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Physicochemical Characteristics and Antimicrobial Efficacy of Soaps Prepared Using *Carica papaya* Extracts

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Abstract

Value-added products harnessing the phytotherapeutic potential of papaya are scarce. There is a paucity of data on the efficacy and safety of such products (if available) from papaya. Thus, the current study attempted to exemplify the utilization of papaya extracts in preparing a value-added product, namely, Toilet Soap. This study is the first of its kind in which the physicochemical characteristics and antimicrobial efficacy of the toilet soaps infused with different papaya extracts were assessed and compared against the market available soaps containing papaya fruit extract. The soap samples had pH values between 8.37 – 9.74 and their moisture content between 2.94 – 11.32 %. Their estimated lather volume ranged between 410 – 780 mL with good foaming power and foam stability. The laboratory-prepared soaps (Samples A to D) had matter insoluble slcohol and total fatty matter contents per Bureau of Indian Standards. They either did not contain or had an insignificant amount of free caustic alkali. The quantitative in vitro assessment findings showed antibacterial efficacy against *Streptococcus aureus* except for Sample E. The in vivo finger imprint test demonstrated the antibacterial efficacy of the soaps against *E. coli* and *S. aureus*.

Keywords: *Carica papaya*; Toilet soap; Physicochemical characteristics; Antimicrobial efficacy; Finger imprint test.

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1. Introduction

Carica papaya, one of the healthiest fruits, exhibits a tremendous therapeutic perspective. It is not just a flavourful treat for the taste buds; it is an Ethnomedicine. It is rich in vitamin C and A, which boost skin health and help treat skin problems. The leaves of papaya are known for their medicinal and healing properties. It is used as a soap substitute that is able to remove stains. Papaya seeds are used to extract oil, and their yield could be up to 34 % [1,2]. Papaya seed oil is rich in oleic acid and triacylglycerol; its nutritional and functional properties are highly similar to olive oil [3]. Thus, papaya seed oil can find

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application in the cosmetic, health, and pharmaceutical industries. Despite the tremendous benefits offered, value-added products that harness papaya's phytotherapeutic potential are scarce. There is a paucity of data on the efficacy and safety of such products (if available) from papaya. Thus, the current study was an effort to exemplify the utilization of papaya extracts in preparing a value-added product, namely, Toilet soap.

Soaps are sodium and potassium salts of fatty acids [4]. Based on the content of TFM (Total Fatty Matter) and insoluble matter in the soap, they are broadly classified as Toilet soaps and bathing bars. Fatty matter content of toilet soaps is 60-80 %, while that of bathing bars is 40-60 %. Toilet soaps represent the most important type of soap due to their turnover and distribution [4]. It is believed that toilet soap possesses multiple benefits and has a higher cleansing ability compared to entry-level bathing bars [5].

The quality of soap is determined by its physicochemical properties, which define soap's efficiency and cleansing properties. The physicochemical characteristic of soap depends on the strength and purity of alkali, the kind of oil used, and the completeness of saponification. Such physicochemical characteristics include moisture content, TFM, pH, free caustic alkalinity, and percentage chloride [6]. Good quality soap for cleansing purposes is the one that strikes a balance in all the mentioned physicochemical parameters [7]. For the current research, the aqueous extracts of the papaya fruit and leaf and the papaya seed oil were used separately during the preparation of the Toilet soaps by the hot process. This study is the first of its kind in which the physicochemical characteristics and antimicrobial efficacy of the Toilet soaps infused with different papaya extracts were assessed and compared against the market available soaps containing papaya fruit extract.

2. Experimental Section

2.1. *Chemicals*

All chemicals and reagents used were of Analytical Grade and obtained from Loba Chemie, Merck, and Sigma Chemical Company. They were used without further purification.

2.2. *Plant material and other ingredients*

Carica papaya, a fully ripened fruit with a firm texture and without bruises or damage, was purchased from the local market in Mumbai, India, on a need basis. The fruit was properly washed with distilled water. Then it was peeled to remove the skin, and its pulp was used. The leaves of papaya were collected from the local gardens in Mumbai. After washing the leaves with distilled water, they were deveined and then used. Both the fruit pulp and leaves were well macerated separately, and 5 g % aqueous extracts of each were prepared. The papaya seed oil was purchased from a local Pharmacy. It was used without any further processing.

Soap needs to have a balance of hard and soft oils subjected to the saponification process in the presence of an alkali. Thus, the value-added product developed using

different papaya extracts also consisted of olive oil (soft oil), coconut oil (hard oil), alkali (lye, also known as sodium hydroxide), water, and sugar (additive). The hot process of soap making (Fig. 1) was followed with slight modifications [8], and the soaps were cured at room temperature for a week.

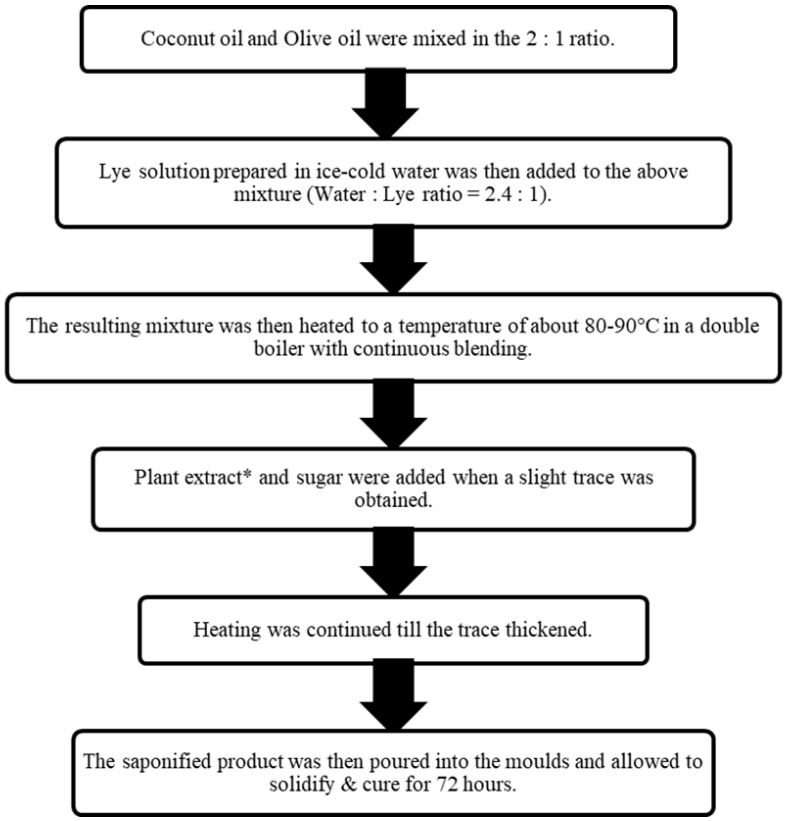

Fig. 1. Hot Process of making soap.

(* Sample A – 10 mL of 5 g % papaya fruit extract**;** Sample B - 10 mL of 5 gm% papaya leaf extract; Sample C – 10 mL of papaya seed oil; Sample D – Mixture of coconut and olive oils to maintain their 2 : 1 ratio)

These prepared soap samples were compared with two market-bought papaya fruit soaps (Samples E and F). Soaps containing papaya leaf extract or papaya seed oil are still not manufactured in the market and hence could not be procured for the comparative study. The above-mentioned Sample D of soap served as a control to assess the effectiveness of the added plant extracts. Thus, a total of 6 soap samples were assessed for their quality defined by the parameters like soap pH; moisture content; alcohol soluble and insoluble content; total fatty matter; free acidity content; free caustic alkali; foam height, and antimicrobial activity (in vivo and in vitro).

2.3. *pH of the soap*

The standard procedure described E. K. Ossai [9], with slight modifications, was followed to assess the pH of the 6 soap samples. In this method, the soap suspension was prepared by shaking 2 g of the soap with 20 mL of distilled water. The pH was measured using a pH meter (Equip-Tronics Digital pH Meter Model EQ-610) after the soap suspension was allowed to stand for at least 12 h at room temperature.

2.4. *Moisture content*

The moisture content of the 6 soap samples was determined by following the standard procedure as described by Ogunsuyi and Akinnawo [10], which was slightly modified. The soap samples were precisely weighed to 5 g each onto clean and dried watch glasses separately. The samples were then dried in an oven set at 60 °C for 2 h and repeated until a constant weight was reached. After cooling the samples at room temperature in a desiccator, they were weighed to determine the weight loss. The percentage moisture for the soap samples was calculated using the following formula –

$$\%\, moisture = \frac{Cs - Cl}{Cs - Cw}\, X\, 100$$

Where: Cw = Weight of crucible,
Cs = Weight of crucible + sample before heating,
Cl = Weight of crucible + sample after heating

2.5. *Alcohol insoluble*

The matter insoluble in alcohol was determined by a method described in reference [11] with slight modifications. 5 g of the soap sample was dissolved in 50 mL hot ethanol and then filtered through pre-weighed Whatman filter paper. The filter paper with the residue was then dried in the oven at 105 °C for 30 min, cooled, and weighed again. The matter insoluble in alcohol (MIA) of the soap samples was then calculated using the formula –

$$MIA = \frac{Ws - FP}{W}\, X\, 100$$

Where: Ws = Weight of sample + filter paper (after treatment),
FP = Weight of filter paper,
W = Weight of the sample

2.6. *Total fatty matter (TFM)*

A method described in reference [12] was slightly modified and used to determine the TFM of the soaps. Briefly, the complete dissolution of 5 g of the soap sample in 30 mL of hot distilled water was carried out. Then 40 mL 1 : 1 HCl was added, and the resulting solution was heated over a boiling water bath until the fatty acids floated as a separate layer. The solution was then cooled on ice which eased the separation of the solidified

fatty acid layer. This solidified fatty acid layer was collected in a pre-weighed evaporating dish. The solution left behind was transferred into a separating funnel, and 50 mL of petroleum ether was added to it. The separating funnel was then shaken for complete mixing of its contents and allowed to stand. The organic layer was collected in the evaporating dish, where a previously separated solidified fatty acid layer was collected. The aqueous layer in the separating funnel was given another treatment with petroleum ether. The organic layers obtained were pooled together, and the contents of the evaporating dish were evaporated to dryness in the oven at 110 °C for an hour and weighed again. The TFM was calculated using the formula –

$$TFM = \frac{Fs - F}{W} X\ 100$$

Where: Fs = Weight of sample after drying + evaporating dish,
F = Weight of evaporating dish, W = Weight of the sample

2.7. *Free caustic alkali*

Free caustic alkali (FCA) was determined by the method described by Vivian *et al*., [7], and Milwidsky and Gabriel [13] with slight modifications. Precisely, 5 g of the soap sample was dissolved in 30 mL of ethanol, and 10 mL of distilled H_2O was added. Using a phenolphthalein indicator, the resulting solution was titrated against 0.1 M HCl. The FCA was calculated using the formula –

$$FCA = \frac{0.31}{W} X\ Va$$

Where: Va = Volume of acid, W = Weight of the sample

2.8. *Lather volume*

Lather volume was determined by the method described in reference [14]. 5 g of the soap were blended with 100 mL of distilled H_2O. The kitchen blender was operated on low speed for exactly 60 sec. Then the lather was quickly poured into a 500 mL measuring cylinder. The lather volume was measured immediately after leveling off the top surface of the foam. Only the top height was read. 100 mL of the 1 % sodium lauryl sulfate solution as a standard was treated similarly.

2.9. *Foaming properties*

The foaming power and foam stability were measured with slight modifications in the method developed by Ross and Miles [15]. Two grams of the soap sample were dissolved in 1 L of distilled H_2O. 50 mL of this soap solution was transferred to a measuring cylinder carefully to prevent foaming in the cylinder. Another 200 mL of the soap solution was taken in a separating funnel and poured from a 45 cm height into the measuring cylinder containing 50 mL soap solution. The resulting turbulence caused the generation of foam. The height of the foam generated in the measuring cylinder was then measured

immediately, indicating the soap solution's foaming power. Again after 5 min. the foam height was measured. The foam stability, given by R5, was calculated as the ratio of the height of the foam at 5 min to that at the zero time [16].

2.10. *In vitro assessment of antibacterial efficacy*

The antibacterial efficacy of the 6 soap samples was assessed quantitatively using a colorimeter. *Streptococcus aureus* and *Escherichia coli* were used as the test organisms. Briefly, the soap solution was prepared by dissolving 1 g of the soap in 100 mL of sterile distilled H_2O. The dissolution was carried out in an electric water bath set at 60 °C. After cooling, 1 mL of each of the resulting soap solution was added to three sets of 9 mL sterile nutrient broth tubes. The tubes in set I was inoculated with 0.1 mL of the *E. coli* suspension in saline, whereas 0.1 mL of the *Strep. aureus* suspension in saline was added to set II tubes. Further, 0.1 mL sterile distilled H_2O was added to the set III tubes, and these tubes served as Negative Control. The two Positive Control tubes contained 9 mL nutrient broth and 0.1 mL of each test organism suspension in the two tubes separately. All the tubes were read colorimetrically at 660 nm against a blank (nutrient broth only) before and after incubation. All the tubes were incubated at 37 °C for 24 h.

2.11. *In vivo assessment of antibacterial efficacy*

In vivo antibacterial efficacy assessment of the soap samples involved the participation of human subjects after obtaining their informed consent. The first step expected the participants to wash their hands following an older method for surgical hand preparation [17]. Precisely, hands were washed for 1 min using Dettol (antibacterial) liquid soap and dried using a clean paper towel. This was followed by the hand disinfection step, which involved rubbing 70 % ethanol solution on the hands until dry. The finger pad of the washed and disinfected thumb was then imprinted onto the surface of a solidified sterile nutrient agar contained in a petri dish (negative control). The same finger pad (thumb) was then purposely contaminated by touching it to the test organism for 1 sec and then immediately imprinted onto the same petri dish (positive control) but at a different location from the negative control. The hands were then washed with the soap sample under study for not more than 1 min and dried using a clean paper towel [18]. Then the same finger pad of the thumb was immediately imprinted onto the same petri dish at a third location (test). Similarly, washing hands with tap water only for 30 sec instead of the soap sample under study was also assessed. The petri plates were then sealed and incubated at 37 °C for 24 h.

3. Results

Carica papaya fruit extract, leaf extract, and seed oil were used separately to prepare the novel products, namely toilet soaps, and the products are shown in Fig. 2. The results for the physicochemical properties of the soap samples A to F are presented in Table 1.

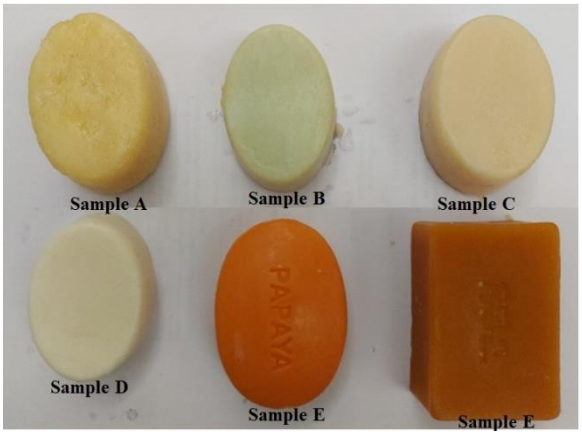

Fig. 2. Toilet soaps prepared using different *Carica papaya* extracts.

[Sample A – Fruit extract**;** Sample B - Leaf extract; Sample C – Seed oil; Sample D – Does not contain any papaya extract (Control sample); Sample E and F – Market bought & contain fruit extract].

The pH values for all the samples fall within the range of 8.37 – 9.74. The moisture content of the soap samples ranged between 2.94 – 11.32 %. MIA values of the laboratory-prepared soaps (Samples A to D) ranged between 9.45 – 9.91 %, and thus, they fulfill the requirement (Table 2) for both Grade 2 and Grade 3 toilet soaps. The market-bought soap Samples E and F gave MIA values of 14.53 % and 47.39 %, respectively. According to Indian Standards for toilet soap, Samples C, D, and E comply with the minimum requirement of TFM for Grade 1 Toilet Soap. TFM values of Samples A and B conform to the Grade 2 toilet soap standards. Samples A, C, and D did not contain any amount of free caustic alkali, while Sample B had an insignificant amount of free caustic alkali.

Free caustic alkali is the free (uncombined) caustic alkali present in soap which mostly results from improper or incomplete saponification. It is one of the parameters that determine the abrasiveness of any given soap. Samples A, C, and D did not contain any amount of free caustic alkali, while Sample B had an insignificant amount of free caustic alkali. Thus, the Soap Samples A to D will not be harsh on the skin. The market-bought Samples E and F contained permitted colors in them, which made it difficult to visualize the color change at the end-point of the titration method.

The lather is the foam created by soap when stirred in water or while bathing or washing hands. It is an important parameter for the acceptability of the soaps. The minimum lather volume requirement for the bathing bar is 200 mL under the test conditions [14]. The Bureau of Indian Standards mentions no such specification for the lather volume of toilet soap. However, a consumer voice report on toilet soap cited that the standard requirement of lather volume is Grade 1: 280 mL, Grade 2: 240 mL, and

Grade 3: 200 mL [19]. According to this, all the Samples A to F belong to Grade 1 quality Toilet Soaps because their lather volume was between 410-780 mL.

Table 1. Physicochemical properties of the soap samples A to F **(**R5 – Ratio of the foam height at 5 min to that at 0 min).

Sample		A	B	C	D	E	F
pH		8.52±0.11	8.89±0.05	8.37±0.08	8.75±0.09	9.74±0.15	9.49±0.09
moisture (%)		4.84±0.06	4.98±0.10	3.20±0.09	4.80±0.08	2.94±0.04	11.32±0.18
Matter insoluble in alcohol (%)		9.74±0.18	9.91±0.08	9.45±0.11	9.58±0.02	14.53±0.16	47.39±0.20
Total fatty Matter (%)		75.89±0.06	72.98±0.13	83.21±0.19	77.98±0.15	89.82±0.08	52.56±0.06
Free Caustic Alkali (%)		0.0	0.009±0.0002	0.0	0.0	Undetectable due to the added colors in the sample	
Lather volume (mL)		670±30	780±10	770±10	750±20	410±20	600±30
Foam height (cm)	0 min	3.1±0.1	5.0±0.0	5.2±0.4	4.2±0.4	5.2±0.2	4.1±0.4
	5 min	2.6±0.2	4.4±0.1	4.8±0.2	3.8±0.3	4.3±0.9	3.0±0.7
	R5	83.87 %	88.00 %	92.31 %	90.48 %	82.69 %	73.17 %

Foam is generated when soap is mixed with water and air. It clings to the surfaces and increases the dwelling time, enabling the cleaning agent to penetrate the dirt, mud, grease, oil, and other grime. Thus, it aids in cleaning but does not have any cleaning power of its own. The foaming power of the soap was measured in terms of foam height which ranged between 3.1-5.2 cm. All the soap samples A to F presented good foam stability because a foam with an R5 value higher than 50 % is considered metastable [16].

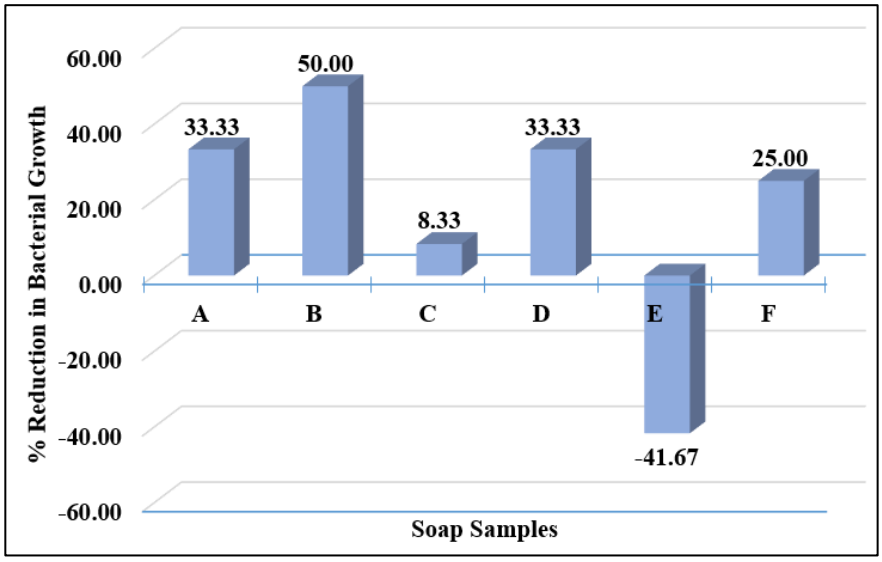

Fig. 3. In vitro assessment of the antibacterial efficacy of the soap samples against *Streptococcus aureus.* (Negative percentage indicates an increase in bacterial growth).

The quantitative in vitro assessment of the antibacterial efficacy of the soap samples against *Escherichia coli* and *Streptococcus aureus* was carried out. It was observed that none of the soap samples were effective against *E. coli*. However, the soap samples showed antibacterial efficacy against *S. aureus* except for Sample E. The findings for percentage reduction in bacterial growth are presented in Fig. 3. Among the samples, Soap B reduced the growth of S. aureus to a greater extent. In contrast, sample E showed an increase in bacterial growth by 41.67 %. This could be due to the high TFM value of Sample E, and the fatty matter in soap is mostly fatty acids which may have supported the growth of *S. aureus*.

Additionally, the soap samples were assessed in vivo for their antibacterial efficacy against *Escherichia coli* and *Streptococcus aureus*. The results obtained by the finger imprint test are illustrated in Fig. 4. It was observed that Sample A significantly prevented the duplication of *E. coli* following its usage. There was a noticeable reduction in the growth of *E. coli* when Sample B was used for handwashing after contamination. But samples C to F were ineffective in eliminating the *E. coli* after their usage. The multiplication of *S. aureus* was almost prevented after the usage of Sample B. However, the other soap samples did not possess antibacterial efficacy against *S. aureus*. Washing hands with only tap water, when contaminated with S. aureus, was ineffective.

4. Discussion

During the hot process of soap making for Samples A to D, it was observed that the saponification process hastened after the addition of sugar, as cited in the literature. The natural fruit sugar present while preparing Sample A soap further accelerated the saponification process, and the soap began to set while the process was still incomplete. Consequently, the texture of the soap was affected, and it was not as smooth as the rest of Samples B, C, and D.

Samples A, B, and C color were due to the natural color of the papaya fruit, leaf, and seed oil, respectively. Sample D was white in color since it did not contain any papaya plant extract and served as a control soap to demonstrate the effectiveness of papaya plant extracts when added to the soap. Samples E and F were bought from the market and contained papaya fruit extract. They possessed intense orange color due to the added permitted colors.

According to Indian Standard Toilet Soap – Specifications [20], Toilet soap shall be of three grades and needs to fulfill the requirements mentioned in Table 2.

Table 2. Requirements for Toilet soap [20].

SL. No.	Characteristics	Grade 1	Grade 2	Grade 3
1.	Total Fatty Matter, percent by mass, *Min*	76.0	70.0	60.0
2.	Free Caustic Alkali, as sodium hydroxide (NaOH), percent by mass, *Max*	0.05	0.05	0.05
3.	Matter insoluble in alcohol, percent by mass, *Max*	2.5	10	10

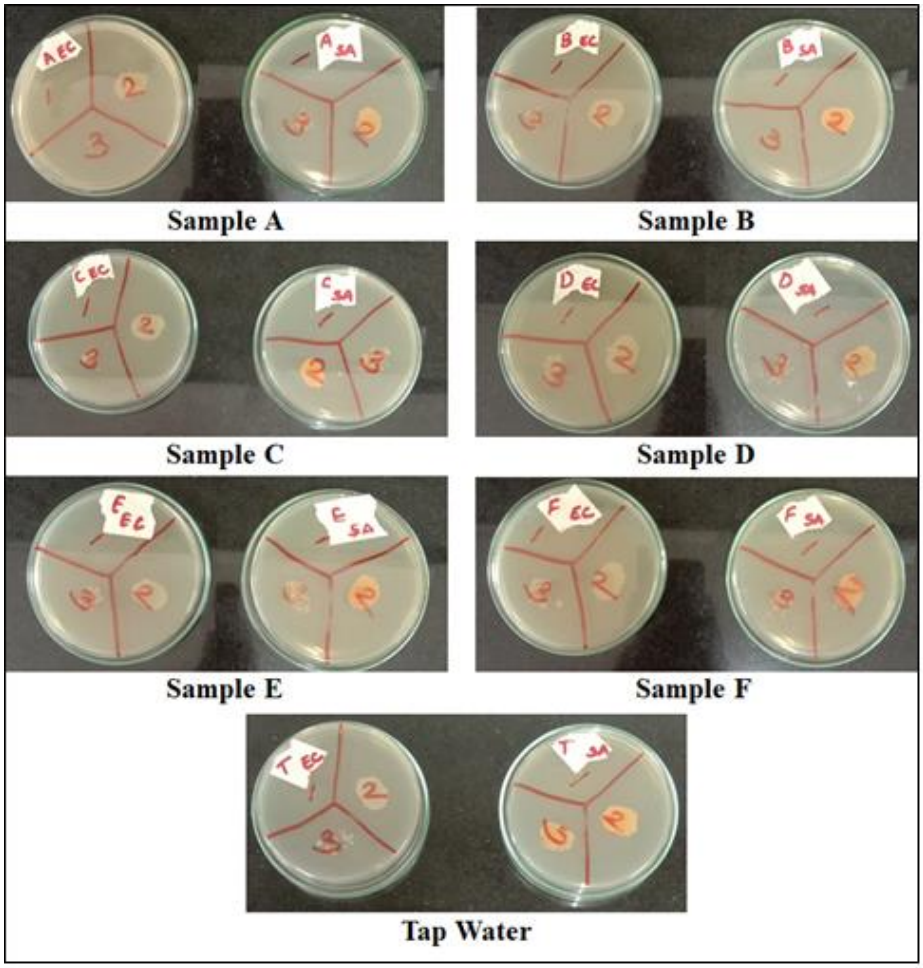

Fig. 4. In vivo assessment of the antibacterial efficacy of the soap samples.
[EC – *Escherichia coli*; SA – *Streptococcus aureus;*
Finger imprints were taken after
1 – Disinfection according to WHO guidelines (Negative control);
2 – Contamination with test organism (Positive control);
3 – Washing with soap samples A to F or tap water (T)]

It is reported that the majority of commercial soaps have pH values between 9 – 10 [21]. However, the laboratory-prepared Samples A to D gave pH values of less than 9. This could be due to the 5 % super-fatting level in the samples, which was pre-adjusted to reduce the harshness of the soap.

The recommended moisture value for soap is 10-20 % [22]. However, the national standard does not mention any specific requirement of moisture content. The soaps with higher moisture levels wear out faster, undergo hydrolysis on storage, favor the growth of microbes and ultimately have low shelf-life. The hydrolysis of soap could probably be due

to the reaction of excess water in soap with the unsaponified neutral fat present (if any) to release free fatty acid and glycerol as the by-products.

The matter insoluble in alcohol (MIA) comprises the non-soap ingredients in the finished product. They are mostly alkaline salts, such as talc, carbonates, borates, silicates, and phosphates, as well as sulfates and starch, which are insoluble in alcohol under the conditions of the test [11]. It reflects the purity of the soap, and high MIA values in the soap indicate a high level of impurities which may be the impurities of alkali used for making the soap [10].

The total fatty Matter (TFM) is defined as the total amount of fatty matter, mostly fatty acids, obtained by decomposing the soap with a mineral acid, usually HCl. It is an important characteristic that describes the primary quality of soap. Additionally, the soaps with high TFM have good moisturizing properties. The lower TFM value in Sample F could be due to additives like fillers, preservatives, color, unreacted NaOH, etc., in the soap.

5. Conclusion

Carica papaya is highly affected by the poor agricultural practices and lack of appropriate processing technologies which incur huge post-harvest losses and generate tonnes of agro-waste. Attempts are made to minimize these losses and agro-waste by manufacturing value-added products using *Carica papaya*, which in turn will have economic benefits. The ripe papaya fruit is commonly used to obtain value-added products which mainly have topical applications, but scientific studies on such products are scarce. The value-added products using papaya leaves and their seed oil are rare. Hence, in the present study the different parts of *Carica papaya* were explored to obtain the value-added product. The laboratory-made Toilet Soap infused with different papaya extracts showed good physicochemical properties and antibacterial efficacy compared to the market-available papaya fruit soap. The findings of this study suggest that these soaps would not be harsh on the skin, possess skin lubrication property and have a longer shelf life. The papaya leaf soap caused a maximum reduction (50 %) in the bacterial growth of *S. aureus* compared to all other soaps under study. To sum up, the ripe papaya fruit, papaya leaves, and its seed oil possess the potential to be used as an ingredient in the preparation of value-added product, namely toilet soap.

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