

ANTI-BACTERIAL HERBAL SOAP FORMULATION AND PREPARATION

Submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in **BIOTECHNOLOGY**

by

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INSTITUTE OF SCIENCE AND TECHNOLOGY
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Accredited with Grade "A" by NAAC

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MAY-2022

DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of C.K Dhana sri (39230012), K.Divya (39230014) and E. Samyuktha (39230060) who carried out the project entitled **“ANTI-BACTERIAL HERBAL SOAP FORMULATION AND PREPARATION”** under our supervision from 9th April 2022 to 23rd April 2022.

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Submitted for Viva voce Examination held on 29-04-2022

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DECLARATION

I **DHANA SRI C.K (REG NO:39230012)**, hereby declare that the Project Report entitled **“ANTI-BACTERIAL HERBAL SOAP FORMULATION AND PREPARTATION”** done by me under the guidance of **Dr. JAYASHREE** (Internal) and **Dr. PUROSHOTHAMAN** (External) at **BIODEAVOUR RESEARCH LAB, PORUR** is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in **BIOTECHNOLOGY**

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SIGNATURE OF THE CANDIDATE

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**ANTI-MICROBIAL HERBAL SOAP FORMULATION AND
PREPARATION USING *Cassia auriculata*
and
Nelumbo nucifera Flowers CRUDE EXTRACTS**

ABSTRACT

Cosmetic industry demands the use of plant-origin ingredients to use in bar soaps. Saponification is the chemical reaction in which triglycerides are mixed with strong base to form fatty acid metals salts during soap preparation. In this study, *Cassia auriculata*, and *Nelumbo nucifera* flower extracts were used to prepare herbal soaps. The herbal extracts were tested for the microbial growth inhibition of *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* by anti-microbial testing. The herbal extracts formulated and prepared herbal soaps were calculated for hardness, cleansing, conditioning, bubbly, creamy, and INS (Iodine and Saponification Value). The *Cassia auriculata* ethanolic crude extract, Anti-Microbial Activity revealed shown potent inhibitory activity with 80 µl in *Escherichia coli* (8 mm), *Staphylococcus aureus* (17 mm) and *Candida albicans* (17 mm).

The *Nelumbo nucifera* ethanolic crude extract, Anti-Microbial Activity revealed shown potent inhibitory activity with 80 µl in *Escherichia coli* (7 mm), *Staphylococcus aureus* (16 mm) and *Candida albicans* (17 mm). The TFM results revealed that the hand-made herbal soap has 76.7 % and proved to be grade 1 soap. The pH of handmade soap has a pH range of 9. Synthetic flower essence was added to soaps to enhance the quality and sensory Pleasants. Results from this study would have superior skin protection against the tested bacteria.

Keywords: Saponification, *Cassia auriculata*, *Nelumbo nucifera*, herbal soap, anti- microbial activity

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CHAPTER - 1

INTRODUCTION

Herbal soap preparation is a medicine or drug it contains Anti-bacterial and Anti-fungal agents which is mainly prepared using of parts of plants such as flowers, stem, root etc., to treatment for an injury or skin diseases. Mostly skin infections are caused by fungi's, *Staphylococcus aureus*, *Escherichia coli*. Crude Extracts from the plants of *Cassia auriculata* and *Nelumbo nucifera* shows the good effectiveness towards these microbes. Hence does not cause any skin problems.

Results from this study would have superior skin protection against the tested bacteria.

CHAPTER -2

MATERIALS USED AND METHODOLOGY

MATERIALS USED:

- Ethanol
- DMSO
- Antibiotics
- Sodium lactate
- Vegetable Oils
- Sodium Hydroxide
- Glass wares
- Soap silicone Mould

SAMPLE PREPARATION

Cassia auriculata and *Nelumbo nucifera* flowers were collected and shade air-dried for 7 days. After that, the dried flowers were powdered using mixer. The grounded powders were subjected to Phyto-compounds extraction by followed maceration method. 20 grams of flowers powder was added to 200 ml of ethanol. The extraction was carried out for 2 days by keeping it in room temperature with intermittent mixing. On 3rd day, the extracts were filtered using Whatman filter paper No.1 to obtain filtrate of crude extracts. The collected filtrate was concentrated by evaporating the ethanol under room temperature for 2 hours. The recovered crude extracts were stored in sample vial at -20°C until further use.

ANTI-MICROBIAL ACTIVITY

PREPARATION OF INNOCULUM:

The antimicrobial properties of flowers crude extract were tested against Gram-positive bacteria *Staphylococcus aureus* (MTCC No 96), Gram-negative bacteria *Escherichia coli* (MTCC NO 1687) and as well as one pathogenic fungus *Candida albicans* (MTCC 227). All the cultures were purchased from Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Sector 39-A, Chandigarh - 160036, INDIA. The Gram-positive and Gram-negative bacteria were cultured in Mueller Hinton broth (MHB) at 37°C. The fungal inoculum was prepared from the 48 h culture of fungal isolates in Potato dextrose broth (PDB) (Nisha et al., 2010).

ANTIMICROBIAL SCREENING

Agar well diffusion method was followed to study the anti-microbial activity of ethanolic crude extract. 100 µl of freshly prepared bacterial or fungi culture was pipetted in the center of solidified Muller Hinton medium for bacteria and PDA for fungi medium in the petri dish. Using sterile 1 ml pipette tips, five wells (6 mm in diameter) were made into agar plates containing microbial inoculums. Then, 40 µl, 60µl, and 80 µl of each extract (100 mg/ml concentration) was added to respective wells. The plates were incubated at 37°C for 24 hours. Antimicrobial activity was recorded by measuring the zone of inhibition appeared around each well. DMSO at a concentration of 100 % was added as a negative control and antibiotics was added as a positive control (Nisha et al., 2010).

Table 1.1: Herbal Soap Formulation

S.No.	Materials	Quantity in gram
1.	Sodium Hydroxide (43 %)	72
2.	Water	95
3.	Coconut Oil	150
4.	Sesame Oil	100
5.	Palm olein Oil	250
6.	Fragrance	7 ml

TOTAL FATTY MATTER (TFM)

TFM is a measure of identifying the amount of fatty matter present in soap. Bureau of Indian Standards (BIS) has categorized bath or toilet soaps as 'normal', 'baby, transparent, and antibacterial soaps. The last three are called specialty soaps targeted to specific users. A toilet or bath soap is a cosmetic by law and it must fulfill the requirements of the relevant Indian standard. 5 gram of soap sample was grated and transferred to the beaker. To that beaker, 100 ml of distilled water and 30 ml of 1:1 Sulphuric acid was added. Then all the contents were digested for 30 minutes using water bath with temperature of 80 °C until formation of clear soap solution. After that, the digested soap solution was transferred to separating funnel. To that, 5 ml of acetone and 4 ml of ethanol was added. The separating flask was shaken manually for 2 minutes. 10 ml of diethyl ether was added to the separating funnel for separation of phases. The extract was collected using Whatman No.1 filter paper. The collected extract was boiled for 30 minutes in water bath with temperature of 80 °C. After 30 minutes, the oil which used to make herbal soap starts to separate [Tarun, J et al 2014]

After oil separation, the flask was transferred to hot air oven to find out the total fatty matter of herbal soap by using the formula:

$$\text{Total Fatty Matter} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Sample weight}} \times 100$$

FOAM FORMING CAPACITY

100 ml of water is taken in 250 ml capacity conical flask and then add 5 gram of soap sample was added. The contents were boiled to dissolve and get a clear soap solution. The conical flask was Shaked vigorously by covering the mouth of the flask by hand. After shaking for 3 minutes, foam will be formed in the conical flask. The time taken for the disappearance of the formed foam was noted down with the help of stop watch. The height of the foam was measured using ruler.

CHAPTER- 3

RESULTS AND DISCUSSION



Fig 3.1: *Nelumbo nucifera*



Fig 3.2: *Cassia auriculata*



Fig 3.3: *Nelumbo nucifera* powder

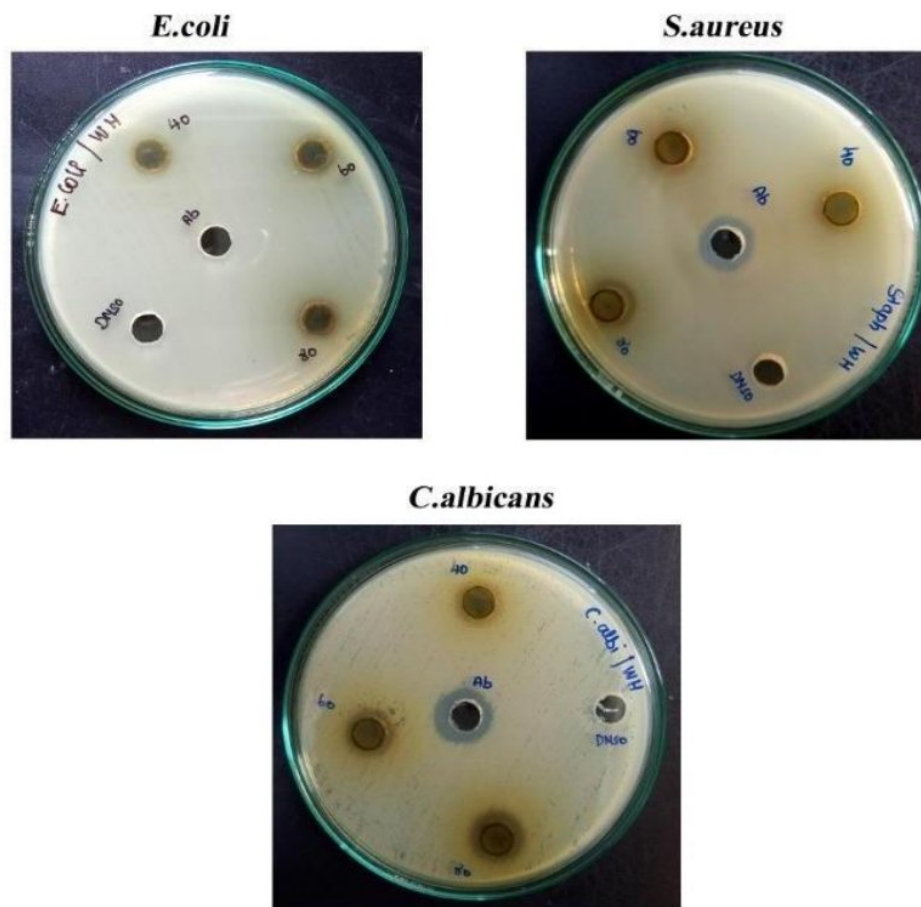


Fig 3.4: *Cassia auriculata* powder



Fig 3.5: *Nelumbo nucifera* and *Cassia auriculata* Ethanolic Crude Extract

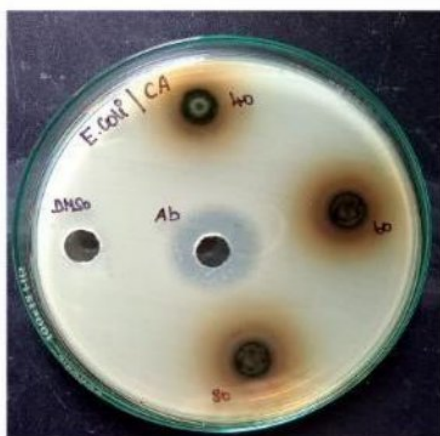
ANTI-MICROBIAL ACTIVITY



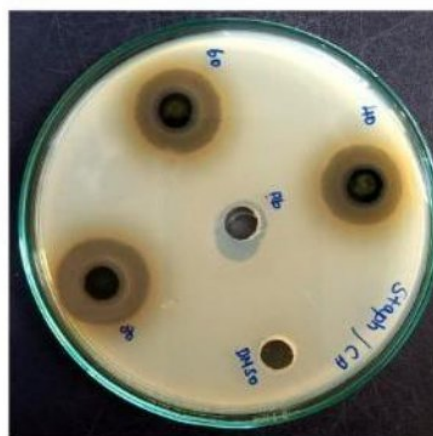
40-40µl- 60-60µl, 80-80µl, AB-10mcg of Ciprofloxacin, DMSO- 100µl

Fig 3.6: *Nelumbo nucifera* Anti-Microbial Activity

E.coli



S.aureus



C.albicans



40-40µl- 60-60µl, 80-80µl, AB-10mcg of Ciprofloxacin, DMSO- 100µl

Fig 3.8: *Cassia auriculata* Anti-Microbial activity

Table 3.1: Zone of Inhibition revealed by *Nelumbo nucifera* ethanolic crude extract

S.No.	Tested Bacteria	40 µl	60 µl	80 µl	DMSO (80 µl) (-Ve)	Chloramphenicol (80 µl) (+Ve)
1.	<i>E. coli</i>	5 mm	6.5 mm	7 mm	No Inhibition	14 mm
2.	<i>S. aureus</i>	13 mm	15 mm	16 mm	No Inhibition	15 mm
3.	<i>C. albicans</i>	12.5 mm	14 mm	17 mm	No Inhibition	15 mm

Table 3.2: Zone of Inhibition revealed by *Cassia auriculata* ethanolic crude extract

S.No.	Tested Bacteria	40 µl	60 µl	80 µl	DMSO (80 µl) (-Ve)	Chloramphenicol (80 µl)
1.	<i>E. coli</i>	6 mm	7.5 mm	8 mm	No Inhibition	15 mm
2.	<i>T. aureus</i>	15 mm	16 mm	17 mm	No Inhibition	14 mm
3.	<i>D. albicans</i>	15 mm	16 mm	17 mm	No Inhibition	14 mm

TOTAL FATTY MATTER (TFM)

The main variable to be considered in cleanser quality of soap is its all out Total Fatty Matter (TFM). Higher the TFM value in the bath soap, better is its quality. According to BIS, Grade 1 soap ought to have 76% least TFM, while Grade 2 and Grade 3 should have 70% and 60 percent least TFM, separately. The TFM results revealed that the hand-made herbal soap have 76.7 % and proved to be grade 1 soap.

EVALUATION OF pH

Typical normal skin has capability of hydrogen (pH) scope of 5.4-5.9 and an bacteria normal flora. Utilization of soap with high pH causes an increase in skin pH, which thus causes an increase in dehydrative impact, irritation and change in bacterial normal flora. The pH of handmade soap has a pH range of 9. In a review done by Tyebkhan G, in which pH paper was utilized for estimating the pH of cleansers/cleaning agents, it was observed that the cleansers regularly utilized by the populace at large have a pH running somewhere in the range of 7 and 9 [Tyebkhan G. 2001].

SOAP PHYSIO-CHEMICAL PROPERTIES

Table 3.2 soap physio-chemical properties

S. No.	Soap Bar Quality	Reference Range	Formulated Soap Bar Range
1.	Hardness	29 - 54	45
2.	Cleansing	12 - 22	20
3.	Conditioning	44 - 69	51
4.	Bubbly	14 - 46	29
5.	Creamy	16 - 48	34

6.	Iodine	41 - 70	57
7.	INS	136 - 165	160

CHAPTER-4

SUMMARY AND CONCLUSION

Hence the prepared herbal soap using *Cassia auriculata* and *Nelumbo nucifera* would have superior skin protection against the tested Gram-positive bacteria *Staphylococcus aureus*, Gram-negative bacteria *E. coli* and pathogenic fungus *Candida albicans*.

The formulated Herbal soap also have the good foaming capacity, cleansing, pH and TFM value according to Bureau of Indian standard.

Therefore, both the flowers have lots of medicinal value, Anti-fungal and Anti-bacterial properties so it doesn't cause any type of skin problems. It maintains moisture and pH level in the skin.

CHAPTER-5

References:

1. Nisha, M. C., Subramanian, M. S., Prathyusha, P., and Santhanakrishnan, R. (2010). Comparative studies on antimicrobial activity of *Artemisia sieversiana* Ehrhart. Ex. Willd. and *Origanum vulgare* L. *Int. J. Pharmtech Res.* 2, 1124–1127.
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3. Tarun, J., Susan, J., Suria, J., Susan, V. J., & Criton, S. (2014). Evaluation of pH of Bathing Soaps and Shampoos for Skin and Hair Care. *Indian journal of dermatology*, 59(5), 442–444. <https://doi.org/10.4103/0019-5154.139861>

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OBJECTIVE:

To extract the crude extracts of *cassia auriculata* and *nelumbo nucifera*.

To perform anti-microbial testing against *E.coli*, *Staphylococcus aureus*, *Candida albicans*.

To formulate and prepare herbal soap.



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PROTOCOLS:

Step 01: Collection of plant samples (flowers),
i.e; *Cassia auriculata* and *Nelumbo nucifera*.



Cassia auriculata

nucifera



Nelumbo



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Step 02 : The collected flowers were subjected to shaded dry
for 7

days to remove moisture content.



Cassia auriculata

nucifera



Nelumbo



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Step 03 : The dried flowers were made in to fine powder using blender.



Cassia auriculata



Nelumbo nucifera



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Step 04 : The flower powder was subjected to ethanolic Crude extraction by following maceration method.

Ethanolic crude extraction followed by maceration method:

20g each flower powder was added to 200ml of ethanol.

The extraction was carried out for 2 days by keeping it in room temperature with intermittent mixing.



cifera



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Step 05 : On 3rd day, extraction were filtered using Whatmann filter

paper no:01 to obtain filtrate of crude extraction.



***Cassia auriculata
nucifera***



Nelumbo



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Step 06 : The collection filtrate was concentrated by evaporating the ethanol under room temperature for 2 hours.



Cassia auriculata



Nelumbo nucifera



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Step 07 : The recovered crude extracts was stored in sample vial at
- 20° C until further use.

The yield of *Cassia auriculata* is 1g.

The yield of *Nelumbo nucifera* is 100mg.



Cassia auriculata



Nelumbo nucifera



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Antimicrobial Activity:

The flower crude extracts were tested against gram positive bacteria *staphylococcus aureus*, Gram negative bacteria *E.coli* and as well as one pathogenic fungus *candida albicans*.

The Gram positive and Gram negative bacteria were cultured in muller Hinton broth (MHB) at 37° C.

The fungal inoculum was prepared from the 48th culture of fungal isolates in Potato dextrose broth (PDB).



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Antimicrobial Screening:

Agar well diffusion method was followed to study the anti-microbial activity of ethanolic crude extract.

100 μ l of freshly prepared bacterial culture or fungi culture was pipetted in the center of muller Histon medium for bacteria and PDA for fungus medium in the petri dish.

Using sterile 1ml pipette tips, five wells were made into agar plates containing inoculum.



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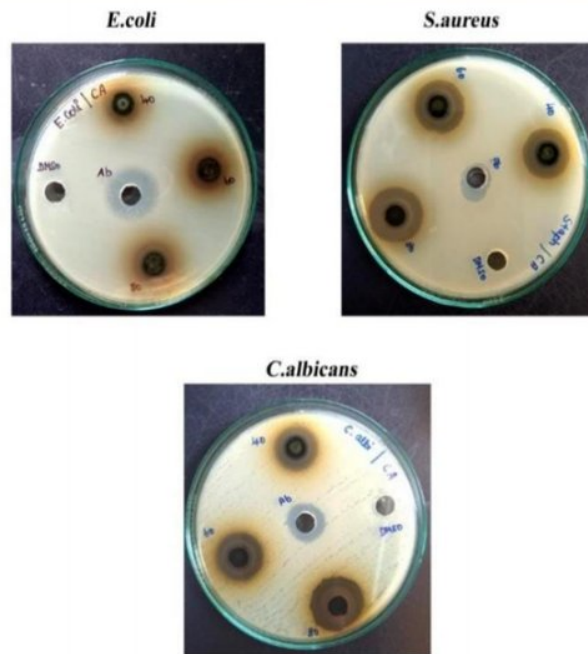
Then 40 μ l, 60 μ l, 80 μ l of each extract (10mg concentration) was added to respective wells.

The plates were incubated at 37°C for 24 hours. Antimicrobial activity was recorded by measuring the zone of inhibition appearing around each wells.

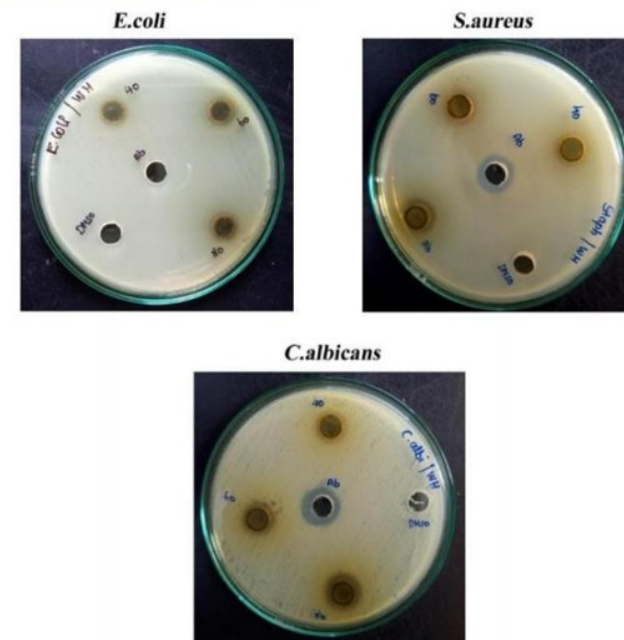
DMSO at a concentration of 100% was added as a negative control and antibiotics was added as a positive control.



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*Cassia auriculata
nucifera*



Nelumbo



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Herbal soap formulation and preparation :-

Materials required :

Sodium hydroxide - 72g

Water - 95g

Coconut - 150g

Sesame oil - 100g

Palm oil - 250g

Fragrance - 7ml

Sodium lactate - 10g

Yield extracts



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All the above given materials, should be weighted.

At first, mix sodium hydroxide and water to dissolve them completely.

Then add all the materials in a thick bottom vessel.

Using hand blender, mix all the content thoroughly.

Then pour the solution in to silicon mould. After drying for 24 hours.

Remove the prepared herbal soaps from mould.



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Total Fatty Matter (TFM) :

TFM is a measure of identifying the fatty matter present in soap.

It is measured by the formula:

$$\text{TFM} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Sample weight}} \times 100$$

Foam Forming Capacity :

5g of grated herbal soap was dissolved in 100ml of water.

The contents were boiled, shaken vigorously.

The disappearance of foam and height of the formed foam were measured.



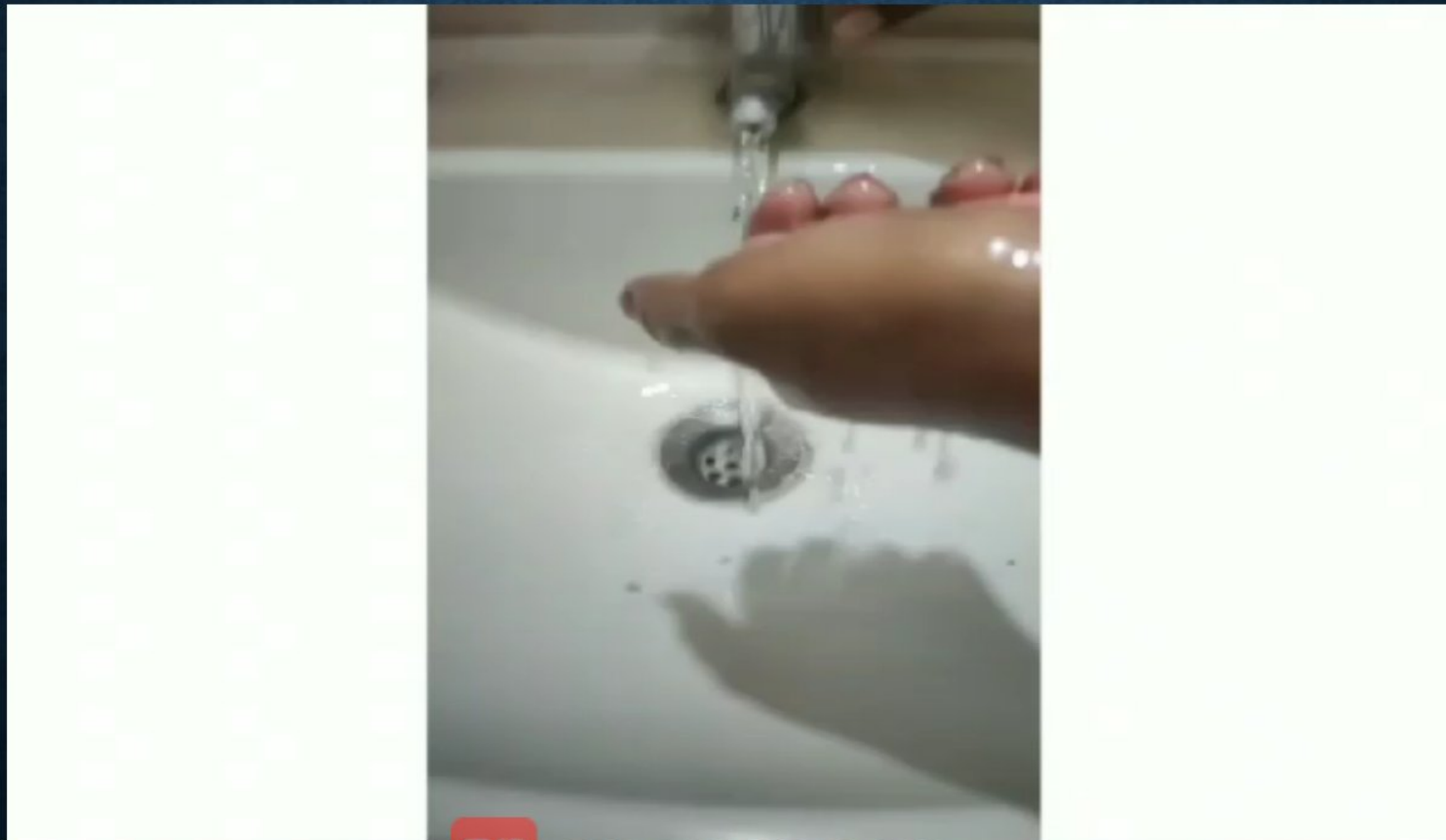
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SOAP SAMPLE:



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Patch test :



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OUTCOME:

We obtained the physio-chemical properties of prepared herbal soap.

Preformed anti-microbial testing against *E.coli*, *Staphylococcus aureus* and *Candida albicans*.

TFM of formulated soap is 76.7%

pH of herbal soap is 9.

Result from this study would have superior skin protection against the tested bacteria



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Thank you!



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