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FORMULATION AND EVALUATION OF ANTIBACTERIAL PAPER SOAP CONTAINING AVERRHOA CARAMBOLA

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ABSTRACT

Maintaining good hand hygiene is crucial to stopping the spread of dangerous diseases, particularly in healthcare and food service settings, and can significantly reduce the transmission of germs and viruses. In school, hand-washing programs have been shown to reduce gastrointestinal and respiratory illness by 42% traditional soaps, while important for hygiene, can cause skin issues, especially when used with hard water. Paper soap offers a practical, portable, hygienic and eco-friendly alternative, being lightweight, mess-free and biodegradable. The study focused on the development of herbal paper soap using *Averrhoa Carambola (Star Fruit*), a tropical with antibacterial properties due to compounds like flavonoids, phenolics and saponins. The research aimed to evaluate the potential of star fruit-based paper soap as an effective antimicrobial solution for hand hygiene. The formulation was evaluated for physical characteristics, Ph, antimicrobial activity, IR-Spectroscopy. This study emphasis the potential of herbal paper soap made from *Averrhoa Carambola* leaf extract as a safe, effective and antimicrobial hygiene product.

KEYWORDS: *Averrhoa Carambola*, Antibacterial activity, Paper soap, Coconut oil, Glycerin, Sodium hydroxide, orange oil.

I. INTRODUCTION

Maintaining cleanliness is essential since the number of illnesses induced on by microorganism is rising. Soap, which is made by combining natural oils or fats with a strong alkali like sodium hydroxide, is used for washing and cleaning. Apart from its cleaning

properties, some soaps are designed to soften the skin or lighten its tone. The type of oil used in soap production depends on the soap's intended function. Hands, being the most exposed part of the body, often carry bacteria from various surfaces. Practicing good hygiene is the simplest, most effective, and cost-efficient method to prevent infections and the spread of antimicrobial resistance.

Different formulations are available for cleansing and antibacterial purpose for hands such as

- Liquid hand wash
- Liquid hand soap
- Hand wash powder
- Solid soaps
- Paper soaps.

Paper Soap

The skin serves as the body's primary defence against harmful pathogens but is frequently exposed to environmental damage. Soap, made from potassium or sodium salts of fatty acids, helps to cleanse the skin and protect it from harmful microorganisms. Soap is basic because it contains both a weak acid (like carboxylic acid) and a strong base (like NaOH).

In addition to its basic ingredients, soaps may contain additives like salt, soda ash, citric acid, perfumes, and other chemicals that enhance qualities like colour, texture, and overall effectiveness. These additives help improve soap performance compared to using only phosphates.

Soaps, especially calcium and lithium soaps, play a vital role in lubricating greases, which are emulsions made by mixing them with mineral oils. Other types of metallic soaps, like aluminium and sodium soaps, are also widely used in various industries.[1]

Benefits of Paper Soap

- **Convenience:** Paper soap is compact and portable, fitting easily into pockets, purses, or backpacks, making it perfect for handwashing while on the go.
- **Single-use Convenience:** Each sheet provides a single serving of soap, eliminating the need to worry about dispensing the right amount.

- **Hygiene:** It reduces the risk of cross-contamination, as there's no need to share a soap bar.
- **Eco-friendly:** Many paper soaps are biodegradable and made from sustainable materials, helping to reduce plastic waste.

Advantages of Herbal Paper Soap

- **Antibacterial:** Herbal paper soaps often contain natural ingredients that help fight bacteria, offering antibacterial benefits.
- **Mild and Gentle:** These soaps are gentle on the skin, making them ideal for those with sensitive skin.
- **Natural Ingredients:** Natural ingredient composed of botanical extract, essential oils, and plant-based oils. herbal soaps provide a natural and holistic approach to skincare.
- **Moisturizing:** Ingredients such as cocoa butter, olive oil, coconut oil and shea butter hydrate and moisturize the skin.
- **Soothing and Calming:** Many herbal soaps feature herbs and extracts known for their effects on the skin that are relaxing and comforting.
- **Cleansing and Detoxifying:** The soaps effectively cleanse the skin by removing impurities and excess oils.[2]

Averrhoa carambola, also known as star fruit, has been used in traditional medicine for treating conditions like skin problems, diarrhea, and fever. It is commonly found in countries such as India, China, and Brazil, and is also used in Ayurveda. This study explores the plant's botanical, phytochemical, and pharmacological properties, along with its potential drug interactions, contraindications, and toxicity. The findings emphasize the importance of additional research to validate its therapeutic benefits and discover the active compounds and their mechanisms of action.[3]

Averrhoa carambola is a tropical Asian fruit tree and furthermore, it is widely grown in China, Malaysia, Singapore, Taiwan, Hawaii, Florida, Brazil and Guyana. It is grown in areas of Brazil that are between the Equator and the tropic of Capricorn. Carambola output must be increased through proper fertilization, particularly in tropical soils with low levels of natural fertility.[4]

Averrhoa carambola (Oxalidaceae) called as Star fruit due to its distinctive shape is a small. slow-growing evergreen plants that typically reaches 5-7 meters in height, though it can grow

up to 10 meters. Its trunk can reach a diameter of 15 cm, with light brown, smooth or slightly cracked bark. The tree has alternate, spirally arranged, pinnate leaves that are 15-25 cm long, with 7-9 leaflets. In traditional medicine both the leaves and fruit of A. carambola are used to treat conditions like imbalanced kapha and pitta, skin diseases, itching, vomiting, diarrhea, worm infestations, general weakness, and intermittent fever. The leaves are known for their anti-itch, fever-reducing, and anti-parasitic properties, and are also used to treat scabies, certain types of poisoning, and intestinal worms.[5]

Fig No. 1: *Averrhoa Carambola L*. Leaf.

Carambola Classification

- Kingdom: Tracheobionta
- Super Division: Spermatophyta
- Division: Magnoliophyta
- Class: Magnoliopsida
- Subclass: Rosidae
- Order: Geraniales
- Family: Oalidaceae
- Genus: Averrhoa Adans
- Species: Averrhoa carambola L.

Human skin serves as a protective layer against harmful pathogens but can be damaged by daily exposure to environmental factors. Soap is commonly used to clean the skin and protect

it from these pathogens. It is made by combining fatty acids with either sodium or potassium hydroxide through a process called saponification. During this process, oils or fats react with the alkali to create soap. The two components of soap molecules are hydrophilic (attracts the water) and hydrophobic (repels the water). This allows soap to break down oils and grease effectively. Some soaps also include additives like colour, fragrance, and sodium bicarbonate to improve their properties. The saponification process produces both soap and glycerine, which can stay in the soap or be extracted for other purposes.[6]

The demand for soap has risen sharply during the COVID-19 pandemic, as washing hands with soap is more effective at eliminating germs and viruses than using hand sanitizers. However, frequent use of soap can dry out and roughen the skin by removing its natural oils. To counteract this, natural ingredients like vitamin E, probiotics, and lauric acid can be added to soap to enhance skin health. Virgin Coconut Oil (VCO) is one such ingredient known for its ability to protect and nourish the skin.[7]

Plant metabolites are influenced by environmental conditions and the plant's elemental composition, and there is increasing interest in their classification, particularly concerning their interactions with ecosystems, chemical groups, and responses to stress. These metabolites serve as indicators of how plants adapt to their environments.

Plant metabolites, often called phytochemicals, are not yet precisely categorized due to their varied forms and compositions. Traditionally, they are split into two main groups depending on their role in plant metabolism.

1. **Primary Metabolites**
2. **Secondary Metabolites**

Primary Metabolites

Primary metabolites are essential for plant functions like photosynthesis, growth, and development. These include amino acids, nucleic acids, and sugars, which are crucial for cell metabolism and reproduction. While the molecular regulation of primary metabolites, which helps in protective biosynthesis, is not fully understood, research suggests that compounds like malate may play a role in plant protection, especially under stress. Variations in malate levels and the presence of malate-transforming enzymes indicate its importance in these processes.

1. Secondary Metabolites

A. Flavonoids

Flavonoids are a large and extensively researched group of plant compounds. These complex molecules consist of two benzene rings connected by a propane unit and are derived from flavones. Flavonoids are water-soluble substances found in plants.

B. Tannin

Tannins are naturally occurring water-soluble compounds with many phenolic groups that can bind to water-soluble proteins. They majority of them identified in plants with vascular structure, especially in woody tissues, leaves, flowers, and seeds. Plants that contain high levels of tannins are often bitter, which helps discourage animals from consuming them.

C. Alkaloids

Alkaloids are naturally occurring substances found in plants, animals, fungi, and bacteria. These compounds contain heterocyclic structures and are named after their alkaline, nitrogen-based composition. First discovered in the 19th century, alkaloids typically have a bitter taste and are commonly found in plants, often referred to as vegetable alkalis.

D. Saponins

Saponins are secondary metabolites that are commonly found in plants. They can form foam when mixed with water, which is why they are named "saponins," similar to soap. These compounds include glycosylated steroids, triterpenoids, and steroid alkaloids, and are characterized by their high molecular weight. Saponins have a bitter taste and a sticky texture, and they can be toxic to fish and other aquatic creatures. In the past, plants containing saponins were used as natural soaps.

E. Phenols

Phenolic chemicals are a well-recognized group of secondary metabolites renowned for their wide range of pharmacological actions. These substances, which fall into three primary groups that are flavonoids, phenolic acid and polyphenols are frequently found in plants. The inclusion of hydroxyl groups (-OH) in their structure makes phenol one of the most basic natural chemicals.

F. Terpenoids

Terpenoids are secondary metabolites that come from the 5-carbon isoprene unit and have diverse cyclic structures. They vary in terms of functional groups and carbon skeletons. Terpenoids are found in all living organisms and represent the largest category of natural compounds. Many terpenoids are of significant economic value because they are used as additives and fragrances in food and cosmetics.[8]

II. MATERIAL AND METHODS

Collection and Identification of plant material

Fresh plant material of *Averrhoa carambola* leaf was collected from Radhanagari. Plants were identified by a Botanical Department of New Collage Kolhapur.

Drying and Extraction

After being thoroughly cleaned with tapping water, the plant materials were allowed to dry in the shade. *Averrhoa carambola* leaf was dried and then powdered into a fine powder.

For extraction, the cold maceration technique was employed. Ethanol was used to soak powdered plant material, which was then stored at room temperature. Three days later, the extracts was vacuum-filtered via Whatman filter paper No.1. The residue was once more immersed in ethanol for three days before being filtered. The filtered was mixed, and a water bath set at 50^0C was used to evaporate the ethanol. Until they could be examined further, the dried extracts were kept at room temperature.[9]

PHYTOCHEMICAL SCREENING OF EXTRACT OF *AVERRHOA CARAMBOLA* LEAVES

Sample solution

5ml of distilled water was used to dissolve 100mg (0.1gm) of the extract, which was then filtered. In the tests that follow, the filtrate was utilized as a sample solution.

Test for alkaloids

1. Mayers test

Using a dropper, 2 drops of Mayer's reagent were added to the sample solution after dissolving 2ml of the sample extract in distilled water.

2. Wagner test

A clean test tube was filled with 2 ml of test solution. Two drops of Wagner's reagent were added to the sample using a dropper.

3. Dragendorff test

A clean test tube was filled with 2 ml of the test solution. Two drops of Dragendorff reagent were added to the sample using a dropper.

4. Hagers test

A clean test tube was filled with 2 ml of the test solution. Two drops of Hager's reagent were added to the sample using a dropper.[10]

A. Test for saponins

1. Ferric chloride test

Filled a test tube with two millilitres of the oil in sample. To the sample, added 2 mL of a 10% NaOH solution. To hydrolyse the ester into its carboxylate and alcohol components, heat the mixture in a water bath for five to ten minutes. Let the mixture cool. Take a portion of the cooled solution. Filled the test tube with a few drops of ferric chloride ($FeCl_3$) solution. Observed the colour change.[11]

2. Foam test

Filled a clean test tube with roughly 2 ml of the test sample. Vigorously shaked the test tube for 30 seconds. Allowed the solution to stand for five minutes. Saponins were present was indicated by the formation of a stable foam that was 1cm tall and lasted for more than ten minutes.[12]

B. Tannin test

Lead Acetate Test

The presence of phenolic compound and tannins was shown by the production of a white precipitate when 2 ml of extract was mixed with 0.5 ml of 1% lead acetate.[13]

C. Amino acid test

Ninhydrin Test

Two millilitres of the sample solution (protein extract) were taken. Two drops of Ninhydrin reagent were added to the test tube. The test tube was heated for approximately five minutes. A deep blue colour indicated the presence of α-amino acids.[14]

D. Test for phenol

Crude extract was combined with two millilitres of a 2% $FeCl_3$ solution. Phenol were indicated the green colour.[12]

E. Test for flavonoid

The crude extract was combined with two millilitres of a 2% NaOH solution. When a few drops of diluted acid were added, the bright yellow colour that had formed—indicating the presence of flavonoids.[16]

❖ PROCEDURE

Formulation Table

Table No. 1: formulation table.

Sr. No.	Ingredient	Quantity
1.	Potassium Hydroxide	5gm
2.	Citric Acid	0.6ml
3.	Dist. Water	100ml
4.	Peppermint Oil	1ml
5.	Glycerine	10ml
6.	Castor Oil	3.3ml
7.	Coconut Oil	16.6ml
8.	Sodium Chloride	5.33gm
9.	Sodium Hydroxide	2:1
10.	Extract	q. s.
11.	Butter Paper	Required Amount

Method of Preparation of Soap Solution

Heat glycerine at 60^0C

↓

Take coconut oil in required quantity, heat and melt it, then add castor oil by continuous stirring

↓

Prepare KOH solution

↓

Pour KOH into glycerine, heat and maintain temperature at 60- 70^{o}C (avoid the overflow of mixture)

↓

The mixture heated for 2-3 hours

Add NaCl into the blend

After being taken out of the water bath, the mixture was cooled for ten to fifteen minutes in ice bath

Method of preparation of Paper Soap[5]

Make a soap solution with different concentrations, such as 5, 10, 15 and 20% w/v

Preventing the formation of foam

The soap that produced the most foam was chosen

Select the paper

Their weight increase and absorption capability were assessed

The best paper was the one with the highest absorption capacity

Extract was incorporated in the prepared formulation

Weigh exactly, combine with 15% soap powder, and add water gradually while moving constantly to create a homogenous soap - extract solution

The paper soap strips were prepared by dipping technique using air dried overnight at 37 $\pm 2^0$C

20 strips were taken

ASSESSMENT OF HERBAL PAPER SOAP

A. Assessment of herbs used organoleptic evaluation

a) Colour –Soft and Medium green in colour

b) Odour – No distinct odour

c) Appearance – good

B. Assessment of liquid soap organoleptic evaluation

a) Colour – Creamy white in colour

b) Odour - Fragrant

c) Clarity – The liquid soap was kept below the black background for the test

C. Assessment of paper soap organoleptic evaluation

a) Size – 4.5/6.5 cm

b) Shape - Rectangular

c) Odour – Pleasant and Fragrant

D. Physical assessment of herbal paper soap

The following characteristics of the herbal paper soap were assessed during formulation

a) PH: Prior to and the development of paper soap, the pH was measured. Initially, the liquid soap was made, and litmus paper was used to measure the pH. The blue litmus paper remained same colour while the paper become view. After the manufacturing of the paper soap, they used a piece of paper soap mixed with water, and allowed to fully dissolve before the pH was measured using a pH meter.[9]

b) Foam Retention: After adding the soap strips to a measuring cylinder filled with water, A hand was placed over the cylinder, and it was shaken ten times. At one-minute intervals, the foam's volume was measured.[19]

c) Antimicrobial Activity of *Averrhoa Carambola* Leaf: *Averrhoa carambola* plant extract was used in number of studies on the antibacterial activity of paper soap. The antibacterial study was done into the Staphylococcus Aureus. Antimicrobial activity of topical agent was determined by agar well diffusion assay, in this various concentration i.e. 25 µg, 50 µg,75 µg and 100 µg of topical agent directly taken from final formulation aseptically by sterile spatula and loaded in wells which are prepared by using sterile cork-borer on Luria Agar plates having diameter 0.7cm. During a 24-hours incubation period at 27^0c, the plants were examined for a zone of inhibition surrounding the well.

d) Foam height: 20ml of water were used to spread the sample. After adding water, it was placed in the measuring cylinder and shaked for a minute. The foam height was then measured and recorded as F1, and after ten to fifteen minutes, it was measured and recorded as F2.

Foam height = F1 – F2.[20]

e) Total moisture content: The weight of the soap's water content and the difference after the paper soap was completely dried at 100-115^{0}c or with a dryer were used to determine the moisture content.

The following formula were used to determine the moisture content:

%Moisture content = Initial weight – Final weight / Final weight*100.[20]

f) Stability test: The formulation undergoes eight days of short-term stability testing. The sample was kept at two different temperatures 37^{0}C at ambient temperature and 2^{0}C to 800^{0}C in refrigerator. The sample was frequently taken out and examined for drug content, clarity, pH and appearance.

g) Primary skin irritation test: At least three individuals were chosen for this, and soap strips were produced, applied and the degree of irritation was measured.[20]

h) Foam stability test: The uniformity of the amount of foam of paper soap creates was known as foam stability. Compared to regular soap, the resulting foam of paper soap was smoother. When combined with water, the foaming agent that contains the surface-active agent will create stable foam. In reality, glycerine has observable impact on foam stability and lacks ant surface active agents.[20]

III. RESULT AND DISCUSSION

1. Authentification

From Radhanagari, fresh *Averrhoa carambola* leaf was collected. The Botanical Department of New Collage Kolhapur recognized the plant.

2. Collection and Identification of Plant Material

From Radhanagari, fresh *Averrhoa carambola* leaf was collected. The Botanical Department of New Collage Kolhapur recognized the plant.

3. Drying and Extraction

After being carefully cleaned with tapping water, the plant material was dried and then powdered into a fine powder.

4. Phytochemical Screening of Extracts of *Averrhoa Carambola* Leaf Extract

Phytochemical Analysis

Table No. 1: Phytochemical Analysis.

Sr. No.	Test	Observation	Result
1.	Saponins (Foam Test)	Produce foam about 1cm	+
2.	Tannin (Lead acetate test)	Formation of white precipitate	+
3.	Amino Acid (Ninhydrin test)	Yellow colour	+
4.	Flavonoids	Initially yellow, it becomes colourless when a few drops of dilute acid were added	+
5.	Phenol	Green or black colouration	+

Test for Alkaloids

Table No. 2: Test for Alkaloids.

Sr. No.	Test	Observation	Result
1.	Dragendorff Test	Orange or red ppt formed	+
2.	Mayers Test	Yellowish or white precipitate formed	+
3.	Hagers Test	Yellow precipitate formed	+
4.	Wagner Test	Reddish or red-brown precipitate formed	+

[**Note:** Positive (+), Negative (-)]

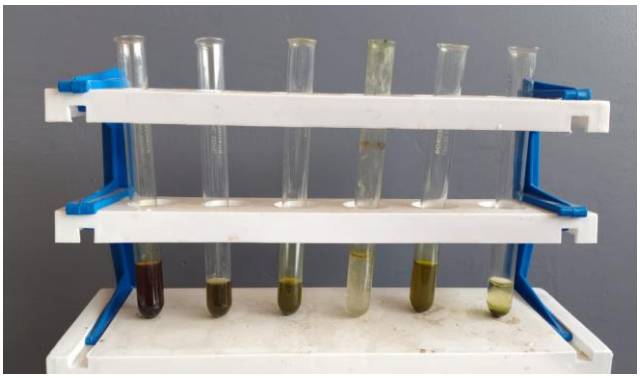

Fig. 1: Phytochemical Analysis.

5. Infrared Spectroscopy

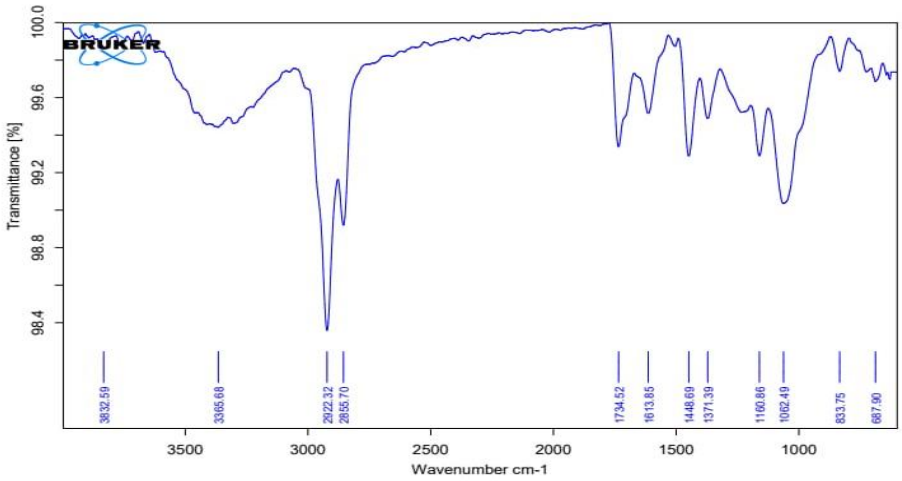

Fig. 2: FTIR of *Averrhoa Carambola.*

It looks like you've uploaded an FTIR (Fourier Transform Infrared) spectrum of *Averrhoa carambola* leaf extract in solid form, measured using a Bruker instrument. Wavenumber (cm^{-1}) is represented by the x-axis, and transmittance (%) is represented by the y-axis.

Key features of the spectrum

- Powerful absorption peaks around 2922.32 cm^{-1} and 2851.70 cm^{-1}, which typically correspond to C-H stretching vibrations (likely from alkanes).
- Broad absorption in the 3600-3200 cm^{-1} range, suggesting the presence of O-H stretching, indicating alcohols or phenols.
- Peaks in the 1700-1500 cm^{-1} region, likely corresponding to C=O (carbonyl) and C=C stretching, suggesting the presence of carboxyl or aromatic compounds.
- Absorptions in the fingerprint region (below 1500 cm^{-1}), which provide characteristic molecular information.

Formulation

Paper soap strips were prepared using the Dipping technique. Various papers were dipped sequentially into a soap solution and then air-dried overnight at room temperature.

Fig. 3: Formulation of liquid soap.

Fig. 4: Paper Soap.

❖ **An Assessment of Herbal Paper Soap**

A. Assessment of Herbs in Organoleptic Analysis

Table No. 3: Evaluation of Herbs.

Sr. No.	Test	Observation
1.	Colour	Green in colour
2.	Odour	No distinct odour
3.	Appearance	Good

B. Assessment of Herbal Soap in Organoleptic Analysis

Table No. 4: Assessment of Herbal Paper Soap.

Sr. No.	Test	Observation
1.	Colour	Creamy white in colour
2.	Odour	Aromatic
3.	Clarity	The liquid soap was left on the black background while the test was conducted

C. Assessment of Herbal Paper Soap Organoleptic Analysis

Table No. 5: Assessment of Herbal Paper Soap.

Sr. No.	Test	Observation
1.	Size	4.5/6.5 cm
2.	Shape	Rectangular
3.	Odour	Flavouring

D. Physical Assessment of Herbal Paper Soap

❖ **pH determination**

Using a pH paper, the pH was determined and found to be basic in nature, that is **5.07**.

Fig. 5: pH Meter.

❖ **Foam Retention**

Foam height measure in minute. The stability of the foam was measured and determined to be 2 cm after the foam was kept for 10 seconds as part of the foam retention test.

Fig. 6: Foam Retention.

❖ **Antimicrobial activity**

Table No. 6: Zone of inhibition of formulation.

Test pathogen	Zone of inhibition in millimeter for sample			
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
S aureus (Carambola)	13	19	18	15

- The zone of inhibition increases from 13 mm at 25 µg/ml to a maximum of 19 mm at 50 µg/ml, suggesting that this concentration is most effective.
- Beyond 50 µg/ml, the zone of inhibition decreases (18 mm at 75 µg/ml and 15 mm at 100 µg/ml), indicating possible saturation or inhibitory effects at higher concentrations.
- This trend implies that the antimicrobial activity of the *Averrhoa carambola* formulation peaks at a moderate concentration and may reduce at higher doses.

❖ **Foam Height**

- Calculating the foam height = F1 – F2

F1 = 8cm

F2 = 5cm

Therefore, the height of the foam was, 3cm.

❖ **Total Moisture Content**

The moisture content was determine using the following formula,

% Moisture Content = Initial weight – Final weight / Initial weight*100

- Initial weight = 0.412gm
- Final weight = 0.400gm

Therefore, % Moisture Content = 2.91%

❖ **Stability**

No changes were observed at room temperature and 35°C. Colour and odour remained unchanged under all conditions.

❖ **Skin Irritation**

Irritation was not found after applied paper soap on hand.

IV. CONCLUSION

This study successfully developed and evaluated herbal paper soap incorporating *Averrhoa carambola* leaf extract. Using phytochemical analysis, the existence of beneficial compounds

such as flavonoids, saponins, tannins, alkaloids, and phenols, which may contribute to the soap's beneficial properties. The formulated paper soap exhibited a pleasant aroma, good foam retention (3 cm foam height), and a pH of 5.07, making it suitable for skin application. Stability testing showed no significant changes in colour or odour at varying temperatures, and skin irritation tests confirmed its safety.

Overall, the results indicate that *Averrhoa carambola* leaf extract is a promising natural additive for eco-friendly personal hygiene products. Future research can explore enhanced formulations and broader antimicrobial testing to further establish its potential in skincare applications.

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Thank You.

Yours Sincerely,

Rohan Bhandigare, Sanika Patil, Nikita Arade, Supriya Bendugade, Shubham Dhavan.

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