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Formulation and Evaluation of Antimicrobial Soap from Piper betle Extract

Dr. Hemalata Sagar Wadkar[1], Aditi Amol Mardhekar[2], Krishna Bhaurao Kendre[3] Tejashree Prakash Patil[4]

'HOD, Department of B. Pharm. (Practice), Abhinav Education Society's College of Pharmacy, Narhe, Pune-411041, Maharashtra, India

[2,3] Students, Abhinav Education Society's College of Pharmacy, Narhe, Pune-411041, Maharashtra, India

***Corresponding author:** Dr. Hemalata Sagar Wadkar*

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ABSTRACT:
Natural soap is produced without synthetic surfactants and incorporates functional ingredients such as natural substances, essential oils, or plant extracts. Soap, a type of surfactant derived from natural oils or fats, is created through the saponification process. This research aimed to analyze solid soap made from coconut oil infused with betel leaf extract and to assess its antimicrobial properties. The solid soap is formulated by immersing coconut oil in a 5% NaOH solution and preparing the betel leaf extract using a decoction method. Currently, we are utilizing Piper betel, commonly known as betel leaf (khaupan). The solid soap formulation includes the addition of stearic acid, propylene glycol, glycerin, ethanol, olive oil, and rose water. These herbal transparent soaps were produced using the hot process method. The physicochemical properties evaluated included moisture content, pH, total ash value, extractive value, and preliminary tests. The antimicrobial efficacy of the herbal transparent soaps was examined using the agar well diffusion method. Multiple batches were created to explore the impact of the soap base during formulation, with the most effective batch selected for the herbal soap formulation.

KEYWORDS: Antimicrobial soap, Betel Leaf, Olive oil, Rose Water

I. INTRODUCTION:

Formulation and evaluation of antimicrobial herbal soap from piper betel extract.A culture of cleanliness embodies individuals' perspectives and actions towards upholding personal and environmental hygiene to prevent diseases, bacteria, and germs. Soap serves as a tool for purifying oneself from dirt, germs, and other contaminants that compromise bodily cleanliness.[1]Research indicates that the leaves of this plant exhibit numerous beneficial bioactivities, and the extract derived from betel leaves holds significant promise for the development of commercial products. Given its many advantages, betel vine is cultivated primarily for its foliage. The optimal conditions for the commercial cultivation of betel vine are found in tropical rainforests, which offer cool shade, high humidity, and sufficient soil moisture, as seen in countries such as Indonesia, Malaysia, the Philippines, Thailand, Cambodia, Vietnam, and India. The betel plant contains a variety of phytochemicals, including chavibetol, chavicol, hydroxychavicol, estragole, eugenol, methyl eugenol, hydroxycatechol, caryophyllene, eugenol methyl ether, cadinene, y-lactone, allylcatechol, p-cymene, cepharadione A, dotriacontanoic acid, tritriacontane, terpinene, eucalyptol, carvacrol, and sesquiterpenes.[2]


Piper betle Leaf Types of betel leaf

The skin is the body's largest sensory organ. It acts as a protective barrier for the internal organs and collects sensory information from the environment. Furthermore, it plays a crucial role in regulating the body's temperature to maintain a healthy state. The skin comprises various unique cells and structures, organized into three main layers: the hypodermis, dermis, and epidermis. Each layer has a distinct function that contributes to the overall operation of the skin. Given that the skin plays a vital role in overall health, it is essential to protect it from diseases and disorders. Skin conditions are a

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common health issue, affecting individuals of all ages, from infants to the elderly, in various ways. Factors such as infections, allergies, sun exposure, injuries, and more can lead to skin problems[3].

Since ancient times, humans have utilized medicinal plants for therapeutic purposes. The leaves, stems, and roots of various medicinal plants have been employed as naturalremedies to address a wide range of ailments and disorders. Although many plant-based treatments have been supplanted by synthetic options, ayurvedic products continue to be recognized for their efficacy and safety[4]. Numerous herbs possess anti-oxidant, anti-bacterial, cytotoxic, anti-microbial, hypotensive, anti-diuretic, anti-inflammatory, anti-spasmodic, anti-diabetic, anti-hemorrhagic, and anti-helminthic properties, along with high nutritional value. Due to their significant medicinal benefits, affordability, accessibility, and compatibility, the integration of natural products into treatments is effective for addressing nearly all diseases and skin issues[5]. The active compounds responsible for the therapeutic effects of these plants are extracted and applied topically in creams, soaps, oils, and ointments to treat skin conditions such as acne, eczema, wounds, and ringworm, as well as for cosmetic and anti-microbial applications. The therapeutic properties of plants are utilized in various formulations for both medical and cosmetic uses[6].

A clean culture signifies the attitudes and behaviors of individuals in upholding personal and environmental hygiene to prevent diseases, bacteria, and germs[7]. Soap serves as a tool for cleansing oneself from dirt, germs, and other contaminants that can soil the body. Soap possesses amphiphilic properties, containing both hydrophobic (polar) and hydrophilic (non-polar) groups.[7] It has the ability to attach to dirt and fat molecules, allowing them to be dissolved in water [8],[9]. The process of soap making typically involves the use of oils and alkali through a saponification process[9]. The saponification reaction, which involves the interaction of triglycerides with alkali (either NaOH or KOH), results in the production of soap and glycerin. Sodium hydroxide (NaOH) is utilized for solid soap, while potassium hydroxide (KOH) is employed for liquid soap [10].

Scrubbing body or hands, particularly with soaps, is the first line of defense against bacteria and other pathogens that can cause colds, the flu, skin infections and even deadly communicable diseases[11]. Conceptually, many people consider that an antimicrobial potion of soaps is effective at preventing communicable diseases. But now researchers highlight that too much of it can have the opposite effect-spreading diseases/infections instead of preventing them[12]. Overutilization of medicated soaps might result in antimicrobial resistance and even rendering an individual more vulnerable to microbial attacks such as opportunistic skin infections[13].

On the other hand, regardless of a wide-spread availability of the so-called medicated soaps; a number of communicable infectious and food-borne diseases as well as poor-hygienic conditions-related health problems are rampant. This can partially be explained by the fact that, occasionally some of these antimicrobial consumer products could have insufficient quantities of antimicrobials. It seems to be more of a marketing phenomenon. Unfortunately, in the long-run may adversely affect the consumers, because overuse of these agents can ascribe to the emergence of drug-resistant microorganisms[14]. This instigated us to embark on evaluation of the antimicrobial effects of the so-called medicated soaps.

II. MATERIALS AND METHODS:

i] PRELIMINARY PHYTOCHEMICAL TESTS:

(a) Ninhydrin test (General test): Heated 3 ml T.S. and 3 drops 5% Ninhydrin solution in boiling water bath for 10 min. Purple or bluish colour appears.

(b) Test for tyrosine: Heated 3 ml T.S. and 3 drops Millions reagent. Solution shows dark red colour.

(c) Test for Vitamins:To 2 ml of a 2% w/v solution added 2 ml of water. 0.1 g of sodium bicarbonate and about 20 mg of ferrous sulphate, shaked and allowed to stand; colour disappears.

(d) Test For Enzymes: To few thick sections of drug, added catechol solution. The sections become yellowish brown. Repeated test with other boiled sections the yellowish brown colour appears.

ii] TOTAL ASH CONTENT:

Weighed and ignited flat, thin, porcelain dish or a tared silica crucible. Weighed about 2 g of the powdered drug into the dish/crucible. Supported the dish on a pipe-clay triangle placed on a ring of retort stand. Heated with a burner, using a flame about 2 cm high and supporting the dish about 7 cm above the flame, heat till vapours almost cease to be evolved; then lowered the dish and heated more strongly until all the carbon is burnt off. Cooled in a desiccator. Weighed the ash

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and calculated the percentage of total ash with reference to the air dried sample of the crude drug.

iii] WATER-SOLUBLE EXTRACTIVE VALUE:

Weighed about 4 g of the coarsely powdered drug in a weighing bottle and transferred it to a dry 250 ml conical flask. Filled a 100 ml graduated flask to the delivery mark with the solvent . Washed out the weighing bottle and pour the washings, together with the remainder of the solvent into the conical flask. Filtered into a 50 ml cylinder. When sufficient filtrate has collected, then 25 ml of the filtrate is transferred to a weighed, thin porcelain dish, as used for the ash values determinations. Evaporated to dryness on a water-bath and completed the drying in an oven at 105° C for 6 hrs. Cooled in a desiccator for 30 minutes and weighed immediately.

iv] MOISTURE CONTENT:

Weighed about 1.5 g of the powdered drug into a weighed flat and thin porcelain dish. Dried in the oven at 100° C or 105° C, until two consecutive weighings do not differ by more than 0.5 mg. Cooled in a desiccators and weighed. The loss in weight is usually recorded as moisture.

III. FORMULATION:

i] FORMULATION TABLE:

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	Category
Betel leaf extract	1 ml	2 ml	3 ml	4 ml	1 ml	2 ml	3 ml	4 ml	Antimicrobial agent
Coconut oil	5 ml	10 ml	15 ml	20 ml	5 ml	5 ml	5 ml	5 ml	Natural fat
Sodium hydroxide	5 gm	5 gm	5 gm	5 gm	5 gm	5 gm	5 gm	5 gm	Lye
Stearic acid	2.5 gm	2.5 gm	2.5 gm	2.5 gm	2.5 gm	2.5 gm	2.5 gm	2.5 gm	Hardening agent
Propylene glycol	2.5 ml	2.5 ml	2.5 ml	2.5 ml	2.5 ml	2.5 ml	2.5 ml	2.5 ml	Moisture retention
Glycerine	4 ml	4 ml	4 ml	4 ml	4 ml	4 ml	4 ml	4 ml	Humectant
Olive oil	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	Natural fat
Ethanol	4.5 ml	4.5 ml	4.5 ml	4.5 ml	4.5 ml	4.5 ml	4.5 ml	4.5 ml	Solvent
Rose water	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	Perfuming agent

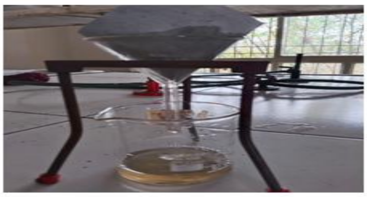
Formulation Table

ii] PREPARATION OF BETEL LEAF EXTRACT:

1. Wash the fresh leaves of Piper betle and Shed dry for one week.
2. After drying the dried leaves are crushed and formed the fine powder.
3. Take a 500ml of water and dissolve 10 g of drug and keep for boiling for atleast 30 minutes.
4. Betel leaf extract is made by the decoction method. This traditional and widely used method involves boiling crude drugs in water

for approximately 15-30 minutes to extract water-soluble and heat-stable components.

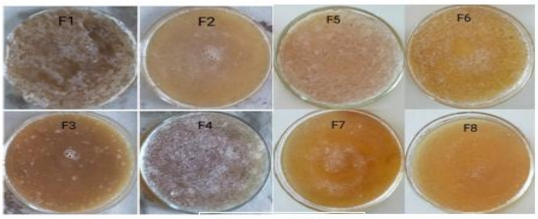
5. After boiling, the mixture is cooled and filtered by filtration method and store at a cool place in air tightened container.



Decoction Method

iii] SOAP MAKING PROCESS:

Dissolved 5% of NaOH in 2.5 ml of distilled water, stirring continuously, and allowed the mixture to rest for 15 to 30 minutes. In a beaker, combined coconut oil and olive oil, heated the mixture in a water bath for 10 to 15 minutes. Gradually added the lye solution drop by drop while stirring continuously until a pudding-like consistency is achieved. Incorporated propylene glycol and glycerin while maintaining continuous stirring. Added ethanol, betel leaf extract, and rose water to the mixture, continuing to stir. After heating the mixture for 5 minutes, poured it into soap molds and set aside for 24 hours to allowed the soap to harden.



Prepared Soap

iv] EVALUATION OF SOAP :

1)Physical Evaluation:

The colour and clarity was done by sensory and visual inspection.

2) Degree of Acidity (pH):

The pH test is a chemical analysis designed to assess the acidity or alkalinity of solid soap. This test is essential as pH is a critical factor in evaluating the stability of solid soap during storage and in determining whether each solid soap formula maintains a pH level that is compatible with skin. An inappropriate pH can lead to skin irritation if the soap is overly acidic, or result in scaly and itchy skin if it is excessively alkaline. A gram of the sample was placed in a glass, to which 10 ml of distilled water was added, and the mixture was stirred until fully dissolved. The acidity (pH) was measured using a pH meter, and the solution was allowed to stand for a moment until a stable pH reading was obtained.[4]

F1	F2	F3	F4	F5	F6	F7	F8
8.45	8.51	8.70	8.71	8.72	8.80	8.90	8.81

pH Table

Determination of pH by pHmeter

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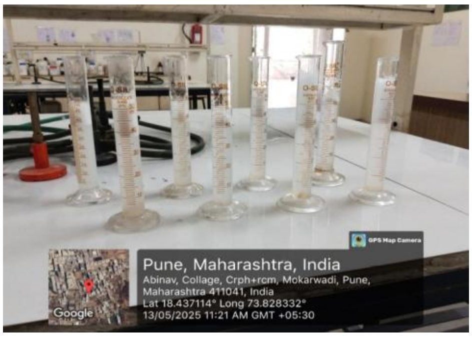
3) Foamability:

50 ml of distilled water were measured, and two grams of the soap sample were thoroughly dissolved by stirring. This solution was then transferred to a 250 ml measuring cylinder, including any washings. The total volume was adjusted to 200 ml by adding more distilled water. The mixture was subjected to 25 uniform strokes and allowed to stand until the water volume stabilized at 200 ml. The height of the foam was subsequently measured from the top of the water volume.[4]

Foamability Table

F1	F2	F3	F4	F5	F6	F7	F8
3cm	4.5cm	4.9cm	5cm	4cm	5cm	6.5cm	7cm



Foamability

4) Foam stability:

The same amount of soap sample and distilled water was used, and the procedure was conducted similarly to that of foamability, allowing the mixture to stand undisturbed for30 minutes. After this period, the time taken for the soap solution to defoam was recorded.[4]

F1	F2	F3	F4	F5	F6	F7	F8
15min	20min	25min	28min	20min	25min	24min	27min

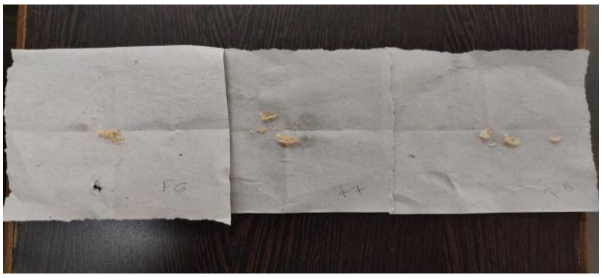
Foam stability Table

5) Alcohol Insoluble Matter:

A volume of 50 ml of warm ethanol was added to a conical flask containing a 5 gm sample of soap to facilitate its dissolution. The mixture was then filtered using tarred filter paper and an additional 20 ml of warm ethanol, followed by drying at 105°C for one hour. The filter paper was subsequently weighed after drying.[4]

F1	F2	F3	F4	F5	F6	F7	F8
1.26 %	1.39 %	1.47 %	1.66 %	2.73 %	2.80 %	2.87%	2.96 %

Alcohol Insoluble Matter Table



Alcohol Insoluble Matter

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%Alcohol Insoluble Matter = Weight of residue x 100
Weight of Soap
= 1.48 / 50 x 100
= 0.0296 x 100
=2.96 %

6) Antimicrobial Activity:

The antibacterial activity was assessed using the agar diffusion method, employing blank paper Initially, 50 µL of 100 ppm Amoxicillin, which serves as the standard antibiotic, was utilized as the positive control, while distilled water was designated as the negative control. Subsequently, bees-layer and seat-layer media were prepared from nutrient agar to provide a suitable environment for the inoculum of each bacterium to proliferate. Following this, the bees-layer medium was poured into a petri dish and allowed to cool until it solidified. Thereafter, the seat-layer medium was introduced with 10 µL of the inoculum solution using a micropipette, permitted to cool and solidify before being placed atop the bees-layer medium. Ultimately, the nutrient agar media, featuring the bees-layer beneath and the seat-layer above, comprised four distinct blank paper discs: two for the extract replications, one for Amoxicillin, and one for distilled water, which were subsequently incubated at 32.5 °C for 24 hours. Finally, the antibacterial activity, indicated by the inhibition of bacterial growth, was evaluated by measuring the diameter of the clear zone formed around the blank paper disc. The measurement of the clear zone's diameter was conducted using a caliper following the conclusion of the incubation period.[6]

Antimicrobial Activity carried for formulation

IV. RESULT AND DISCUSSION:

i] Preliminary Phytochemical Tests:

TEST	OBSERVATION	INFERENCE
1)Test For Amino acid		
a. Ninhydrin Test: Heat 3 ml T.S. sections and 3 drops 5% Ninhydrin solution in boiling water bath for 10 min.	Purple or bluish colour appears.	Amino acid present
b. Test For Tyrosin: Heat 3 ml T.S. and 3 drops Millions reagent	Dark red colour appears	Tyrosine present
2) Test for Vitamins:To 2 ml of a 2% w/v solution add 2 ml of water. 0.1 g of sodium bicarbonate and about 20 mg of ferrous sulphate, shake and allow to stand;	Colour disappears	Vitamins present
3) Test For Enzymes: To few thick sections of drug, add catechol solution. The sections become yellowish brown. Repeat test with other boiled.	Yellowish brown colour	Enzymes present

Phytochemical Tests Table

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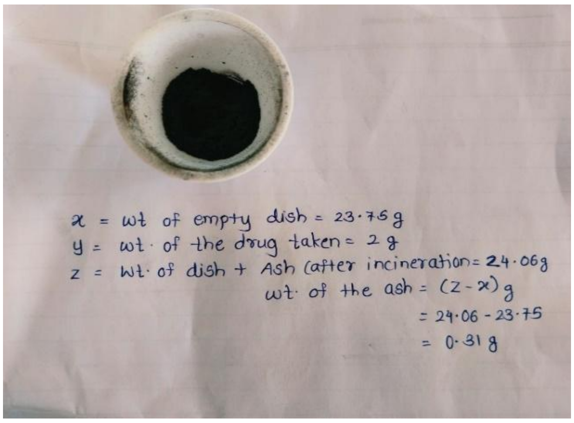
ii] TOTAL ASH CONTENT:

Chemical Tests

Calculation:
Weight of the empty dish = x
Weight of the drug taken = y
Weight of the dish + Ash(after complete incineration) = z
Wt. of the ash = (z – x) g
'y' g of the crude drug gives (z – x) g of the ash
∴ 100 g of the crude drug gives 100/y × (z –x) g of the ash
Total ash value of the sample = $\frac{(z - x)}{y}$ 100%
therefore, the total ash value of the Piper betle powder is 0.31 g.



Total Ash Value

iii] WATER-SOLUBLE EXTRACTIVE VALUE:
Calculation:
25ml of water extract gives = 1.20 g of residue
100ml of water extract gives = 4 x 1.20 g of residue
∴ 100ml of water extract gives = 4.80 g of residue
5 g of air dried drug gives = 4.80 g of water soluble residue
100 g of air dried drug gives = 80 x 1.20 g of water solute residue
Water soluble extractive value = 80 × x %
= 80 × 1.20 %
= 96 %

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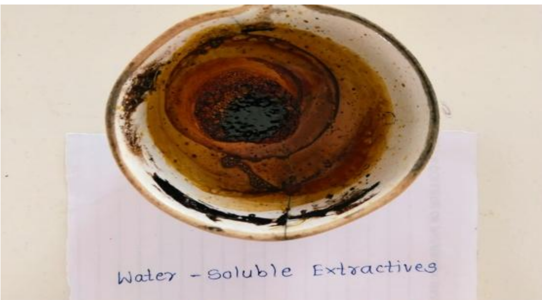
Therefore, Water soluble Extractive value of the given sample is 96 %



Water- Soluble Extractive Value

iv] MOISTURE CONTENT:

TIME	WEIGHT READINGS
0 minutes	77.98 gm
15 minutes	77.94 gm
30 minutes	77.92 gm
45 minutes	77.92 gm

Moisture Content Table

Calculaton:

Weight of drag = 2gm
Weight of empty porcelain dish = 75.56gm
Weight of empty porcelain dish+ Weight of drug (A) =77.98 gm
Weighs of empty porcelain dish+ Weight of drug (after 15min) =77.93 gm
Weight of empty porcelain dish+ Weight of drug (after 3min) =77.92 gm
Weight of empty porcelain dish+ Weight of drug (after 45 min) = (B) = 77.92 gm

Moisture content = w – d / w × 100%
= 77.98 – 77.92 / 2 × 100 = 0.06 / 2 × 100 = 3%

Parameters	**Prepared herbal soap**
Physical parameters (Colour, Odor, Clarity)	Colour: Yellow whitish Odor: Pleasant Clarity: Crystal clear
pH	8.90
Foambility	7cm
Foam stability	14min
Alcohol insolube matter	3.06%
Total Ash Value	0.31 g
Water Soluble Extractive Value	96 %
Moisture Content	3 %

Parameters and Results Table

V. CONCLUSION:

Based on the information provided, one can gather that betel leaf extract is suitable for creating herbal soap with excellent physical properties, namely having a dense, transparent shape, a distinctive aroma of betel leaves, and a pH range of 8-9, foam height of 57-65mm, and water content less than 15%. Eight batches were performed during experiment and from that F8 batch is optimized for best results.The betel leaf used in the glycerine soap bar production have a significant effect on soap bar moisture content, free alkali and pH values, as well as consumer preference for aroma, colour, and transparency. Green betel soap appears to have higher moisture content, free alkali level, and pH, as well as consumer preference for its aroma, colour, and transparency. Moreover, higher concentrations of betel leaf extract added to the soap led to higher free alkali level and pH, but a weaker preference from consumers for its colour and transparency. Based on all the measured parameters and its antimicrobial activity with Stapylococcus aureus and E.coli, the addition of betel extract up to 2.5% is considered to be the optimal formula to produce a glycerine soap bar which is qualified to meet the national standards for soaps.

VI. FUTURE PERSPECTIVE:

The formulation of antimicrobial soap using Piper betle extract presents a promising avenue in the development of natural, sustainable, and effective personal hygiene products. With rising global concerns about antibiotic resistance and the adverse effects of synthetic chemicals in cosmetics, herbal-based alternatives are gaining momentum. In the future, further research could focus on: Standardization of extract concentration for consistent antimicrobial efficacy, incorporation with other synergistic herbal agents to broaden the spectrum of activity, scaling up production through eco-friendly and cost-effective methods, clinical testing and dermatological safety trials for commercial viability, formulation of different product types (liquid soap, handwash, face wash) using Piper betle extract.

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