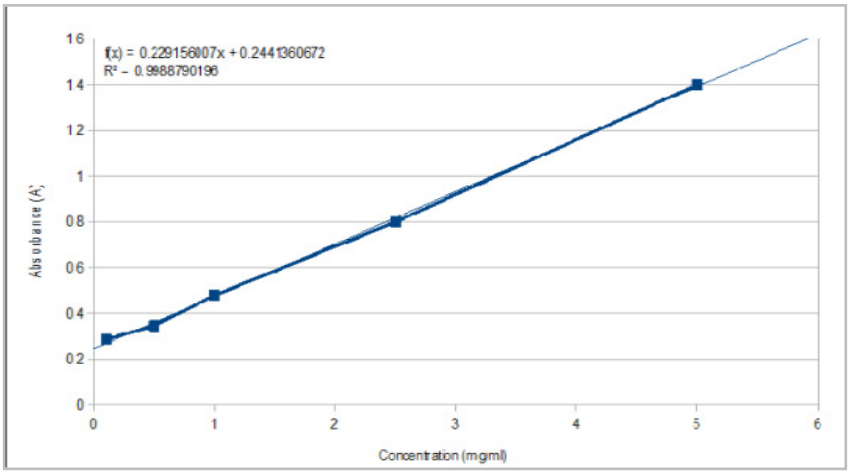


Figure 2 : *Calibration curve of Quercetin*

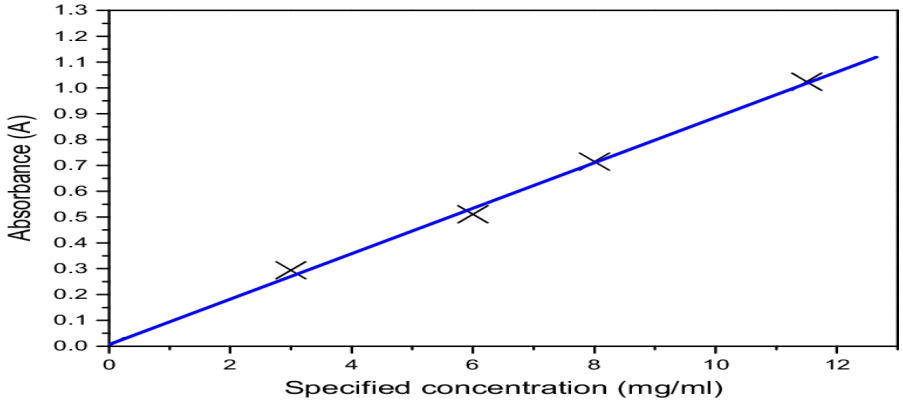


Figure 3 : *Calibration curve of Atropine*

Table 3:Effect of ethanolic extract prepared ointment of *Basella alba stem* by using oleaginous ointment base for healing in burn wound model

Groups	Post wounding days								Period of epithelialisation
	0 day	3rd day	6th day	9th day	12th day	15th day	18th day	21st day	
Control (Oleaginous Ointment base)	2.65±0.01	2.57±0.01	2.24±0.02	1.95±0.01	1.54±0.02	0.89±0.02	0.75±0.03	0.54±0.01	23.69±0.12
Standard (Betadine)	2.67±0.01	2.41±0.04	1.74±0.02	0.95±0.02	0.11*±0.01				13.67±0.38
Ethanolic extract of *Basella alba stem* (10%w/w ointment)	2.02±0.02	1.85±0.06	1.62±0.025	1.26±0.3	1.00±0.28	0.80±0.04	0.28*±0.02		17.66±1.5
Ethanolic extract of *Basella alba stem* (20%w/w ointment	2.43±0.2	2.04±0.04	1.53±0.035	1.4±0.045	0.98±0.04	0.25*±0.03			15.33±0.83

Valuesareexpressed interms ofmean± SEM;n=3animals ineachgroup;
**p<0.05.Statisticallysignificant differenceincomparison with control group.*

plates with extracts and standard were kept in a refrigerator at 2 to 8 °C for a period of 2 h for effective diffusion of test compounds and standards. Later, they were incubated at 37 °C for 24 h. The assay was carried out in triplicate. The diameter of the zone of inhibition was measured and recorded[23]. The control used in antimicrobial activity is DMSO.

Statistical Analysis

Statistical analysis was carried out by using either one-way analysis of variance (ANOVA) or unpaired t-test and then followed by post tests like Dunnett's test for multiple comparisons by using Graph Pad Prism Software .

RESULTS

Preliminary and Quantitative Phytochemical Screening

The ethanolic extract was dark brown in colour. The results of preliminary phytochemical analysis of ethanolic extract of *Basella alba stem* indicated the positive result for the secondary metabolites likePhenols, alkaloids,flavanoids,Steroids,Triterepenoids (Table 1).

Wound Healing Activity

When compared to control group, the wound healing ability of ethanolic extract of *Basella alba stem* of different concentrations by using oleaginous ointment base on burn wound models was significantly greater. Similarly, when compared to standard drug (povidone iodine ointment) treated group the ethanolic stem extracts ointment treated groups showed wound healing on 3rd day onwards in burn wound models.The results were indicated in tables 3&4.

Burn Wound Model

The epithelialisation period was greater with 20% dose of *Basella alba stem extract* and 100% wound

Table 4:Wound Healing Percentage by *Basella alba* Ethanolic Stem Extract Oleaginous Ointment Base in Burn Wound Model

Groups	Percentage(%) ofwoundhealing ontheday						
	3rd day	6th day	9thday	12th Day	15th day	18th day	21st Day
Control (Oleaginous Ointment base)	3.01	15.47	26.41	41.88	66.41	71.69	79.62
Standard (Betadine)	9.73	34.83	64.41	95.88			
Ethanolic extract of *Basella alba stem* (10%w/w ointment)	8.41	19.80	37.62	50.49	60.39	86.13	
Ethanolic extract of *Basella alba stem* (20%w/w ointment	16.04	37.03	42.38	59.67	89.71		

Values are expressed as mean percentage in each group;

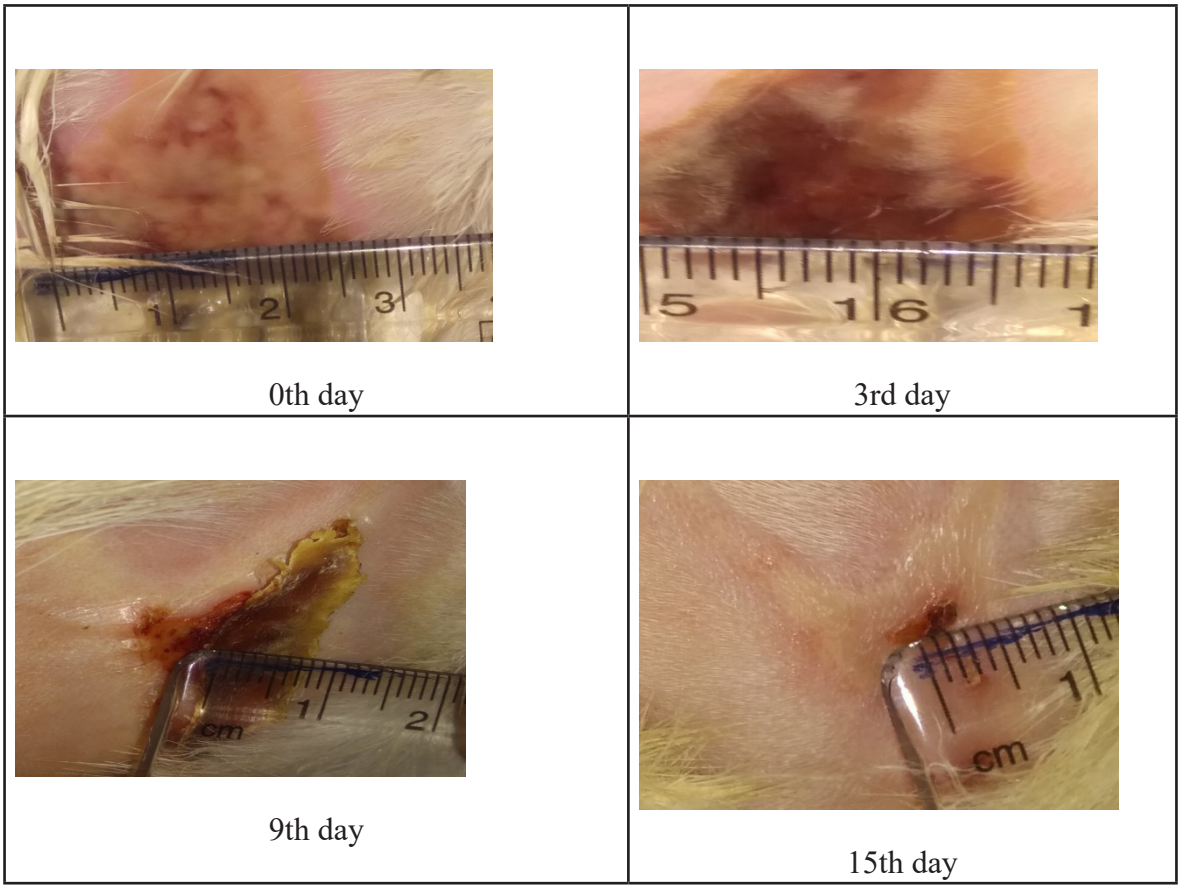


Figure 4: *Effect of ethanolic extract prepared ointment of* ***Basella alba stem*** *on burn wound healing model*

Table 5: Zone of inhibition of ethanolic extract of *Basella alba* stem

Type of bacteria	Name of microorganism	Control (DMSO)	Ethanolic extract (100mg/ml)	Ethanolic extract (200mg/ml)	Gentamycin (25ug/ml)
Gram positive bacteria	***Staphylococcus aureus***	7.2±0.45	11.5±0.5	13.36±0.55	15.2±0.2
	Bacillus subtilis	6.2±0.36	13.56±0.41	15.53±0.4	19.23±0.25
Gram negative bacteria	***Escherichia coli***	8.1±0.35	12.43±0.51	14.56±0.49	22.13±0.15
	Pseudomonas aerugenosa	6.45±0.54	8.26±0.31	18.5±0.4	15.1±0.1

contraction was observed for ethanolic extract on (15.33±0.83) days respectively, which were almost similar to that of povidone iodine ointment treated group (13.67±0.38) days.The group of rats treated with ointment prepared with 10% of ethanolic extractshowed wound contraction on 6th day onwards and achieved 100% wound healing in (17.66 ±1.5) days, as shown in Table 3&4 and Figure 4.

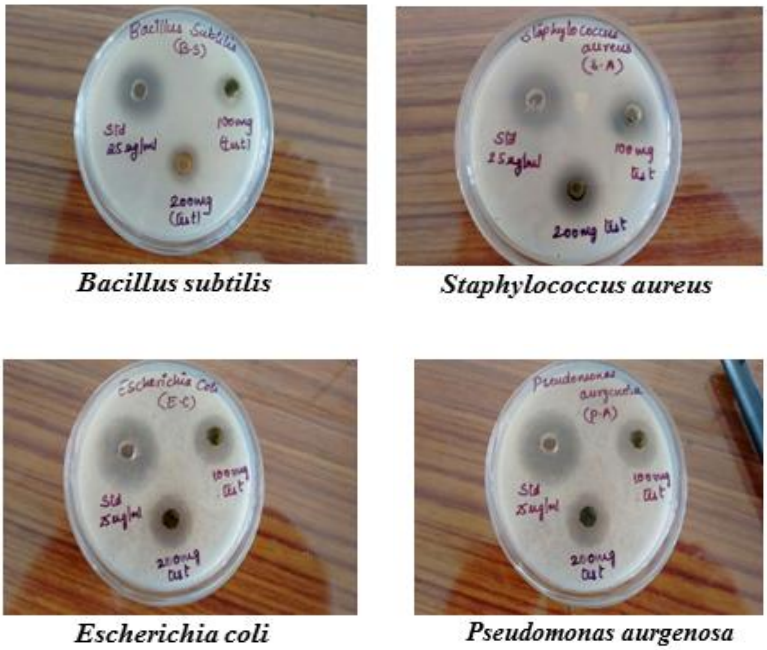
Antibacterial Activity

The present study was carried out to evaluate antimicrobial activities of various extracts of *Basella alba stem*.Thestem extract was prepared using 95%ethanol. The extract of *Basella alba stem*was tested for their antimicrobial efficiency against gram positive bacteria which includes *Staphylococcuaureus*,*Bacillus subtilis,* gram negative bacteria which includes *Escherichia coli, Pseudomonas aerugenosa*at a dose of 100mg/ml and 200mg/ml. The standard drugused for comparison was Gentamycin. The ethanolic extract at the dose of 100mg/ml showed the inhibition zone of *Staphylococcus aureus* equal to 11.5±0.5, of *Bacillus subtilis*13.56±0.41,of *Esericchia coli* 12.43±0.51 and of *Pseudomonas aerugenosa*8.26±0.31,Extract of 95% ethanol at a dose of 200mg/ml showed the inhibition zone of *Staphylococcus aureus* equal to 13.36±0.55, of *Bacillus subtilis* 15.53± 0.40*, of Escherichia coli*14.56±0.49 and of *Pseudomonasaerugenosa* (18.5± 0.4).These results, along with those of the control group and the standard drug are presented in Table 5 and Figure 5.

DISCUSSION

The preliminary qualitative and quantitative phytochemical screening of the ethanolic extract shows presence of alkaloids and flavonoids (Table 1 & 2). In the present study, burn wound model was used to assess the wound healing activity of *Basella alba* stem extract on various phases of wound healing. The main aim of wound healing is to close the wound without causing discomfort and less pain to the patient in the short time. After 8days of injury,redness and swelling was reduced this indicates that the prepared ethanolic extract ointment hastissue debride effect.

The phytochemical constituents present in *Basella alba stem* may be responsible for wound-healing activity of *Basella alba stem***.**The accelerated process of wound healing may be due to the individual or the additive effects of the phytoconstituents function. The increased epithelialisation period observed in our study may be contributed by the phytoconstituents present in *Basella alba stem.*Groups treated with ointment prepared with ethanolic extract ex-



Bacillus subtilis *Staphylococcus aureus*

Escherichia coli *Pseudomonas aurgenosa*

Figure 5: *Zone of inhibition of Ethanolic Extract of* **Basella alba** *stem*

hibited increasedepithelialisation period and the phytoconstituemts present helps to increase in collagen and results infibres stabilization[24].

Basella alba

Stemextract exhibited promising and somewhat better wound healing promoting activity similar to that of the standard povidone iodine ointment(Betadine).Ethanolic extract prepared ointment was found to show antibacterial efficacy against gram positive and gram negative organism which include *Escherichia coli, Bacillus subtilis, Staphylococcus aureus*and*Pseudomonas aerugenosa*. The extract has an antibacterial effect against the selected organisms in this study suggesting that the wound healing activity of *Basella alba stem* extract may be by an antibacterial activity mechanism.The presence of various phtochemicals like phenols ,flavonoids and alkaloids are responsible for the therapeutic potential exerted by *Basella alba stem*

CONCLUSION

The present study has demonstrated that the ethanolic extract of *Basella alba stem* has properties that arecapable of promoting increased wound-healing activity compared to that of placebo control.The plant has shown also significant antimicrobial activity. It may be due to the presence of major active constituents present in the plant. Further studies are required to confirm the main active constituents responsible for the activity. The above results could justify the inclusion of the plant in the wound healing management in folk[25]. □

REFERENCES

1. Evans P. The healing process at cellular level: a review. *Physiotherapy*.66(8), 256-9, 1980.
2. Nayak B.S., Anderson M., Pereira L.P. Evaluation of wound-healing potential of Catharanthus roseus leaf extract in rats. *Fitoterapia.* 78(7-8), 540-4, 2007.
3. Biswas T.K., Mukherjee B. Plant medicines of Indian origin for wound healing activity: a review. *Int. J. Low Extrem. Wounds.* 2(1),25-39, 2003.
4. Czemplik M., Zuk M., Kulma A., Kuc S., Szopa J. GM flax as a source of effective antimicrobial compounds. *Sci. Microb.Pathog.Commun.Curr. Res. Technol. Adv.76*, 39-47, 2011.
5. Zuk M., Dorotkiewicz-Jach A., Drulis-Kawa Z., Arendt M., Kulma A., Szopa, J. Bactericidal activities of GM flax seedcake extract on pathogenic bacteria clinical strains. *BMC Biotechnol. 14*, 70, 2014.
6. Thang P. T., Teik L. S., Yung C. S. Anti-oxidant effects of the extracts from the leaves of Chromolaena odorata on human dermal fibroblasts and epidermal keratinocytes against hydrogen peroxide and hypoxanthine–xanthine oxidase induced damage. *Burns*, *27*(4), 319-327, 2001.
7. Pier-Giorgio P. Flavonoids as antioxidants. *J. Nat. Prod. 63*(7), 1035-1042, 2000.
8. Cushnie T. T., Lamb, A. J. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents*, *26*(5), 343-356,2005.
9. Procházková D., Boušová I., Wilhelmová N. Antioxidant and prooxidant properties of flavonoids. *Fitoterapia*, *82*(4), 513-523, 2011.
10. Ren W., Qiao Z., Wang H., Zhu, L., Zhang L. Flavonoids: promising anticancer agents. *Med. Res. Rev. 23*(4), 519-534 ,2003.
11. Kim H. P., Son K. H., Chang H. W., Kang S. S.Anti-inflammatory plant flavonoids and cellular action mechanisms. *J. Pharmacol. Sci. 96*(3), 229-245, 2004.
12. Chirumbolo S. Flavonoids in propolis acting on mast cell-mediated wound healing. *Inflammopharmacology*, *20*, 99-101, 2012.
13. Ambiga S., Narayanan R., Gowri D., Sukumar D., Madhavan S. Evaluation of wound healing activity of flavonoids from Ipomoea carnea Jacq. *Anc. Sci. Life*, *26*(3), 45, 2007.
14. Zulkefli N., Che Zahari C. N. M., Sayuti N. H., Kamarudin A. A., Saad N., Hamezah H. S., Sarian M. N. Flavonoids as potential wound-healing molecules: Emphasis on pathways perspective. *Int. J. Mol. Sci. 24*(5), 4607, 2023.
15. Ketuly K. A. Acute toxicity study and wound healing potential of Gynura procumbens leaf extract in rats. *J. Med. Plant Res. 5*(12), 2551-2558, 2011.
16. Palada M.C., Chang L.C. International Co-operators guide: "Suggested cultural practices for Basella", AVRDC. 2004. AVRDC Report 2003. T.Kalb ed. AVRDC Publication Number 04-599. Shanhua, Taiwan: AVRDC—The WorldVegetableCenter,2003.
17. Prance G., Nesbitt M. (eds) (2005) Cultural history of plants. Routledge, New York. p. 114.
18. Savithramma N., Rao M. L., Suhrulatha D. Screening of medicinal plants for secondary metabolites. *Middle-East J. Sci.Res. 8*(3), 579-584, 2011.
19. Stratil P., Klejdus B., Kubáň, V.Determination of total content of phenolic compounds and their antioxidant activity in vegetables evaluation of spectrophotometric methods. J. *Agric. Food Chem.54*(3), 607-616,2006.
20. Chang C. C., Yang M. H., Wen H. M., Chern J. C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal. 10*(3), 178-182, 2002.
21. Shamsa F., Monsef H., Ghamooshi R., Verdian-Rizi M. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. *Thai J. Pharm. Sci. 32*, 17-20, 2008.
22. Rashed A. N., Afifi F. U., Disi A. M. Simple evaluation of the wound healing activity of a crude extract of Portulaca oleracea L.(growing in Jordan) in Mus musculus JVI-1. *J. Ethnopharmacol. 88*(2-3), 131-136, 2003
23. Pharmacopoeia, I. Controller of publications. *New Delhi*, *2*, 764.,1996.
24. Udupa A. L., Kulkarni D. R., Udupa S. L. Effect of Tridax procumbens extracts on wound healing. *Int. J. Pharmacogn. 33*(1), 37-40, 1995.
25. Ehrlich, H. P., & Hunt, T. K.Effects of cortisone and vitamin A on wound healing. *Ann. Surg. 167*(3), 324-328,1968.