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# Development of Formulation Containing *Shorea robusta* and *Acorus calamus* Extracts and Evaluation of its Antimicrobial Potential Against Burn Wound Pathogens

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**Abstract:** This research focuses on the extraction, formulation, and evaluation of *Shorea robusta* resin and *Acorus calamus* rhizome extracts for their antibacterial and wound healing properties in the context of treating burn wounds infections. The study involved the collection, authentication, and preparation of plant specimens followed by the isolation of bacterial strains from burn wounds. Methanolic extracts of *S. robusta* and *A. calamus* were prepared and tested for their minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) against various bacterial strains commonly associated with burn infections. Individual extracts showed effectiveness at certain concentrations, while a combination of both extracts exhibited potent inhibitory activity against multidrug-resistant strains. Further, ointment formulations were developed using these extracts and various bases. The formulations demonstrated significant antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus niger*. Physicochemical evaluations, including organoleptic properties, pH, spreadability, acid value, saponification value, dye test, irritancy test, and stability studies, were conducted on the formulated ointments. These evaluations confirmed the suitability, stability, and safety of the formulations for topical application. Overall, the research outlines a promising approach for developing herbal-based ointments using *Shorea robusta* and *Acorus calamus* extracts, exhibiting substantial antimicrobial efficacy and favorable physicochemical characteristics for potential application in burn wound management.

**Keywords:** *Acorus calamus*, *Shorea robusta*, Anti-microbial, Burn wound, Ointments, Formulation, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Candida albicans*, *Aspergillus niger*

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## Introduction

Burn injuries present a significant global health challenge, contributing to substantial morbidity and mortality rates worldwide. These injuries often result in infections, posing complex issues in their management, particularly with the rise of multidrug-resistant bacterial strains. The conventional approaches to treating burn wound infections are increasingly limited due to the escalation of antimicrobial resistance among pathogens (WHO, 2018). Consequently, there is a growing interest in exploring alternative therapeutic avenues, particularly those derived from natural sources.

Herbal medicine, characterized by its diverse array of bioactive compounds, offers a promising solution to address the complexities of burn wound infections. Plants have a long history of utilization in traditional medicine for their antimicrobial and wound healing properties, providing a rich resource for potential therapeutic agents (Bakkali *et al.*, 2008). Among these botanicals, *Shorea robusta* and *Acorus calamus* stand out for their historical use in indigenous medicinal practices and their documented pharmacological activities.

*Shorea robusta*, commonly known as the Sal tree, holds cultural and medicinal significance across various Asian regions, including India. This species has been traditionally employed in folk medicine for its wound healing properties, attributed to its diverse chemical constituents such as flavonoids, tannins, and terpenoids (Singh and Kumar, 2018). Conversely, *Acorus calamus*, referred to as Sweet Flag or Vacha, is esteemed in Ayurveda for its antimicrobial effects and its use in managing various infections, owing to its rich content of essential oils, alkaloids, and phenolic compounds (Pawar *et al.*, 2020).

This study aims to explore the therapeutic potential of these two medicinal plants, *S. robusta* and *A. calamus*, in combating burn wound infections. By harnessing their bioactive components, the study seeks to extract, formulate, and evaluate the efficacy of these plant-derived compounds in topical ointments for the management of burn injuries.

The primary objective involves the extraction of active compounds from *S. robusta* and *A. calamus*, followed by the assessment of their antimicrobial activities against clinically relevant bacterial strains commonly associated with burn infections. This evaluation has encompassed determining the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC), crucial parameters to assess the potency of these herbal extracts against the targeted pathogens.

Furthermore, the study aimed to formulate these extracts into topical ointments, optimizing their concentrations and bases for enhanced antimicrobial efficacy and favorable physicochemical characteristics suitable for clinical application. In essence, this research endeavors to bridge traditional herbal knowledge with modern scientific approaches, aiming to develop innovative therapeutic interventions derived from *Shorea robusta* and *Acorus calamus* to address the challenges posed by burn wound infections, especially in the context of emerging antibiotic resistance.

## Materials and Methods

### *Collection and authentication*:

*Shorea robusta* plant specimens were acquired from the Bastar district Forest (19.1071oN, 81.9535oE) in the current study, which is located in the southern region of Chhattisgarh and has an area of 4029.98 km (Pandey *et al.*, 2018). Another species, *Acorus calamus*, was selected in the Pathare fields in Ratnagiri, Maharashtra, India. The plants' resin and rhizome were collected using a conventional procedure according to the C.C.R.A.S guidelines (The Ayurvedic Pharma-copoeia of India). Botanists identified and validated both plant specimens, which were then stored in the Blatter herbarium at St. Xavier's College in Mumbai, India. This study made use of fresh plant material. The raw rhizomes and resins

were carefully cleaned. For drying, a known amount of rhizome and resin (50 g each) were stored at room temperature. Rhizomes and resins were ground in a mixer grinder after being dried. The powdered components were kept in sealed polythene bags until they were used.

*Sample collection and isolation*:

Clinical samples of burn wounds were obtained with sterile swabs from patients with burns and transferred in sterile Ringer's solution (Srinivasan *et al.*, 2009), which was obtained from the Bai Rukminibai KDMC Hospital in Kalyan, Maharashtra, India. Samples were streaked on sterile Nutrient agar, MacConkey agar, and Sabouraud's agar plates. The plates were kept at 37°C for 24 hours. The gram origin of all isolates was also determined (Karah *et al.*, 2020).

*Preparation of extracts*:

Cold individual alcoholic herbal extracts prepared by 50 g of powdered herb material were combined with 500 ml of 50% methanol and shaken for 72 h. At room temperature, this was filtered and evaporated to dryness. The dried extracts were kept in the refrigerator until they were used (Ashafa *et al.*, 2010).

*Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)*:

The minimum inhibitory concentrations (MIC) of the plant extracts of *S robusta* and *A calamus* were measured by broth dilution method. The different dilutions 0.5 mg/ml to 20 mg/ml of both crude plant extracts were prepared in 0.5 ml Mueller-Hinton Broth (MHB) before inoculating with 0.5 ml of *S.aureus* and *P.aeruginosa* bacterial culture. The test tubes were incubated at 37°C for 24 h under anaerobic condition (Parvekar *et al.*, 2020). Using a spectrophotometer set at 530 nm, the growth of the bacterial isolates in the test tubes was detected as turbidity after incubation. MIC was recorded as the lowest concentration of crude extracts in which bacterial growth was inhibited.

For MBC evaluation (Kowalska–Krochmal and Dudek-Wicher, 2021), the tubes with no growth were streak-plated on Mueller-Hinton Agar (MHA) and incubated under the mentioned condition for both bacterial strains. The MBC was recorded as the lowest concentration showing no visible growth of bacterial strains.

*Antibacterial screening*:

The bactericidal activity of *S robusta* and *A calamus* plant extracts was investigated using the agar ditch plate method (Nayak *et al.*, 2014). A 6.0 cm X 2.0 cm ditch was formed in the sterile nutrition agar plate (12 ml). Both extracts (individually and in combination) were weighed to determine concentrations such as 1 mg/ml, 5 mg/ml, 7 mg/ml, 10 mg/ml, and 20 mg/ml before being dissolved in 10% DMSO and discharged into the ditch. The cultures were streaked perpendicular to the ditch and parallel to one another. The plates were incubated at 37°C for 24 hours. There was an inhibition of organism growth after incubation.

*Selection of drug ratio*:

For formulation, ratio of *Acorus calamus* Rhizome and *Shorea robusta* resin extract was selected based on its antimicrobial activity in the form of zone of inhibition against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Staphylococcus aureus.*

*Acorus calamus* (1.0%): *Shorea robusta* (1.0%) were mixed in ratio (1:2, 1:1, 2:1) and mixture were checked for its antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Staphylococcus aureus* by Zone of inhibition method (Kale *et al.*, 2020). Ratio showing a comparative zone with commercially available formulation (Silver sulfadiazine) was selected for further study (Nímia *et al.*, 2019).

*Formulation*:

Ointments are semisolid preparations for external application of such consistency that they may be readily applied to the skin by injection. They serve as vehicles for topical application of medicinal

substances and also function as protectives and emollients for the skin (Matzen *et al.*, 2018). To determine the antibacterial activity of the supportive base, the Agar Cup method was chosen. Honey and Clarified butter, aloe gel, oil base and emulsifying wax were chosen for the study. Different bases were tried out with different concentrations and according to the results obtained a better base was selected for further studies.

*Preparation of ointments*:

Accurately weighed 2 g of extracts (1 g of *Acorus calamus* + 1 g of *Shorea robusta*) was taken in a beaker. In another beaker, distilled water (25%) of batch size was transferred and gradually added emulsifying agent and aloe vera gel was added and boiled for 15 min in a water bath. Then Butylated Hydroxytoluene (BHT) as an antioxidant was added while it was stirred continuously and mixed well till gelling agents were completely wetted and dispersed and made free from lumps. Clarified butter (Ghee) was added in the above beaker and stirred till it was completely dispersed. Oily component (Coconut oil) was mixed and added into the solution obtained. Honey was then transferred to the solution and mixed well. Rose water was added to the prepared solution and stirred till it was completely mixed. Then both extract solutions were transferred into the beaker, having the solution obtained and stirred for 15 min. Phosphate buffer was transferred in above solution to obtain the desired pH between 3.2 to 6.8 of the solution obtained was adjusted by adding alkalizing agent (Sodium bicarbonate) while stirring continuously. Remaining purified water was added to adjust the weight of the batch. Final product was obtained.

*Antimicrobial efficacy*:

The following bacterial and fungal strains were used for the efficacy study in the experiment (Bhalodia and Shukla, 2011) namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger.* The tests were done against the most strains known to be isolated from burn infections. The antibacterial and antifungal activity of the formulations was determined by the Agar well diffusion technique using Muller Hinton Agar for *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and Sabouraud's Dextrose agar for *Candida albicans* and *Aspergillus niger*. Wells were filled with the three different formulations. The diameter of the wells bored was 6 mm which was loaded with F1, F2, F3 and positive control (Silver sulfadiazine Ointment). Incubation (24 h at 37°C) was done and recorded the zones of inhibition.

*Physicochemical evaluation:*

Ointment formulations were evaluated for physicochemical properties and optimized formulas were evaluated for antimicrobial efficacy and *in vitro* wound healing properties.

*Organoleptic properties*:

Physical appearance, colour, texture, phase separation, and homogeneity were all evaluated for blank formulations (no active ingredients or preservatives) and drug-loaded formulations. These characteristics were evaluated by visual observation. The homogeneity and texture of the produced ointment were evaluated by pressing a little quantity between the thumb and index finger (Chen *et al.*, 2016). The consistency of the formulations and the presence of tiny particles were used to assess the texture and homogeneity of the formulations. Immediate skin feeling (including stiffness, grittiness, and greasiness) was also evaluated.

*pH values*:

The pH strips were used with the help of standard buffer solution. Weight 0.5 g of ointment dissolved it in 50 ml of distilled water and its pH was measured with the help of standard colour chart of pH (Lukić *et al.*, 2021).

*Spreadability*:

Spreadability of the formulations was determined

by measuring the spreading diameter of 1 g of sample between two horizontal glass plates (10 cm) after one minute. The standard weight applied to the upper plate was 25 g. Each formulation was tested three times (Ranganathan *et al.*, 2023).

Spreadability = Weight attached to upper plate X Length/Time taken to separate from the fixed slide

Weight attached to upper plate = 25 g; Length = 10 cm

*Acid value*:

Prepared ointments were used for determination of acid Value. To find the acid value 0.1 N KOH standard solution is used as standard (Sarmila *et al*., 2023).

The acid value for ointment can be calculated using following formula:

Acid value = ml of 0.1 N KOH used X 56.1 / Weight of the ointment taken in g

*Saponification value*:

Introduce about 1 g of ointment refluxed with 25 ml of 0.5 N alcoholic KOH for 30 min, to this 1 ml of phenolphthalein added and titrated immediately, with 0.5 N HCl (Fatima *et al.*, 2017).

The saponification value for ointment can be calculated using following formula:

Saponification value = volume of ml of blank – titre value X 28.05/ Weight of ointment taken in g

*Dry test*:

The scarlet red dye is mixed with the ointment (Chavan *et al.*, 2020). A drop of the cream was put on a microscopic slide then covered with a cover slip, and examined under a microscope. If the disperse globules appear red the ground is colorless. The cream is oil-in-water (o/w) type. The reverse condition occurs in water-in-oil (w/o) type cream i.e. the disperse globules appear colorless.

*Irritancy test*:

Marked an area (1sq.cm) on the left-hand dorsal surface (Sekar *et al.*, 2017). The cream was applied to the specified area and time was noted. Irritancy, erythema and edema were checked if any for regular intervals up to 24 h and reported.

*Stability study*:

All formulations was compared for samples stored at room temperature (25°C) and in the refrigerator (4°C) as well as those packaged into glass containers versus plastic containers. In addition to the pH values, color, physical appearance, and texture were also tested during the 8 weeks with the above-described methods (Kim *et al.*, 2019).

## Results and Discussion

*Sample Collection and isolation*:

A total of 5 isolates (2 Gram positive and 3 Gram negative organisms) responsible for burn wound infections were obtained from pathological samples. These included *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus subtilis* and *Escherichia coli*. Antibiotic Sensitivity testing of these isolates showed resistance to more than 30% of the antibiotics but were intermediate in nature. The inoculum of the test bacteria was prepared using the colony suspension method. Colonies picked from 24 hold cultures grown on nutrient agar were used to make suspensions of the test organisms in saline solution to give an optical density of approximately 0.1 at 600 nm. The suspension was then diluted 1:100 by transferring 0.1 ml of the bacterial suspension to 9.9 ml of sterile nutrient broth before being used.

*Determination of minimum concentration (MIC) and minimal bactericidal concentration (MBC)*:

In this study, MIC and MBC of methanolic extracts of *S robusta* and *A calamus* against *S. aureus* and *P.aeruginosa* were determined by both dilution method and both were found to be effective at 10 mg/ml and 12.5 mg/ml, respectively. Table 1 shows the MIC and MBC of 1 gram positive and 1 gram negative isolate for methanolic extracts of *A. calamus* and *S. robusta* individually, lower MIC

Table 1: Minimun inhibitory concentration (MIC), turbidity for different concentrations of methanolic extracts of *S robusta* and *A calamus* after 24 h

| Plant Extract | Concentration mg/ml | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 20<br>2% | 17.5<br>1.75% | 15<br>1.5% | 12.5<br>1.25% | 10<br>1% | 7.5<br>0.75% | 5<br>0.5% | 2.5<br>0.25% | 1.0<br>0.1% | 0.5<br>0.05% |
| ***S. aureus*** | | | | | | | | | | |
| *A.calamus* | - | - | - | - | - | + | + | + | + | + |
| *S. robusta* | - | - | - | - | + | + | + | + | + | + |
| *S. robusta + A.calamus* | - | - | - | - | - | - | + | + | + | + |
| ***P. aeruginosa*** | | | | | | | | | | |
| *A. calamus* | - | - | - | - | + | + | + | + | + | + |
| *S. robusta* | - | - | + | + | + | + | + | + | + | + |
| *S. robusta + A. calamus* | - | - | - | - | - | - | + | + | + | + |

Positive (+): Turbidity indicating growth; Negative (–): No turbidity indicating absence of growth

Table 2: Minimun bactericidal concentration (MBC) for different concentrations of methanolic extracts of *S robusta* and *A calamus* after 24 h

| Plant Extract | Concentration mg/ml | | | | |
|---|---|---|---|---|---|
| | 20<br>2% | 17.5<br>1.75% | 15<br>1.5% | 12.5<br>1.25% | 10<br>1% |
| ***S. aureus*** | | | | | |
| *A. calamus* | - | - | - | - | - |
| *S. robusta* | - | - | - | - | + |
| *S. robusta +* | - | - | - | - | - |
| ***P. aeruginosa*** | | | | | |
| *A. calamus* | - | - | - | - | + |
| *S. robusta* | - | - | - | + | + |
| *S. robusta + A. calamus* | - | - | - | - | - |

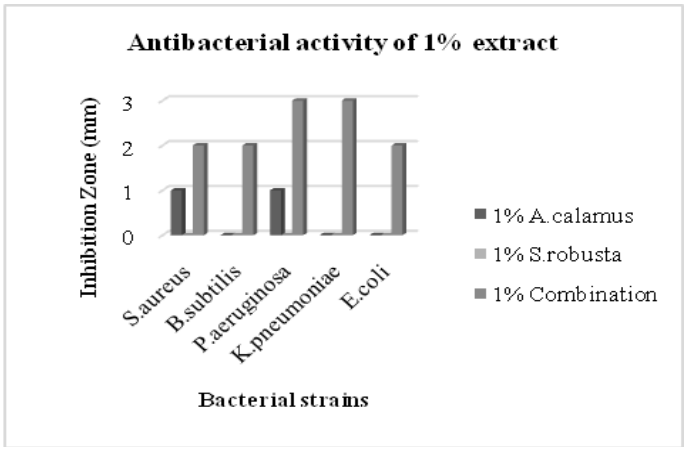
Positive (+): Indicating growth; Negative (–): Indicating absence of growth

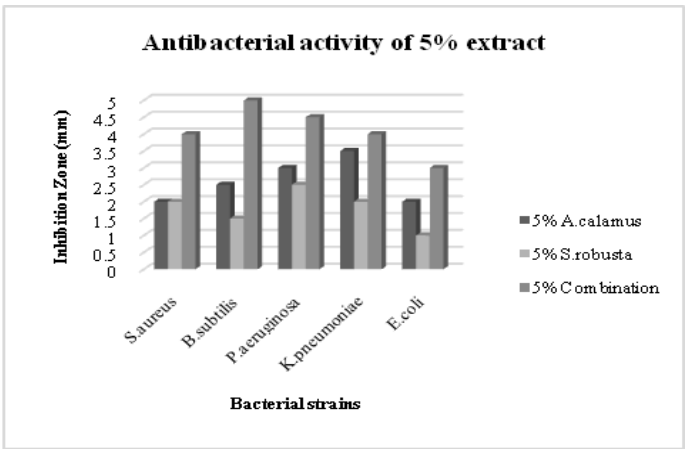
values were observed for *S.aureus* and *P.aeruginosa* with 10 to 12.5 mg/ml and 12.5 to 17.5 mg/ml, respectively. Combination extracts showed MIC value at 7.5 mg/ml for both isolates. Table 2 shows the MBC of the extracts presented 12.5 mg/ml against *S. aureus* and 15 mg/ml against *P. aeruginosa.*

*Antibacterial screening*:

The MIC study showed the inhibitory activity of methanolic extracts of *S. robusta* and *A. calamus* against *P.aeruginosa* and *S.aureus* strains. Inhibitory activity of the combinations of both extract was studied against the 5 strains by agar ditch method. Figure 1 depicts that individual *A. calamus* extract showed inhibition at 1 mg/ml concentration against *P. aeruginosa* and *S. aureus* strains and at 5 mg/ml concentration against further strains. Similarly, individual *S.robusta* extract showed inhibition at 5 mg/ml against *P.aeruginosa* and *K.pneumonia* and at 7 mg/ml concentration against further strains.

Combination extract showed bactericidal effect against all isolates at 1 mg/ml concentration. By the study of combination



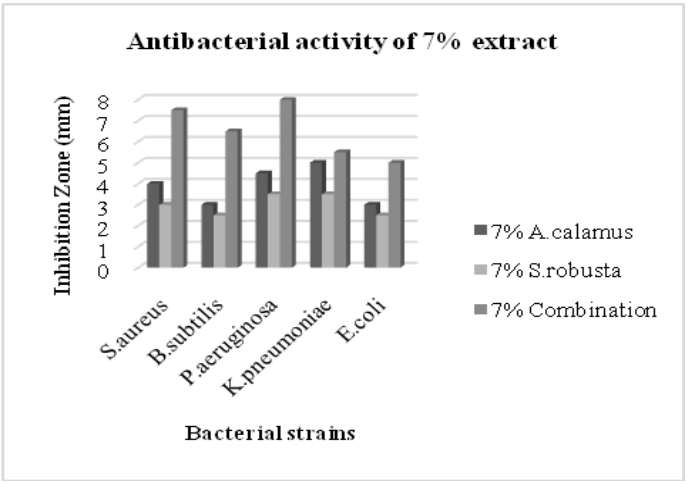




Fig. 1: Antibacterial activity of *A. calamus, S. robusta* and their combination at 1%, 5% and 7% concentration against bacterial strains.

Table 3: Quantitative composition of methanolic *Acorus calamus* Rhizome extract and *Shorea robusta* resin extract of extract ointments formulation

| Composition | F1 | F2 | F3 |
|---|---|---|---|
| Plant extracts (*Acorus calamus + Shorea robusta*) | 0.5% + 1.0% | 1.0% + 1.0% | 1.0% + 0.5% |
| Aloe Vera gel | 18% | 18% | 18% |
| Honey | 3% | 3% | 3% |
| Coconut oil | 3% | 3% | 3% |
| Clarified butter (Ghee) | 3% | 3% | 3% |
| Emulsifying Agent | 1.5% | 2.5% | 1.5% |
| Butylated Hydroxytoluene (BHT) | 1.85% | 1.85% | 1.85% |
| Rose water | 0.8% | 0.8% | 0.8% |
| Glycerol | 11.5% | 11.5% | 11.5% |
| Phosphate buffer | 0.76% | 0.76% | 0.76% |
| Sodium bicarbonate | 1.75% | 1.75% | 1.75% |
| Distilled water | Q.S. 100% | Q.S. 100% | Q.S. 100% |

extract, it has proven that the synergistic antimicrobial activities of cold extract of the both plants at up to 1 % exhibited the potent inhibitory activity against the most of the multidrug-resistant strain.

*Selection of drug ratio*:

Selection of Drug ratio of *Acorus calamus* rhizome extract is conventionally used as topical antibacterial in concentration of 1% in the form of ointment and *Shorea robusta* resin is reported to be effective as wound healing agent in concentration of 1%. To formulate combination topical formulation having these both drugs, concentration of drugs were selected by checking its antibacterial efficacy against commercially available formulation Silver sulfadiazine.

*Experimental design of formulation*:

During formulation emulsifying wax used at two different concentrations (Table 3) resulting in three different batches of ointments for rhizome and resin extract, total three batches prepared. In this case Honey, Coconut oil and Clarified butter, these three types of gelling agents were taken. Three gelling agents were used as follows: Honey (at concentration 3%), Coconut oil (at concentration 3%), Clarified butter (at concentration 3%). All the batches were prepared according to the experimental design (El-Kased *et al.*, 2017).

*Antimicrobial efficacy*:

It was observed that in the agar well diffusion method, the ointment topical preparations exhibited inhibitory activity against the tested bacterial strains - *E. coli*, *P. aeruginosa*, *K. pneumonia* Gram (-) and *S. aureus*, *Bacillus subtilis* Gram (+) and fungal strain *C. albicans, A. niger*. Ointments formulations produced a prominent zone of inhibition against the strains. The anti-microbial and anti-fungal activity of ointment formulations is shown in Table 4.

*Physicochemical evaluation*:

*Organoleptic properties*:

The organoleptic properties, including physical appearance, color, texture, phase separation, homogeneity, and immediate skin feel of the selected topical formulations are displayed in Table 5. Results showed that the ointments had a cosmetically appealing appearance and smooth texture, and they were all homogenous with no signs of phase separation. All formulations were pale yellow whitish due to sodium bicarbonate.

*pH value*:

The pH values of all prepared formulation ranged

Table 4: Antimicrobial activity of Ointments formulations

| Antimicrobial activity of Ointments formulations | | | | |
|---|---|---|---|---|
| **Bacterial strains** | **Zone of Inhibition (mm)** | | | |
| | **F1** | **F2** | **F3** | **Standard (Silver sulfadiazine)** |
| *S.aureus* | 20 | 28 | 24 | 22 |
| *B.subtilis* | 22 | 30 | 26 | 25 |
| *P.aeruginosa* | 23 | 33 | 29 | 28 |
| *K.pneumoniae* | 19 | 31 | 25 | 22 |
| *E.coli* | 22 | 32 | 27 | 25 |
| **Fungal strains** | **Zone of Inhibition (mm)** | | | |
| | **F1** | **F2** | **F3** | **Standard (Silver sulfadiazine)** |
| *C.albicans* | 23 | 33 | 28 | 22 |
| *A.niger* | 21 | 30 | 25 | 20 |

(F1) – Formulation 1, (F2) – Formulation 2, (F3) – Formulation 3

Table 5: Physicochemical evaluation of selected topical formulations

| Formulation | Physical appearance | Colour | Texture | Phase separation | Homogeneity | Immediate skin feel |
|---|---|---|---|---|---|---|
| F1 | Opaque | Pale yellow | Smooth | No | Homogeneous | Moisturizing, no grittiness, light, not greasy |
| F2 | Opaque | Whitish | Smooth | No | Homogeneous | Moisturizing, no grittiness, light, not greasy |
| F3 | Opaque | Pale yellow | Smooth | No | Homogeneous | Moisturizing, no grittiness, light, not greasy |

Table 6: pH of plants extracts formulation

| Formulation | pH |
|---|---|
| F1 | 5.7 |
| F2 | 6.8 |
| F3 | 6.5 |

from 5.5-7 which are considered acceptable to avoid the risk of irritation upon application to the skin because adult skin pH is 5.5 (Table 6).

*Spreadability, acid value and saponification value:*

The spreadability test showed in Table 7, depicts that the formulated cream has good spreadable property. The results of acid and saponification value of all formulation of cream are presented in Table 8 which showed satisfactorily values.

*Dye test*:

The scarlet red dye is mixed with the formulation. After examination under a microscope, the

Table 7: Spread ability of Ointment formulation

| Spread ability of formulations | | |
|---|---|---|
| **S. No.** | **Formulation** | **Spread ability coefficient at 60 sec.** |
| 1 | F1 | 18.33 |
| 2 | F2 | 20.38 |
| 3 | F3 | 19.44 |

| Spread ability of marketed ointment | |
|---|---|
| **Marketed Ointment** | **Spread ability** |
| Silver sulfadiazine | 22.80 |

Table 8: Test applied for acid value and saponification value

| S. No. | Parameter | Formulation | | |
|---|---|---|---|---|
| | | **F1** | **F2** | **F3** |
| 1 | Acid value | 5.5 | 6.8 | 5.9 |
| 2 | Saponification value | 26.8 | 27.4 | 25.7 |

disperse globules appears colorless in the red ground i.e. water-in-oil (w/o) type cream.

*Irritancy test*:

The formulated ointments showed no redness, edema, irritation and inflammation during studies. Ointment is safe to use.

*Stability study*:

In all storage circumstances, all formulations retained their pale yellow and white colour and intensity of colour for 8 weeks. Similarly, towards the conclusion of the storage period, the physical appearance, homogeneity, and texture of all formulations had not changed. In any of the containers or temperatures, none of the formulations showed evidence of physical or chemical instability.

## Conclusion

The comprehensive investigation into *Shorea robusta* and *Acorus calamus* extracts presents a compelling case for their role in managing burn wound infections. Through meticulous isolation, extraction, and formulation processes, these extracts exhibited potent antimicrobial activity against common pathogens, including multidrug-resistant strains. The determination of MIC, MBC, and subsequent ointment formulations validated their efficacy, offering promising prospects for alternative therapeutic interventions.

The study's findings emphasize the potential of these herbal extracts to serve as valuable assets in combating challenging burn wound infections, especially in the context of increasing antibiotic resistance. However, while the results are encouraging, further exploration, including *in vivo* studies and clinical trials, is essential to ascertain their safety, efficacy, and applicability in real-world medical scenarios. The integration of traditional herbal knowledge with scientific methodologies presents a promising avenue for developing sustainable, effective treatments for burn wound care.

In brief, this research lays a strong foundation for continued investigation, offering a glimpse into the promising future of natural remedies in addressing the complexities of burn wound infections and providing potential solutions in the era of evolving healthcare challenges.

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