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Evaluation of wound healing and antimicrobial activity of root and whole plant of *Byttneria herbacea* Roxb. (*Samarakhadyam*)

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Byttneria herbacea Roxb., family Sterculiaceae, a folklore herb commonly known as *Samarakhai* by the tribes of Odisha, is traditionally used in treating wounds, wound infections and related ailments. The present study was carried out to evaluate the antimicrobial and wound-healing activity of the root and whole plant of *B. herbacea*. The antimicrobial potential of *B. herbacea* root (BHR) and *B. herbacea* whole plant (BHW) was screened against four Gram-positive and Gram-negative bacterial strains: *Staphylococcus aureus* (ATCC 6538), *Streptococcus pyogenes* (ATCC 12384), *Pseudomonas aeruginosa* (ATCC 9027), *Klebsiella pneumoniae* (ATCC 10031) and one fungal strain: *Candida albicans* (ATCC 10231) which are predominantly responsible for wound infection. The antimicrobial activity was assessed by the cylinder plate method. The wound-healing efficacy of BHR and BHW was evaluated in the excision wound model in albino rats. BHR and BHW showed a good zone of inhibition against respective pathogens at higher concentrations. Both BHR and BHW were found to possess significant wound-healing activity, evidenced by the increase in the rate of wound contraction and skin-breaking strength, decrease in the period of epithelialisation, and increased hydroxyproline content. This study revealed the wound-healing potential of *B. herbacea* in albino rats.

Keywords: Antibacterial, Excision wound model, Gandhamardan hills, Pharmacological activity, *Samarakhai*

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Introduction

Wounds signify one of the foremost global health challenges that put great economic, financial, and social stress on health organizations, healthcare providers, patients, and their families[1]. Wounds are defined as physical, chemical, or thermal injuries that result in an opening or breaking in the integrity of the skin or the disruption of the anatomical and functional integrity of living tissues[2]. Wound healing is an intricate process initiated in response to damage that restores the function and integrity of broken tissues. The healing process might be broadly classified into three stages: the inflammatory phase, consisting of the establishment of homeostasis and inflammation; proliferative phase (such as granulation, contraction, and epithelialization); and lastly, the remodeling phase, which in the end determines the strength and appearance of the healed area[3].

Infectious diseases have accelerated to a remarkable extent through recent years. It is observed by Paul and Singh that the prevalence of infectious diseases in outpatient care increased nearly 3 times, from 8 to 26 per 1,000 in two decades (1995 to 2014)[4]. These are the diseases caused by pathogenic microorganisms, such as bacteria, viruses, fungi, protozoa, and parasites. Microorganisms are identified as causative agents of food spoilage and various diseases. The clinical efficacy of several existing antibiotics is being prone with the assistance of the emergence of multidrug-resistant pathogens[5]. The incidences of novel and relapsing infectious ailments and antibiotic resistance have prominently accelerated the vulnerability of delayed healing. So, it is necessary to discover a newer and safer ethnomedicine for wound healing that has an antimicrobial impact.

Byttneria herbacea Roxb., family Sterculiaceae, is a reputed folklore plant commonly observed in peninsular India from Gujarat southwards to Tamil

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Nadu and in Bihar and Odisha[6]. It is locally known as Samarkhoi, Samarakhai, Samarkai, Sambarkhai, and Samar Kayee by the tribes of Odisha and Bengal, and different parts of the plant have been mentioned for their usage in 32 various ailments by 65 tribes of 12 states of India[7]. Its vernacular name indicates that the plant is favourite to *Samara*, a type of deer. Traditionally, its root and the whole plant are being used for wounds, fractures, dislocations, swelling, cuts, ulcers, and sprains[8]. The paste of roots is used topically in wound healing[9]. Few pharmacological activities have been mentioned on *B. herbacea*, including anti-oedemogenic[10], anti-inflammatory[11], and anti-oxidant activity[12]. Because of those cited activities, observations, and traditional uses of the plant, a study was undertaken to discover *in-vitro* antimicrobial evaluation and *in-vivo* excision wound healing potential of root and whole plant of *B. herbacea*. The intention behind selecting *B. herbacea* plant was to cure both wounds and inhibit the growth of microorganisms at the site of the wound.

Materials and Methods

Collection and authentication of plant material

The plant *Samarakhadyam* (*B. herbacea* Roxb.), identified by a traditional healer, was collected from its natural habitat, Gandhamardana hill ranges (20.9269° N, 82.8136° E) of the Bargarh district of Odisha. Plant herbarium was authenticated by the Botanical Survey of India (BSI), Kolkata (CNH/2016/Tech.II/68). A voucher specimen (No. Phm: 6200/16-17) of the plant herbarium has been stored in the Pharmacognosy laboratory of the Institute for future reference.

Sample preparation

Root along with whole plant, was washed under running water, made to dry in shade and ground to a coarse powder througha mechanical grinder. Samples were filtered through a fine mesh (120#) and preserved in air-tight glass bottles for animal experiment. For local application in excision wounds, paste was prepared by adding distilled water.

Antimicrobial study

Bacterial and fungal strains

A total of five pathogenic microbial strains were used in the study: four Gram-positive and Gram-negative bacterial strains: *Staphylococcus aureus* (ATCC 6538), *Streptococcus pyogenes* (ATCC 12384), *Pseudomonas aeruginosa* (ATCC 9027), *Klebsiella pneumoniae* (ATCC 10031), and one fungal strain: *Candida albicans* (ATCC 10231). These microbes were selected based on their potential to cause wound infections. All bacterial and fungal strains were purchased and procured from The American Type Culture Collection (ATCC).

Precautions and safety

All the Glass wares and media were properly sterilized. A dedicated inoculating loop was used for each culture. Sub-culturing was done under aseptic condition and performed within biosafety cabinet near the Burner flame. All Persons were on sterile gown while sub culturing. Glycerol vialonce after thawing was not frozen since it will reduce cell viability. No any culture was found decontaminated/dried. Freshly prepared slants of *S. aureus*, *S. pyogenes*, *P. aeruginosa*, *K. pneumonia* and *C. albicans* were used and washed the slants by using 10 mL of sterile normal saline solution.

Cylinder plate method

The cylinder plate method[13] was used for the study. Mueller Hinton Agar was used for determining the activity of respective bacterial and fungal strains. Media was prepared as per the manufacturer's Instruction. Then, the media were autoclaved at 121°C temperature and 15 lbs pressure for 20 min. Exactly 2 g of root and whole plant powder samples were taken in two specific conical flasks and labeled Sample -1 and -2. 10 mL of DMSO (Dimethyl sulfoxide) was added to samples and sonicated for 10 min. After sonication, samples were positioned on a water bath at a temperature of 800ºC for 30 min. Samples were filtered by Whatman filter paper. The filtrate becomes used as a stock solution for activity. From the stock solution, 100, 150, and 200 µL samples were inoculated on the plate.

Ceflox-CF Cream (ciprofloxacin, fluocinolone acetonide, clotrimazole, and neomycin sulphate cream) was selected as the standard for the study. Exactly 1 g of Ceflox - CF cream was taken in 100 mL of the conical flask. Precisely 5 mL of methanol and 5 mL of DMSO (dimethyl sulfoxide) were added. The sample was heated in a water bath at a temperature of 800ºC for 30 min. The standard solution was filtered by Whatman filter paper. The filtrate was used for the activity.

Testing procedure for antibacterial and antifungal activity

Sterile media were cooled to a maximum of 55°C. Total 15 mL of MHA (Mueller Hinton Agar) for antibacterial activity and 15 mL of SDA (Sabouraud Dextrose Agar) for antifungal activity were poured through a sterile measuring cylinder into sterile Petri plates. Plates were allowed to solidify on a smooth surface. In rest of the media, 5 µL bacterial and 5 µL fungal cultures were added to plates and combined slowly. Then, the media was poured on the above MHA and SDA-containing plates. The plates were solidified, after which the required wells in MHA and SDA plates categorized them as blank, std. and test at a proper distance through the sterile borer. Added blank, std. and test samples in respective labelled wells. When the samples were diffused completely in a well, the MHA plate was incubated for 24 hours at 35°C in a Bacteriological incubator, and the SDA plate was incubated for 72 hours at 25°C in a BOD (Bio-Oxygen Demand) incubator. Then, the zone of inhibition was evaluated.

Animals

Charles foster albino rats (220±30 g) of either sex were utilised in experimental study. All the chosen rats were kept under acclimatization for one week prior to the experiment. Animals were housed in polypropylene cages with stainless steel top grills. Cages were wiped clean each day in the morning hours. Animals were exposed to a 12-hour light and 12-hour darkish cycle. The ambient temperature changed to 22±03°C, and the relative humidity changed to 50 to 60%. VRK brand rat pellet feed supplied by Keval Sales Corporation, Vadodara, Gujarat, was provided during the study. Drinking water was given *ad libitum* in polypropylene bottles with a stainless-steel sipper tube. Experiments were conducted in conformity with AIEC (Institutional Animal Ethics Committee) after acquiring its approval (IAEC/21/2016/18) following the guidelines formulated by CPCSEA (The Committee for the Purpose of Control and Supervision of Experiments on Animals), India.

Test for wound healing activity

Healthy Charles Foster rats of either sex were selected for the experimentation and randomly divided into four groups of six animals each. Group 1 served as the control, with no local application. Group 2 was treated by locally applying *B. herbacea* root (BHR) paste. Group 3 was treated by local application of *B. herbacea* whole plant (BHW) paste. Group 4 served as standard and was treated with the standard drug, Betadine (BT) ointment locally. Before the operative procedure, all instruments (forceps, scissors, etc.) were autoclaved, and the operative procedures were carried out under aseptic conditions. The experimental animals were anaesthetized under mild ether anaesthesia by inhalation method. The rats were inflicted with excision wounds, as defined by Morton and Malone[14]. The dorsal fur of the animals was shaved by scissors earlier than the procedure without inflicting any abrasions. The area of the wound to be created (at the back portion of the rat-suprascapular region) was outlined on the animals back with a permanent marker. A complete thickness excision wound of a circular area of 140 mm^2 and 2 mm depth is created alongside markings with a curved scissor. Then, the animals were kept in separate cages on a normal diet. Drugs were applied untill 95% epithelialisation was completed from the day of operation.

The wound contraction rate is assessed by tracing the wound on alternate days using transparent tracing paper and a permanent marker. The wound zones recorded were measured using graph paper. Epithelialization begins to be considered at the moment at which the eschar came off without leaving any residual of raw wound[15]. Thus, the number of days required for 95% epithelialisation and percentage of contraction periodically were recorded[16] (% wound contraction = Healed zone× 100 / Total wound zone, where healed zone = original wound zone - present wound zone). On the 15th day, the blood becomes collected by retro-orbital puncturing under mild ether anaesthesia. After collecting the blood, it was allowed to clot by leaving it at room temperature for 15-30 minutes. The clot was removed by centrifuging at 1000-2000× g for 10 min. in a refrigerated centrifuge, and then serum was obtained for biochemical estimations. Serum hydroxyproline[17] and Orosomucoid[18] levels were estimated. After that, rats were euthanized under a higher dose of ether by inhalation method. The tensile strength of the excised skin of the healed wound zone and granulation tissue was recorded and then subjected to biochemical parameters.

Statistical analysis

The data are expressed as mean±standard error of the mean for six rats per experimental group. One-way analysis of variance (ANOVA) was used to compare the mean values of quantitative variables among the groups, followed by Dunnett multiple ‘t’ test and Students ‘t’ test for paired and unpaired data by using Sigma stat software to decide the significant difference between groups at $P < 0.05$.

Results

Antimicrobial activity

BHR and BHW showed a good zone of inhibition against respective pathogens in 150 and 200 µL concentrations. It was observed that the whole plant sample (ranging from 21-26 mm) showed more zone of inhibition than the root sample (ranging from 18-21 mm) against the *P. aeruginosa* bacterial strain. The root sample (ranging from 19-21 mm) showed more zone of inhibition in comparison to the whole plant sample (ranging from 15-16 mm) against the *S. aureus* bacterial strain (Fig. 1).

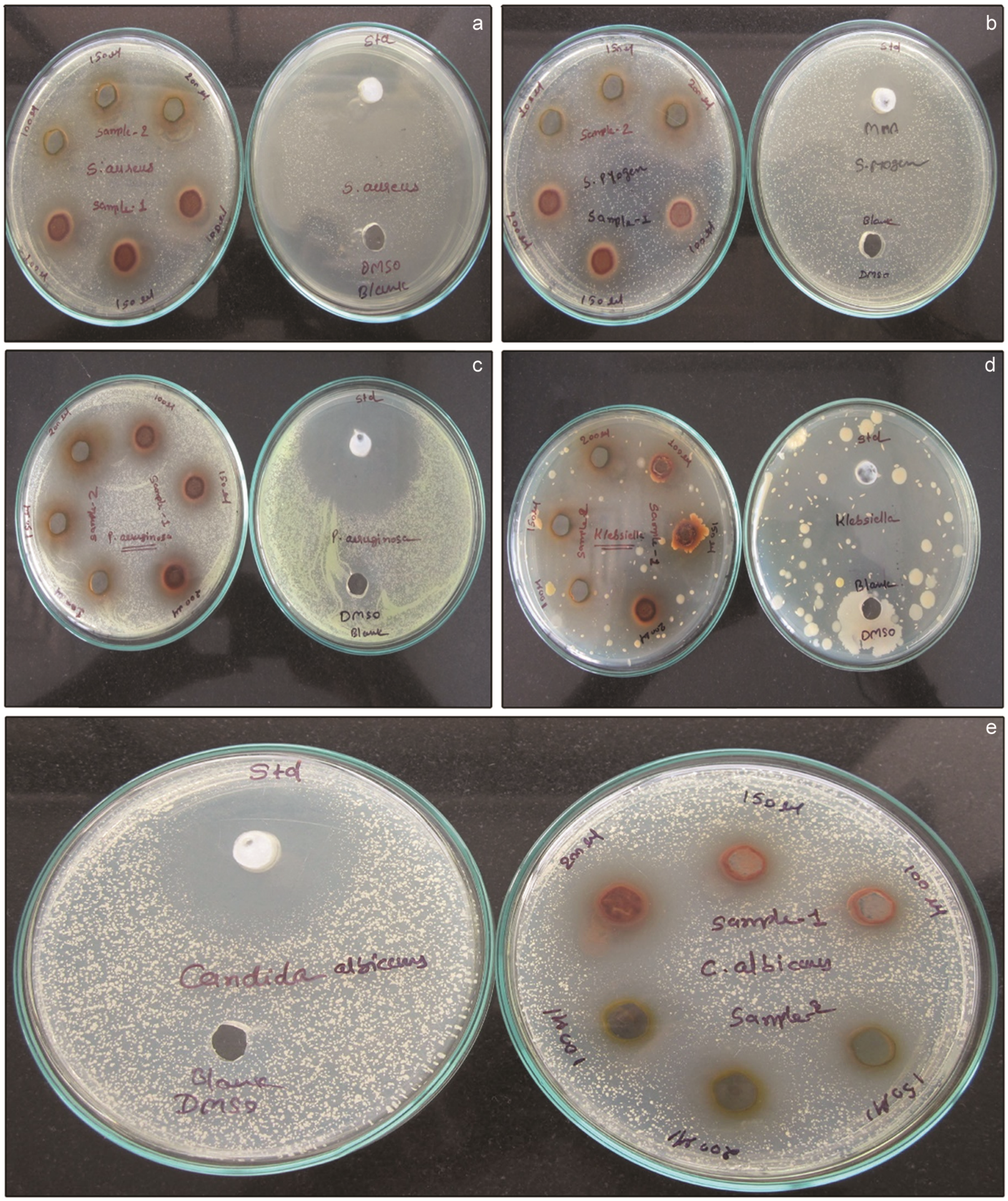

Fig. 1 — *In-vitro* culture plates (Cylinder plate method) of standard drug (Ceflox CF cream), BHR (sample-1) and BHW (sample-2) showing zone of inhibition (ZI) against respective strains. a) *Staphylococcus aureus*; b) *Streptococcus pyogenes*; c) *Pseudomonas aeruginosa*; d) *Klebsiella pneumoniae*; and e) *Candida albicans* at 100, 150 and 200 µL concentration.

In terms of the antimicrobial spectrum, both test samples were found to be effective against gram-positive and gram-negative bacterial strains, as well as fungal strains, in a concentration-dependent manner. When comparing the zone of inhibition of both test samples at each concentration level for all individual strains, it was found that BHW exhibited antimicrobial efficacy with a maximum zone of inhibition (ZI) against *S. pyogenes* and *P. aeruginosa* at 200 µL. BHR was significantly more effective (Maximum ZI among both test samples) against *S. aureus* at the same maximum concentration level (200 µL). On the other hand, BHR and BHW were found to have almost similar ZI against *K. pneumoniae* and *C. albicans* at 200 µL (Fig. 2). Ceflox-CF cream shows its maximum antimicrobial efficacy as a standard drug compared to BHR and BHW (Fig. 3).

The results of the present study shows that both test samples exhibited inhibition against respective pathogens, and it was also found that the zone of inhibition of all samples increased linearly with an increase in concentration.

Excision wound-healing activity

The effect of the test drug samples was assessed on percentage of contraction, complete epithelialization period, tensile strength of the skin and granulation tissue, and serum parameters. A significant increase in the percentage of wound contraction was observed in

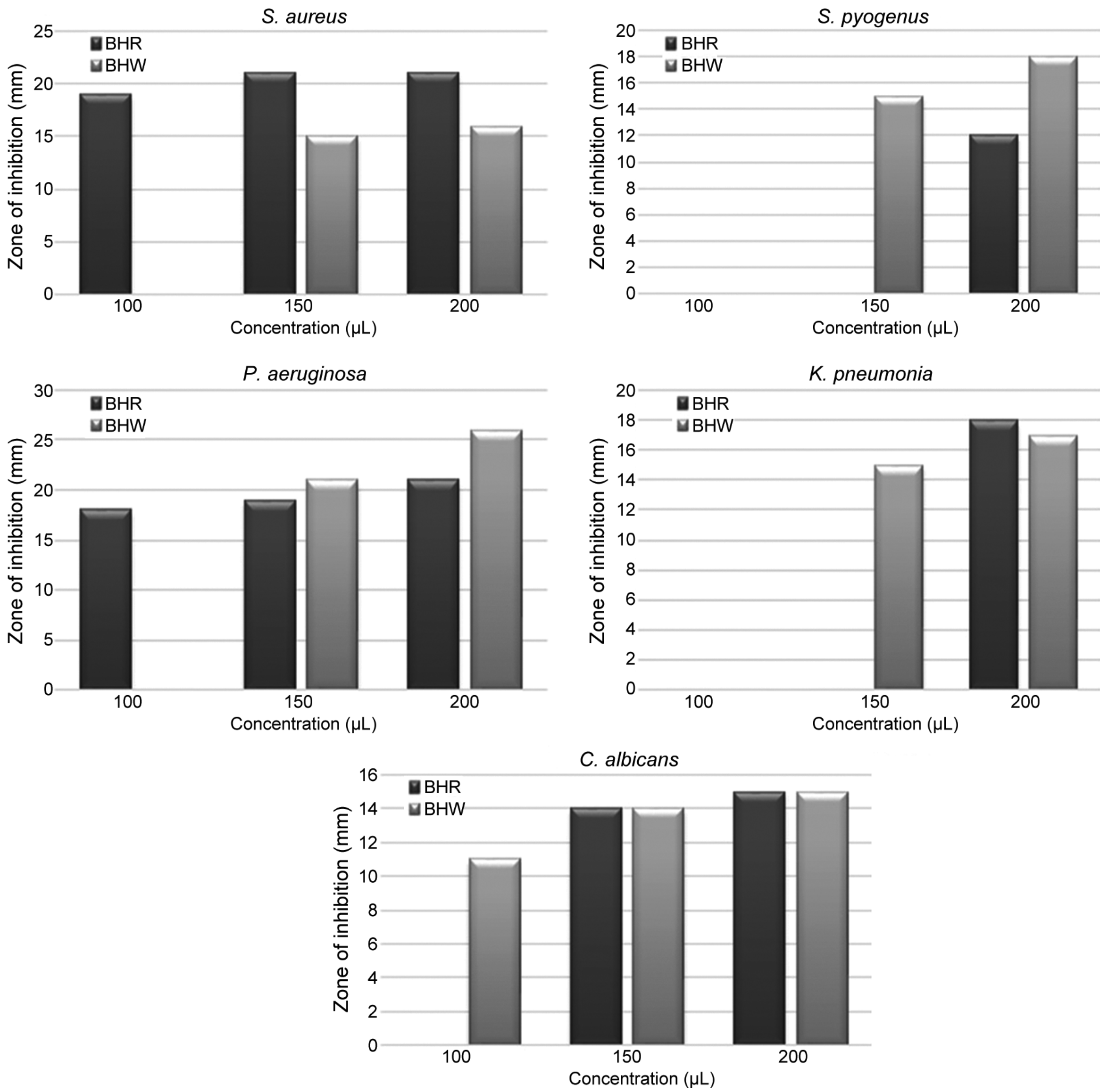

Fig. 2 — Antimicrobial efficacy of BHR and BHW against microbial strains at different concentrations.

the root powder-treated group (BHR) on the 12th and 13th day, while the whole plant powder-treated group (BHW) on the 11th and 12th day when compared with the normal control group. It was found that the root-treated group, whole plant-treated group, and the standard group showed a similar pattern in the day-wise wound contraction graph (Fig. 4).

The effect of test drug samples on 95 per cent epithelialization and tensile strength of skin in the excision wound healing model has been depicted in Table 1. The epithelialization period (95%) was found to be significantly (P <0.05) decreased in all treated groups when compared with the normal control group. A non-significant increase in tensile strength (partial thickness) of skin was observed in the BT-treated group compared to the normal control group. BHR and BHW-treated groups produce no effects on the tensile strength of wounded skin in comparison with the normal control group.

The effect of test drug samples on serum hydroxyproline and orosomucoid levels has been depicted in Table 2. A highly significant increase in serum hydroxyproline level was observed in the BHR and BHW-treated group. In contrast, a non-significant increase was observed in the betadine ointment-treated group compared to the normal control group. A non-significant increase in serum orosomucoid content was observed in all drug-treated groups compared to the normal control group.

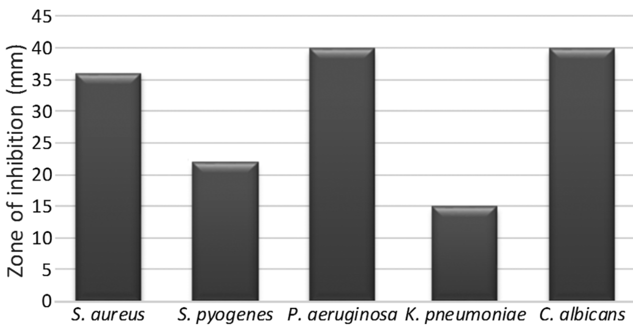

Fig. 3 — Antimicrobial activity of Ceflox CF cream against different microbial strains.

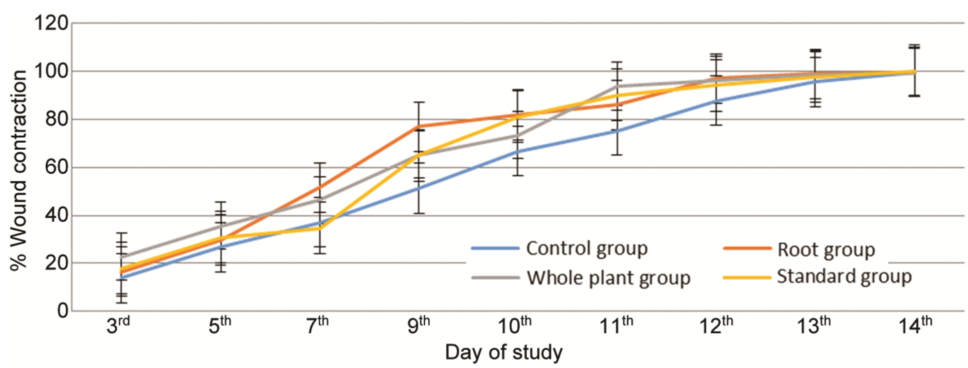

Fig. 4 — Day-wise wound contraction of all four groups in excision wound healing activity in rats.

Table 1 — Effect of test drug samples on 95% epithelialization and tensile strength of skin in excision wound healing activity in rats

Groups	95% epithelialization period (days)	% change	Tensile strength (kg)			
			Partial-thickness	% change	Full-thickness	% change
Control	13.50±0.34		0.74±0.09	---	0.87±0.05	---
BHR	12.00±0.36*	11.11↓	0.78±0.05	4.30↑	0.90±0.06	4.14↑
BHW	12.17±0.48*	9.87↓	0.78±0.05	4.97↑	0.85±0.04	2.65↓
BT	12.17±0.40*	9.87↓	0.91±0.05	22.58↑	0.93±0.04	7.25↑

Data: Mean±SEM, ↑-increase ↓-decrease
*P <0.05, compared to the normal control group (unpaired 't' test)

Table 2 — Effect of test drug samples on serum hydroxyproline and serum orosomucoid level in excision wound healing activity in rats

Groups	Hydroxyproline (mg/dL)	% change	Orosomucoid (mg/dL)	% change
Control	2079.52±114.78		113.29±20.76	
BHR	2778.86±105.24@	33.63↑	107.26±20.85	5.31↑
BHW	2892.82±90.16@	39.11↑	168.50±45.38	48.73↑
BT	2176.20±41.34	4.64↑	148.19±4.60	30.81↑

Data: Mean±SEM, ↑-increase ↓-decrease
@P <0.05 compared to the normal control group (Annova followed by Dunnett'smultiple't' test)

Discussion

As a result of the high incidence of antibiotic resistance, estimating the antibacterial effect of herbal drug for treating skin and wound infections is crucial in addressing both human and animal health issues[19]. Some preceding studies reviewed[20,21] that *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* are the most commonly accountable pathogens for wound infection. The above consequences display that root and whole plant have an effective zone of inhibition against *S. aureus, P. aeruginosa*, and *C. albicans*; therefore, *B. herbacea* may be a useful drug in the management of wound infection, as referred to in the ethnomedicinal claims.

Antibacterial properties of the plants may be because of the presence of various bioactive chemical agents within the extracts, which are regarded to act by a different mechanism to employ an antibacterial action. Some studies have proven that plants with antimicrobial activity contain bioactive constituents like tannins, flavonoids, alkaloids, and saponins[22]. From an analytical study, it was found that tannins, alkaloids, phenolic compounds, and flavonoids were found in sufficient amounts in root and whole plant sample of *B. herbacea*[23].

Wounds are physical injuries to the skin. Wound-healing carries a homeostasis process that encompasses re-epithelialization and reconstruction of tissue matrix collagen. It is an inherent immunological activity occurring on its own. However, factors like inflammation and microbial contamination not only delay the healing process but also increased the possibility of secondary infection[24]. Plant-primarily based total bio-actives were suggested for their beneficial impact on wound care by promoting epithelialisation with the least scarring of skin tissue[25].

In Ayurveda, numerous drugs originated from medicinal plants are used for wound healing under the parlance of *Vranaropaka* (wound healer)[26,27]. Many studies endorsed that wound healing may be improved through herbal drugs having antibacterial, anti-oxidant, and anti-inflammatory properties[28]. Plants are stronger healers because they promote the repair mechanisms naturally. Herbal drugs in wound management comprise disinfection, debridement, and providing a wet surrounding to encourage the established order of the appropriate environment for the natural healing process[29]. The wound-healing action of *B. herbacea* is probably due to synergistic or individual activity of the phytoconstituents present within the plant.

The time required for complete epithelialization of the excision wound is a vital parameter to assess the wound healing process. The increased rate of wound contraction in both test groups might be due to improved epithelialization. In recent years, oxidative stress has been associated with various degenerative processes and diseases; those including acute and chronic inflammatory conditions such as wounds[30]. The anti-oxidant activity of the plant appears to be due to the presence of flavonoids, alkaloids, and tannins in ample quantity. This can be accountable for the wound-healing activity of *B. herbacea.*

The healing duration is predominantly based upon the wound contraction rate, which increases and allows wound closure through fast re-epithelialization by shortening the keratinocyte's migratory distance[31]. The epithelialization involves the migration and proliferation of the newly formed epithelial cells in the direction of broken wound beds[32]. A shorter epithelialization period by both test samples is probably due to the sufficient viability of epithelial cells as compared to normal control.

Collagen is a chief protein of the extracellular matrix that subsequently contributes to wound strength[33]. The healing process relies upon the regulated biosynthesis and deposition of the latest collagen and its subsequent maturation[34]. Hydroxyproline, an amino acid found in collagen, is directly proportional to the formation of collagen, and its estimation allows clinically to understand the rate at which the healing process proceeds in the wound tissue[35]. Serum hydroxyproline level was found to be significantly higher in both test groups in comparison to normal control. The presence of increased hydroxyproline content in both test groups might be an indicative pharmacological event related to improved proliferation and migration of fibroblasts with significant collagen depots in injured tissue[36]. It is recommended that the observed short epithelialization period and rapid contraction should be a result of the synergistic capability of both test groups, which improved the collagen turnover[37].

Plant based bioactive compounds were considered a potential therapeutic intervention for the effective remedy and control of wounds accompanied by effective antimicrobial and anti-oxidant activities[38]. Susceptibility of bacterial growth within the wound region responding to contamination is another reason for hindered wound healing. Antimicrobial activity may be correlated with wound restoration as contamination can dramatically hinder the healing process through the impaired formation of granulation tissue[39]. Henceforth, the plausible mechanism by which the healing was triggered is probably attributed to broad-spectrum antimicrobial action proven by the test drug. It is observed that root and the whole plant produced a remarkable zone of inhibition towards *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Candida albicans*, which supports the impact of *B. herbacea* on wound healing and can be used to prevent the bacterial and fungal contamination in any cuts and wounds.

Conclusion

Byttneria herbacea root and whole plant extracts reveal promising antibacterial and antifungal activity against tested pathogens. Excision wound healing study showed that *B. herbacea* root and whole plant effectively stimulate wound contraction in albino rats. This justifies the use of its root and a whole plant for the treatment of wounds, as claimed in the folklore literature. The antimicrobial effect of the drug supported the wound-healing process by accelerating the remodelling of injured tissue.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgement

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