



FORMULATION AND STANDARDISATION OF ANTIMICROBIAL HAND WASH FROM PIPER BETLE LEAF EXTRACT

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ABSTRACT

Hand hygiene plays a critical role in preventing the transmission of infectious diseases. This study focuses on the formulation and standardization of an antimicrobial hand wash using *Piper betle* leaf extract. The *Piper betle* plant, commonly known as betel leaf, possesses various bioactive compounds with antimicrobial properties. The leaf extract was prepared using the Soxhlet extraction method with a suitable solvent, such as methanol, and its antibacterial activity was assessed using the agar well plate diffusion method with *Staphylococcus aureus*. The formulation of the antimicrobial hand wash involved incorporating the *Piper betle* leaf extract into a suitable base formulation comprising surfactants, emollients, and preservatives. Physicochemical parameters such as pH, viscosity, and stability were also assessed to ensure product quality and shelf-life. Gram-positive organism were significantly inhibited by the standardised anti microbial hand wash composition. the hand wash showed good stability and skin compatibility. Though the result showed that formulation and standardisation of an antimicrobial hand wash from *Piper betle* leaf extract offer a promising approach to developing a natural and effective product for hand hygiene. This research contributes to the growing field of natural product-based antimicrobial formulations and highlights the potential of *Piper betle* as a valuable source for developing novel antimicrobial agents.

KEYWORDS: *Piper betle* leaf, leaves extract, hand wash, agar well plate diffusion method.

INTRODUCTION

Herbal plants are the untapped natural resources for traditional medicine, folk medicine, modern medicine, nutraceuticals, food supplements, pharmaceutical intermediates, and chemical precursors for synthetic medications. 90 to 95% of the formulations used in the traditional systems of medicine, including Ayurveda, Homoeopathy, Unani, and Siddha, are plant-based. *Piper betle* Linn's leaves are grown in South India, Bihar, Bengal, Orissa, and Karnataka because of their value in Indian medicine and the hot, humid climate. They are a type of evergreen perennial creeper known as "Paan."^[1,2]

For a variety of functions, the *Piper Betle* makes use of leaves, roots, stems, stalks, and fruits. Numerous pharmacological effects of *Piper betel* leaves exist, such as anticancer, antimutagenic, and antihelminthic ones. They also have antioxidant and antifertility activities, as well as antibacterial, therapeutic, and protecting qualities. Alkaloids, sugars, amino acids, tannins, and steroidal substances are all present in the leaves. They include essential oils, vitamins, and minerals. its leaf is used in folk medicine to treat a variety of illnesses.^[3,4,5,6]

Agents that limit bacterial development, stop microbial colonies from forming, and eradicate or kill microorganisms are referred to as having antimicrobial activity. Both bactericidal and bacteriostatic agents fall within this category. Disc-diffusion, broth or agar dilution, and poisoned food techniques are among the laboratory techniques for testing or screening in vitro antimicrobial activity. For further investigation of an agent's antimicrobial action, identifying the inhibitory effect (bactericidal or bacteriostatic), and assessing cell damage brought on by the test microorganism, time-kill, and flow cytometric methods are advised.^[7,8]

Maintaining good hand hygiene is crucial for protecting the skin from harmful microorganisms and preventing the spread of infections and bacteria. Hand washing is a vital step in protecting the skin from microorganisms. Proper fingernail brush usage is the best technique for eradicating transient microorganisms from hands and fingertips. Hand washing involves thorough scrubbing followed by rinsing, reducing the amount of hazardous microorganisms on hands, and removing visual filth. This practice is especially important for professionals in the medical, restaurant, and other industries where hand

cleanliness is essential.^[9,10,11,12]

MATERIALS AND METHODS

Scientific Names: *Piper betle* Linn

Family: Piperaceae

Collection of plant material

The fresh leaves of *Piper betle* was used in the present study are collected from the local market of Kadungallur, Malappuram district, Kerala, India in March 2023. Other ingredients were collected from the chemical store of the college. *Piper betel* leaf was identified and authenticated by senior botanist Dr. A.K Pradeep, Assistant Professor, Department of Botany, University of

Calicut. Fresh leaves was washed with distilled water to remove the dirt and other foreign particles. After that, the leaves were allowed to dry for two weeks at room temperature in a shady place. After that, a regular grinder was used to grind the dried leaves. Then, a soxhlet device was filled with the leaf powder.

Extraction of plant material

15gm of dried powder was subjected to Soxhlet extraction with 300ml methanol as solvent, extraction was carried out for 3 hrs, 10 cycles and temperature was maintained at 65°C. Colour of extract was dark green.^[13,14]

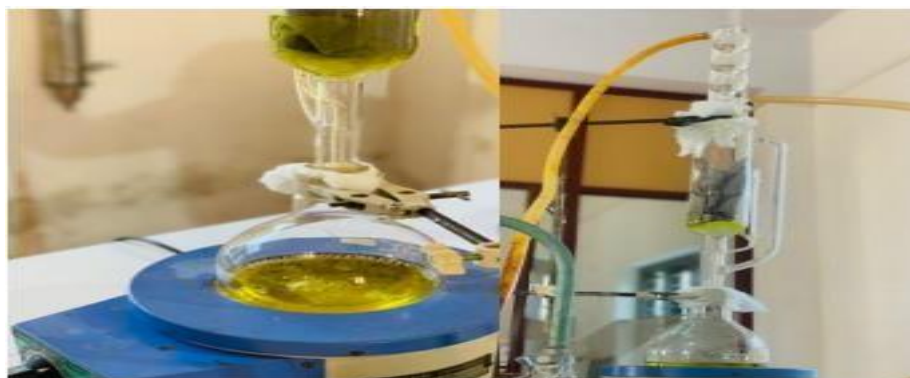


Figure 1: Soxhlet extraction.

Formulation of hand wash

In this formulation, weigh the required quantity of active ingredients such as 20 ml of *Piper betle* leaves extract filtrate. Other ingredients such as 3.5 g of SLS, 40 ml glycerine were added. 0.3 g of methyl paraben is dissolved in a beaker containing distilled water and mixed well. 2 g of carbopol 940 is added and stirred constantly

for few minutes to form gel. 1g of sodium chloride is mixed with boiling water and added to the formulation. Required quantity of perfume is added as fragrance and colourant as colouring agent. Then the quantity is made sufficient to 100ml by adding distilled water. Finally, it was placed in a tightly sealed container and appropriately labelled for subsequent investigation.

Table. 1: Composition of formulated hand wash.

Sr.no	Ingredients	Quantity	Uses
1.	Piper betle leaves extract	20ml	Antimicrobial agent
2.	Sodium lauryl sulphate(SLS)	3.5g	Foaming agent
3.	Glycerine	40ml	Softening agent
4.	Methyl paraben	0.3g	Preservative
5.	Carbopol 940	2g	Thickening agent
6.	Sodium chloride	1g	Stabilising agent
7.	Distilled water	Up to 100ml	Vehicle
8.	Perfume	q.s	Fragrance
9.	Colourant	q.s	Colouring agent

Evaluation of hand wash

1) Organoleptic evaluation

Parameters like colour, odour, texture was evaluated. Colour and texture were evaluated by visual inspection and touch sensation respectively. Odour was inspected by sensing inspection.

2) Appearance and Homogeneity

Appearance and homogeneity was evaluated by visual method.

3) Grittiness

1ml of formulation was taken on fingertips and rubbed between two fingertips, then the hand wash was evaluated.

4) pH

1g of hand wash was dissolved in 100ml of distilled water. The sample solution was measured using digital pH meter and it should be calibrated before and every pH measurements.

5) Viscosity

The viscosity of formulation was evaluated using Ostwald viscometer.

6) Spread ability

0.5gm of hand wash was pressed between two slides and left for about 5 minutes, were no more spreading was expected. Diameter of spreaded circle was measured in cm.

7) Stability

The stability studies was carried out by storing the sample for a week. During the stability studies no change in colour and no phase separation were observed in the formulation.

8) Foam Height

1 g of hand wash was dissolved in 50 ml distilled water. The solution was transferred into a measuring cylinder. Volume was made up to 100 ml with distilled water. This solution is taken in 3 test tubes in a series of successive portion of 1, 2, 3 ml and remaining volume is made up with 10 ml of water. Then the test tubes were shaken for 15 seconds. It should be allowed to stand for 5 minutes. And the foam height was determined.

9) Foam Retention

The sample was prepared as per the foam height procedure. The volume of foam at 1 minute intervals for 4 minutes was recorded

10) Antimicrobial Study of *Piper betle* hand wash

- The screening of antimicrobial efficacy of the formulated hand wash was aseptically performed on gram positive bacteria *Staphylococcus aureus* by using agar well plate diffusion method.
- Two sterile petri plates were taken for testing the

antimicrobial activity.

- A well was prepared in the plates containing 15 ml of Muller Hinton agar medium and allowed for solidification.
- 100µl of the test sample was introduced into the well. The standard antibiotics like ciprofloxacin injection (5µg/50µl) were used as standard.
- The plates were incubated at 37°C in order to determine the activity.
- After 24 hours of the Plates were observed for the zone of inhibition.
- Efficiency of formulation was determined by measuring the diameter of zone of inhibition.

RESULTS AND DISCUSSION

A tiny amount of synthetic chemicals and natural *Piper betle* leaf extract were included in the formulation of the hand wash. Due to issues with pH, viscosity, foamability and spreadability in some batches, different batches were prepared for the formulation trial phase. Make one final batch after discarding the previous batches. The *Piper betle* leaf extract that was created was dark green in colour, non-gritty, and had strong antibacterial properties. The prepared *Piper betle* hand wash was shown in figure 2.



Figure. 2: Formulated *Piper betle* hand wash.

Evaluation of piper betle hand wash

Table 2: Physico chemical parameters.

Sr.No.	Evaluation Parameters	<i>Piper betle</i> handwash
1.	Colour	Dark Green
2.	Odour	Lemon like
3.	Texture	Smooth
4.	Appearance and homogeneity	Translucent
5.	Grittiness	Non-Gritty
6.	pH	7.66
7.	Stability	Stable

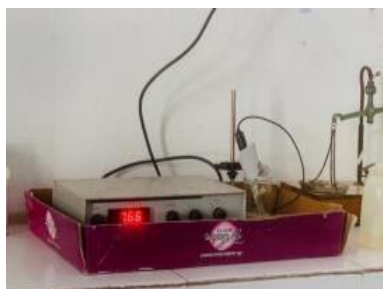


Figure. 3: Determination of pH by pH meter.



Figure. 4: Stability of hand wash

Viscosity**Table 3.** Viscosity of *Piper betle* hand wash.

Sr.No	Time (Min)
1.	05:30
2.	06:12
3.	05:36

Table 4: Spread ability of hand wash.

Sr.No	Diameter(cm)	Radius(cm)
1	3.5	1.75
2	4.2	2.1
3	4.0	2.0

Foam height**Table 5:** Foam height of hand wash.

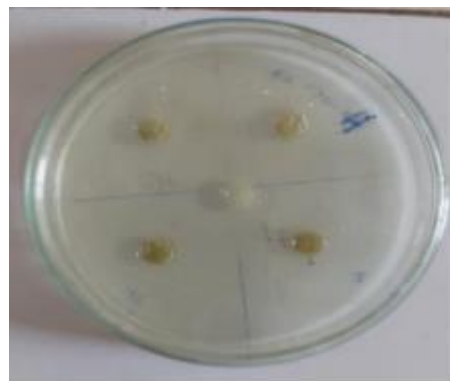
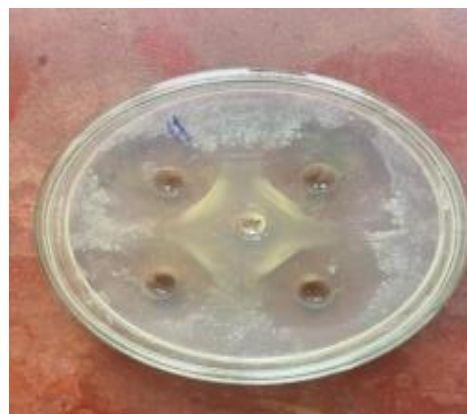
Sr.No	Sample :Water	Foam Height(cm)
1.	1:9	2
2.	2:8	3.5
3.	3:7	4.5

**Figure. 5:** Determination of Foam height.**Foam Retention****Table 6:** Foam retention of hand.

Sr.No	Time(min)	Foam Retention(cm)
1.	1	2.4
2.	2	2.0
3.	3	1.8
4.	4	1.5
5.	5	1.0

**Figure. 6:** Determination of Foam retention.**Antimicrobial Activity****Table 7:** Anti microbial activity of *Piper betle* hand wash

Zone of Inhibition (mm)	
Microorganism (<i>Staphylococcus aureus</i>)	
Formulated <i>Piper betle</i> hand wash	Standard (<i>Ciprofloxacin</i>)
19 mm	20 mm

**Figure .7:** Before incubation of formulated *Piper betle* hand wash.**Figure. 8:** After incubation formulated *Piper betle* handwash**CONCLUSION**

The development and standardisation of handwash products containing *Piper betle* leaf extract may have a number of advantages. Here is a summary of how handwash made from *Piper betle* leaf extract was created and standardised. In this work, "formulation and standardisation of antimicrobial hand wash from *piper betle* leaf extract," the extraction, formulation, and evaluation of hand wash were carried out. To extract the active ingredients or elements from *piper betle* leaf, a soxhlet extraction method is usually used. Several evaluation criteria, including colour, smell, appearance, homogeneity, grittiness, pH, viscosity, spreadability, stability, foam height, foam retention, and antimicrobial activity were assessed in this formulation. *Staphylococcus aureus* was utilised as the test organism in a *in vitro*- antimicrobial activity, and the results showed that *Piper betle* had good anti- microbial action.

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