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Research Paper

Formulation and Evaluation of Antimicrobial Herbal Hand Wash Using Betel Leaf

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ABSTRACT

In the present study ethanolic extracts of leaves of Piper betel were screened for antibacterial properties against pathogenic bacteria namely *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The extract were effective against the used pathogens. Ethanolic extracts. Showed a maximum zone of inhibition 14mm against *Staphylococcus aureus* and 16mm against *Pseudomonas aeruginosa*. The Increasing prevalence of antimicrobial resistance and the adverse effects of synthetic chemicals have driven the need for natural alternatives in personal hygiene products. This research explores the development of an antimicrobial herbal hand wash using Betel leaf (Piper betel), a plant known for its potent antimicrobial and antioxidant properties. Betel leaves contain bioactive compounds such as alkaloids, phenols, and flavonoids, which contribute to their ability to inhibit microbial growth. The study investigates the formulation of a hand wash that combines Betel leaf extract with other natural ingredients to enhance its effectiveness while maintaining skin-friendly properties. The antimicrobial activity of the hand wash was evaluated against common pathogens, including *Staphylococcus aureus*, and *pseudomonas aeruginosa*. Results demonstrated significant inhibition of microbial growth, indicating the potential of Betel leaf as a natural and effective antimicrobial agent. Additionally, the formulation was assessed for skin irritation, pH balance, and user acceptability. This study suggests that Betel leaf-based herbal hand washes can serve as a viable alternative to synthetic hand sanitizers, providing an ecofriendly and skin-safe option for daily use.

INTRODUCTION

This research paper focuses on the antimicrobial potential of Betel leaf, a natural ingredient known

for its antiseptic, antimicrobial, and anti-inflammatory properties. Betel leaf contains bioactive compounds such as alkaloids, flavonoids, and essential oils, which have

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demonstrated significant antimicrobial effects. This study aims to explore the use of Betel leaf extracts in the development of an eco-friendly, herbal hand wash formulation, highlighting its effectiveness as an antimicrobial agent. The paper examines several aspects of the formulation process, including the antimicrobial activity of Betel leaf, potential synergistic effects when combined with other herbal ingredients, and the

overall effectiveness of the resulting hand wash. The goal is to demonstrate how Betel leaf's natural properties can provide a safer, chemical-free alternative to conventional hand sanitizers, benefiting both personal health and the environment. Through this research, the study contributes to the growing body of knowledge about plant-

Classification	
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Piperales
Family	Piperaceae
Genus	Piper
Species	Betel

based ingredients in hygiene products, promoting the shift toward more sustainable, health-conscious alternatives in personal care. The findings could lead to the development of more natural and effective hand wash products, with wider applications in health and hygiene practices.

MATERIAL AND METHODS

Collection of plant material Betel leaf were obtained in fresh form from the local market. The taxonomical classification of plant is given above in the table

Plant Material Extraction

The process of preparing an ethanolic extract from betel leaf using reflux condensation involves heating dried and powdered betel leaves (Piper betle) in ethanol under controlled conditions to isolate its active compounds. The reflux

condensation technique allows the ethanol solvent to vaporize and condense back into the reaction vessel, promoting efficient extraction. Below is a detailed procedure for the extraction:

Required Materials:

Dried and powdered Piper betle leaves
Ethanol (typically 95% or 100%)
Reflux apparatus (round-bottom flask, condenser, heat source)
Heating mantle or Bunsen burner
Glass wool or clamp to secure the setup
Filter paper or separatory funnel
Evaporating dish or rotary evaporator (optional, for solvent removal)

Procedure:

1. Preparing the Raw Material (Betel Leaves):

Ensure that the betel leaves (Piper betle) are clean and free from impurities.



Dry the leaves either in the shade or in a low-temperature oven to avoid damage to the active compounds. Once completely dried, grind the leaves into a fine powder using a mortar and pestle or a mechanical grinder.

2. Assembling the Reflux Apparatus:

Reflux System Setup

Round-Bottom Flask: Use a round-bottom flask as the reaction vessel. This allows the ethanol to circulate efficiently.

Condenser: Attach a condenser to the top of the flask. The condenser will allow the ethanol to condense after evaporating and return to the flask, ensuring the solvent remains in the system.

Water Source: Ensure that the condenser is connected to a water source for cooling. The water should be flowing through the condenser to keep it cold and maintain efficient condensation.

Heating Source: Use either a heating mantle or a Bunsen burner as the heat source for the flask. Make sure to place the flask on a heat-resistant surface to avoid accidents.

Stability: Optionally, secure the flask using glass wool or a clamp to ensure it remains stable throughout the reflux process.

3. Extraction Process

Weighing the Betel Leaves: Weigh 20-30 g of powdered betel leaves (this amount may vary depending on the desired scale of extraction).

Adding the Powdered Betel Leaves: Add the powdered betel leaves into the round-bottom flask.

Adding Ethanol: Pour ethanol (95% or 100%) into the flask to completely cover the powdered betel leaves. The typical solvent-to-plant material ratio is 3:1 (solvent to plant material), but this can be adjusted depending on the concentration needed.

4. Refluxing

Heating: Begin heating the flask gently. As the ethanol heats up, it will evaporate. The condenser will then cool the vapor and condense it back into the flask, ensuring that ethanol is continuously recirculated through the system.

Refluxing Time: Allow the reflux process to run for 2-4 hours, depending on the desired extraction. The active compounds, such as alkaloids, essential oils, flavonoids, and polyphenols, will be extracted into the ethanol during this time.

5. Temperature Control

Maintaining Temperature: The boiling point of ethanol is 78.37°C. Ensure that the temperature of the system stays below this point to avoid excessive evaporation or the loss of ethanol. Proper temperature control is essential for a smooth and effective extraction process.

6. Monitoring the Process

Check Condenser: Periodically check that the condenser is working properly and that the cooling water is circulating efficiently.

Check Ethanol Level: Ensure that the solvent is circulating well and that ethanol does not evaporate too quickly, leading to a loss of solvent.

Reflux Duration: After 2-4 hours, monitor the extract for the desired concentration. You may need to continue refluxing or filter the solution depending on the extraction results.

7. Post-Reflux

After completing the reflux, you can remove the round-bottom flask and allow the solvent to evaporate under reduced pressure if needed. This will leave you with the extracted compounds from the betel leaves. By following these steps carefully, you will effectively extract the bioactive compounds from the betel leaves using the reflux method.



8. Filtration and Separation:

After completing the extraction process, filter the mixture through filter paper to eliminate the solid plant material. This can be done using a basic Buchner funnel or any suitable filtration system. The filtrate, which contains the ethanol extract from the betel leaves, should be collected.

9. Concentration (Optional):

If you wish to concentrate the extract or remove excess ethanol, you can use a rotary evaporator or

gently heat the extract in an evaporating dish under reduced pressure to evaporate the solvent.

What remains will be a more concentrated form of the bioactive compounds from the betel leaf.

10. Storage:

Store the ethanolic extract in an airtight container, preferably in a dark glass bottle, to protect it from light and air, which could cause degradation of its active compounds.



Extraction of Betel leaf by using reflux condensation

Formulation of herbal hand wash

Ingredient	Role
Betel leaf extract	Antimicrobial property
Glycerine	Moisturize the skin
Alovera juice	Soothing property
Sodium lauryl sulphate	Foaming agent that helps breakdown oil and dirt on the skin
Hydroxy propyl methyl cellulose	Gelling agent
Methyl paraben	Preservative
Rose water	Fragrance
Distilled water	Vehicle

Procedure for herbal hand wash

Procedure:

1. Phase A (Water Phase) Preparation:

Heat distilled water to around 70-75°C. Dissolve water-soluble ingredients like glycerin or aloe vera if used.

2. Phase B (Surfactant Phase) Preparation:

In a separate container, combine the surfactants (e.g., SLS, SLES, or mild surfactants like Decyl Glucoside). Mix them gently to avoid foaming. If necessary, heat slightly to ensure the surfactants are fully dissolved (around 40-50°C).

3. Thickening & Stabilizing:

Add thickening agents like Hydroxyethylcellulose or Carbomer to the water phase or surfactant phase. If using Carbomer, neutralize with a small amount of Sodium Hydroxide or Triethanolamine to thicken the gel.

4. Combine Phases A and B:

Slowly combine the water phase with the surfactant phase, mixing gently to avoid foam

formation. Stir the mixture to ensure uniform consistency and ensure all components are well dissolved.

5. Add Preservatives & Fragrance:

Once the mixture is uniform and cooled to below 40°C, add the preservative and fragrance. Stir well to disperse them evenly.

6. Adjust pH:

Test the pH of the formulation (ideal range is 4.5 to 6.0 for skin safety). If necessary, adjust the pH by adding small amounts of Citric Acid or a similar pH adjuster.

7. Cooling and Packaging:

Let the mixture cool completely. Once cooled, transfer the formulation into bottles or dispensers.

8. combine phases A and B

Slowly combine the water phase with surfactant phase, mixing gently to avoid the foam formation stir the mixture to ensure uniform consistency and ensure all components are well dissolve.



Antimicrobial herbal hand wash using betal leaf

Evaluation parameters of herbal hand wash

Physicochemical Evaluation

These tests assess the physical and chemical characteristics of the hand wash to ensure quality and consistency.

pH Level: The pH of the hand wash should be skin-friendly, typically ranging from 4.5 to 7, to prevent skin irritation.

Viscosity: Ensures the product has an appropriate consistency for ease of use, TVneither too runny nor too thick.

Foam Formation: A good hand wash should produce enough lather for effective cleansing, as foam helps in removing dirt and microbes.

Stability Testing: Assess the stability of the product under different temperature and humidity conditions to ensure its longevity and effectiveness throughout the product's shelf life.

Color and Appearance: Ensure the hand wash maintains a consistent appearance and color. Natural herbal formulations may experience slight variations, but significant changes might indicate formulation issues.

Toxicological Evaluation

These tests ensure that the hand wash is safe for human use, especially for prolonged or frequent use.

Skin Irritation Test: Conduct patch testing or irritation tests on human skin to ensure the product does not cause irritation, dryness, or discomfort.

Allergenic Potential: Evaluate the potential for allergic reactions, especially in individuals sensitive to specific herbs or plant-based ingredients.

Toxicity Testing: Ensure that the ingredients, especially concentrated herbal extracts, do not pose any toxic risk when applied to the skin or absorbed.

Fragrance: The product should have a pleasant, not overpowering fragrance. It should not cause discomfort or irritation, especially for individuals with sensitive noses or skin.

Feel and Texture: Evaluate how the hand wash feels during and after application, whether it leaves the skin feeling clean, moisturized, or dry.

Ease of Use: The product should be easy to dispense, apply, and rinse off without leaving residues on the hands.

Evaluation parameter	Result
Appearance	Greenish to dark green
Odour	Characteristics, aromatic and strong odour
Texture	Smooth, and slightly viscous.
Clarity	Clear
Viscosity	Intermediate
pH	4.6 -6.5
Foaming ability	Good
Cleansing ability	Good
Stability	Stable

Antimicrobial susceptibility Assay Procedure:

1. Preparation of Agar Plates:



Prepare nutrient agar plates by sterilizing the agar medium and pouring it into sterile Petri dishes.

Allow the agar to solidify at room temperature

2. Inoculation of Bacterial Culture:

Using a sterile cotton swab, swab the surface of the nutrient agar plate with the bacterial culture to form a uniform lawn of bacteria.

3. Making Wells:

Using a sterile cork borer, make 2-3 wells in the solidified agar plates. The wells should be equidistant from each other and large enough to hold the extract.

4. Application of Piper betel Extract:

Using a sterile pipette, fill the wells with different concentrations of the Piper betel leaf extract. For control, you may use a well containing only the solvent (ethanol or water). For test you add piper betel leaf extract.

5. Incubation:

Incubate the plates at 37°C for 24-48 hours, depending on the growth rate of the bacteria.

6. Observation:

After incubation, check for zones of inhibition around the wells. A clear zone around the well indicates that the Piper betel leaf extract has antibacterial activity against the test bacteria. Measure the diameter of the inhibition zone (in mm) using a ruler or caliper.

7. Interpretation of Results:

If the zone of inhibition is observed, the extract has antibacterial activity.

The larger the inhibition zone, the stronger the antibacterial activity of the extract. Compare the antibacterial activity of Piper betel leaf extract test with a standard

RESULT

Antimicrobial susceptibility Assay of ethenolic extraction of Betel leaf Antimicrobial susceptibility Assay was performed for the ehanolic extract against various pathogens and the result of the same can be seen in table below. It can be seen that maximum zone of inhibition was seen against staphylococcus aureus and pseudomonas aeruginosa.

Test organism	Zone of inhibition
Staphylococcus aureus	14
Pseudomonas aeruginosa	16



Staphylococcus aureus

pseudomonas aeruginosa

DISCUSSION

Discussion: Herbal medicines are a valuable and readily available resource for primary health care and complementary care system. They can be the best alternative for the available antibiotics against which the pathogens are adapting resistance. Piper betel used as an antimicrobial herbal handwash can be a very good substitute for the available drugs after proper pharmacological investigation. Plants extract were prepared from dried sample in this research work as has been reported earlier by Ethanolic extract of P. betel leaves were taken for the antibacterial studies in the present research work, earlier [4-6] have reported antimicrobial properties of aqueous, ethanolic extracts of Piper betel. Agar well diffusion method was used here in order to determine the antibacterial properties of plant extracts against pathogens as has been performed

CONCLUSION

The antimicrobial activity of Piper betel leaf extract, particularly the ethanolic extract, demonstrates promising potential as an effective antimicrobial agent against various bacterial species. The presence of clear zones of inhibition in the antimicrobial susceptibility assays indicates that the extract possesses antibacterial properties, likely attributed to its bioactive compounds such as phenols, flavonoids, and alkaloids. These compounds appear to disrupt bacterial cell structures or inhibit essential metabolic processes, leading to bacterial growth inhibition.

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