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Phytochemical and antimicrobial screening of *Crinum asiaticum* extract of herbal hand wash tablets

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Abstract
This study evaluates the phytochemical composition and antimicrobial activity of *Crinum asiaticum* leaf extract for developing herbal hand wash tablets. Ethanolic extraction followed by qualitative screening confirmed the presence of alkaloids, flavonoids, tannins, phenols, glycosides, and saponins. The extract was tested against *E. coli*, *Bacillus subtilis*, and *Aspergillus niger* using the agar well diffusion method, showing clear, concentration-dependent zones of inhibition. A herbal hand wash tablet was formulated using the extract and assessed for pH, appearance, foamability, viscosity, and skin irritation, all of which met acceptable standards. The formulation displayed strong antimicrobial action comparable to marketed products. Overall, *Crinum asiaticum* extract demonstrates promising potential as a natural and effective antimicrobial ingredient for herbal hand wash tablets.

Keywords: *Crinum asiaticum*, Phytochemical screening, Herbal hand wash, Antimicrobial activity, Ethanolic extract, Zone of inhibition

Introduction
The Crinum genus in the Amaryllidaceae family comprises 180 species, distributed across Asia, Australia, Africa, and America, with significant alkaloid content. *Crinum asiaticum* Linn., found in tropical regions worldwide, is traditionally used to treat pain, inflammation, wounds, swellings, and as an antidote in Southeast Asian medicine [1]. In Thai medicine, its leaf is used to treat inflammatory joints and support postpartum care [2]. Traditional Thai medicine uses *Crinum asiaticum* hot leaf compresses for musculoskeletal discomfort [3]. The leaf extract contains alkaloids, phenolics, terpenoids, and aldehydes [4], showing anti-inflammatory and pain-relieving effects [5]. Lycorine is its major active compound with anti-inflammatory properties [6]. The Crinum L. genus comprises 130 flowering species in the Amaryllidaceae family [7], growing in tropical climates across continents [8]. These species contain 118 distinct alkaloids [9] with various pharmacological effects, including weight loss, sedation, and antinociceptive activities [10]. *Crinum asiaticum* is a perennial bulbous herb growing up to 2m tall [11], known as poison bulb for its emetic properties [12] and spider lily [13]. It has medicinal and ornamental value [14]. *Crinum asiaticum* (Amaryllidaceae) possesses anticancer, immune-stimulating, analgesic, antiviral, antimalarial, and antimicrobial properties. The study aimed to prepare and examine alcoholic leaf extracts for phytoconstituents. The plant was collected from Bapatla college of pharmacy, Andhra Pradesh, India and authenticated by Dr. D. Raja kumari. Botany, The Bapatla College of Arts & Science, Bapatla district, Andhra Pradesh.

Fig 1: *Crinum asiaticum*

Materials And Methods

Materials Used

Materials required

Collection of plant material: The plant *Crinum asiaticum* was collected from its natural habitat i.e., from the compound surroundings of Bapatla college of pharmacy, Bapatla district, Andhra Pradesh, India and was authenticated by Dr. D. Raja kumari Head of the Department of Botany, The Bapatla College of Arts & Science, Bapatla district, Andhra Pradesh.

Chemicals used

Sodium lauryl sulfate (SLS), Glycerin, Ethylenediaminetetraacetic acid (EDTA), Isopropyl Alcohol, Sodium benzoate, Citric acid, Sodium Bicarbonate, Peppermint oil, Agar, Beef extract, Peptone, NaCl, Distilled water, Dextrose, and all the above chemicals are collected from the Bapatla college of Pharmacy, Bapatla district, Andhra Pradesh, India.

Method

2.1Preparation of plant material

Collect fresh leaves and bulbs of *Crinum asiaticum* Wash and mince the bulbs into small pieces, then air-dry at room temperature for 10 days. Spread on a clean surface in a shaded, ventilated area. The leaves were shade-dried to remove moisture and facilitate component identification. Once completely dried, grind into coarse powder.

Fig 2: Shade drying of leaves of *Crinum asiaticum*

2.2 Experimental Method

Solvent Selection and Extraction Procedure Soxhlet apparatus Ethanol was selected as extraction solvent to target bioactive compounds. The extracts were concentrated and stored appropriately. Ethanol (C2H5OH): Polarity: Polar solvent with hydroxyl group, suitable for polar compounds. Applications: Used in extraction, chromatography, and reactions with polar functional groups. Safety: Flammable, use in ventilated areas.

2.3 Extraction Procedure

The dried powder was extracted with ethanol by soxhlation for 3h. Concentrated extract was evaporated to paste on water bath at 50°C. Plant material was extracted using Soxhlet apparatus and water by cooled maceration for 24hrs at room temperature. Extracts were filtered, concentrated at 45°C using rotary vacuum evaporator, and vacuum dried. Formulation of Herbal Handwash Tablets The tablet contains *Crinum asiaticum* extract with antimicrobial properties effective against germs and skin problems like fungal infection, ringworm, eczema, and dermatitis. The formulation is skin-suitable and offers therapeutic benefits. The study explores formulation and evaluation of *Crinum asiaticum* Herbal Hand Wash for antibacterial activity

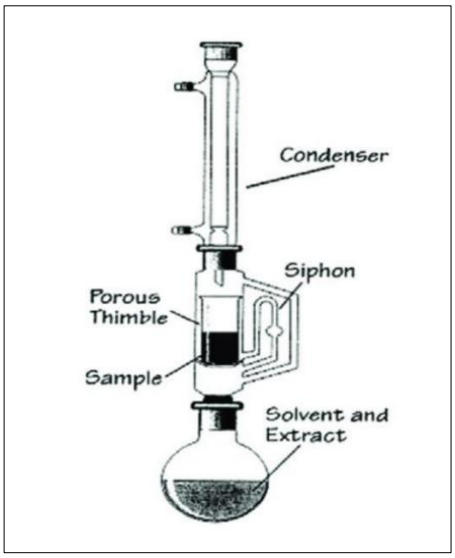


Fig 3: Soxhlet apparatus

Fig 4: Soxhlet Procedure

Quantitative chemical examination for phytoconstituents

Phytochemical analysis is an essential preliminary step in evaluating the medicinal potential of plant extracts. It helps identify important bioactive groups such as alkaloids, flavonoids, tannins, phenols, glycosides, and saponins. Each class of compound responds in a unique way to specific chemical reagents, producing characteristic color changes or precipitates.

The procedures shown below represent standard qualitative tests that were performed on the ethanolic leaf extract of *Crinum asiaticum* These tests help confirm the presence or absence of major secondary metabolites responsible for antimicrobial, antioxidant, and therapeutic activities. The table summarizes the procedure, expected observation, and inference exactly as described in your material.

Table 1: Phytochemical Tests for *Crinum asiaticum*

Phytochemical Class	Test Name	Procedure (As Given in Image)	Observation	Inference
Alkaloids	Mayer's Test	1 ml extract + 4-5 ml dilute HCl → shake → add Mayer's reagent	White or pale yellow precipitate	Presence of alkaloids
	Dragendorff's Test	1 ml extract + 4-5 ml dilute HCl → shake → add Dragendorff's reagent	Orange precipitate	Presence of alkaloids
	Wagner's Test	1 ml extract + 4-5 ml dilute HCl → shake → add Wagner's reagent	Brown precipitate	Presence of alkaloids
Phenolic Compounds	Phenol Test	1 ml extract + ferric chloride solution	Yellow precipitate	Presence of phenols
	Ellagic Acid Test	1 ml extract + few drops of 5% glacial acetic acid + 5% sodium carbonate	Muddy yellow, olive brown, Niger brown, chocolate color	Presence of phenols
Tannins	Ferric Chloride Test	1 ml extract + 1% ferric chloride solution	Blue-green or brownish-green color	Presence of tannins
	Gelatin Test	Extract + 3 drops of 1% gelatin (10% NaCl)	White precipitate	Presence of tannins
Saponins	Foam Test	1 ml extract + shaken well with water	Honey-comb-like foam	Presence of saponins
Glycosides	Keller-Kiliani Test	1 ml extract + glacial acetic acid → heat 1 min → cool → add ferric chloride → transfer to tube with conc. H_2SO_4	Reddish-brown ring at junction	Presence of glycosides
	Molisch Test	1 ml extract + Molisch reagent + conc. H_2SO_4 along tube side	Reddish-violet ring at junction	Presence of glycosides
Flavonoids	Flavonoid Test	1 ml extract + few magnesium turnings + conc. H_2SO_4	Magenta, scarlet, or deep cherry color	Presence of flavanols/flavones/flavonoids
	Ferric Chloride Test	1 ml extract + neutral ferric chloride	Blackish-green color	Presence of flavonoids
	Lead Acetate Test	1 ml extract + lead acetate	Yellow precipitate	Presence of flavonoids
	Shinoda Test	1 ml extract + conc. HCl + magnesium turnings	Magenta color	Presence of flavanone/flavone
Carbohydrates	Molisch Test	1 ml extract + Molisch reagent + conc. H_2SO_4	Reddish-violet ring	Presence of carbohydrates

From above table preliminary screening is performed and various phytoconstituents identified in leaf extracts of *Crinum asiaticum* by qualitative chemical examination.

Table 2: Preliminary Phytochemical Screening of *Crinum asiaticum* Leaf Ethanolic Extract

S. No	Phytochemical Test	Specific Tests Performed	Result (Ethanolic Extract)
1	Carbohydrates	(i)Molisch Test (ii) Anthrone Test	-
2	Proteins & Amino acids	(i) Ninhydrin Test	-
3	Steroids	(i)Salkowski Test (ii) Liebermann-Burchard Reaction	-
4	Phenolic Compounds	(i)Ellagic Acid Test (ii)Phenol Test	+
5	Glycosides	(i)Keller-Kilani Test (ii) Sulphuric Acid Test	+
6	Saponins	(i) Foam Test	+
7	Tannins	(i) Ferric Chloride Test	+
8	Alkaloids	(i)Dragendorff's Test (ii) Mayer's Test	+
9	Flavonoids	(i) Lead Acetate Test	+
10	Bitters	(i)Vanillin Sulphuric Acid Test (ii) Serial Dilutions	-

Inference

The leaf extract of plant *Crinum asiaticum* was qualitatively tested for the presence of different phytoconstituents and The alcoholic extract of *Crinum asiaticum* gave positive for glycosides, phenols, saponins, tannins, alkaloids, and flavonoids.

Anti-Microbial Screening of the extract

Anti-Microbial Activity

The herbal handwash tablet is prepared using *Crinum asiaticum* extract, a plant well-known for its strong antimicrobial and antifungal properties. The natural bioactive compounds present in the extract help protect the skin from common infections such as fungal diseases, eczema, ringworm, and contact dermatitis.

Because these plant-based ingredients are gentle and effective, the formulation offers a safe alternative to chemical handwashes so making it suitable for everyday use without causing irritation. Overall, the extract supports healthy skin while effectively reducing harmful microbes.

Anti-Bacterial Activity

The antibacterial potential of *Crinum asiaticum* was evaluated using the Agar Well Diffusion Method, a standard technique for measuring how well a substance can inhibit bacterial growth.In this method, the plant extract is introduced into

small wells created on agar plates that have been previously inoculated with bacteria.The antibacterial activity is assessed based on the zone of inhibition and the clear area around the well where bacterial growth is suppressed.The extract showed activity against:

- **Gram-positive bacteria:** Bacillus species
- **Gram-negative bacteria:** *Escherichia coli*

A clear zone around the wells indicates that the extract can effectively slow down or kill bacterial cells, supporting its use in antimicrobial handwash tablets.

Table 3: Formulation Table of nutrient agar medium

Ingredients	Quantity
Agar	20 g
Beef extract	1.5 g
Peptone	5 g
NaCl	5 g
Distilled water	1000 ml

Wells of 6-8 mm diameter were made using a sterile cork borer, and different concentrations of plant extract were added into the wells. The plates were incubated for 24 hours at 34°C, and the zone of inhibition (ZOI) around each well was measured to determine antibacterial activity.

Observations of the zone of inhibition in different bacteria

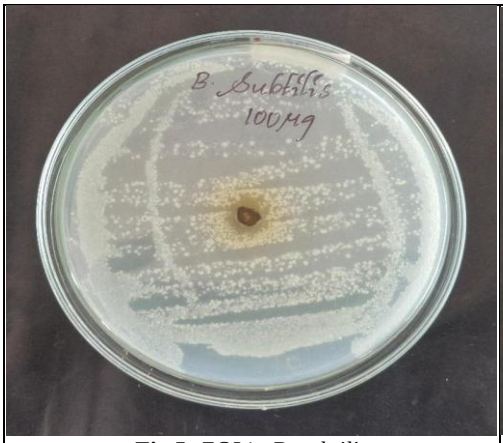


Fig 5: ZOI in *B. subtilis*

Fig 6: ZOI in *B. subtilis*

Fig 7: ZOI in *B. subtilis*

Fig 8: ZOI in *B. subtilis*

Fig 9: ZOI in *B. subtilis*

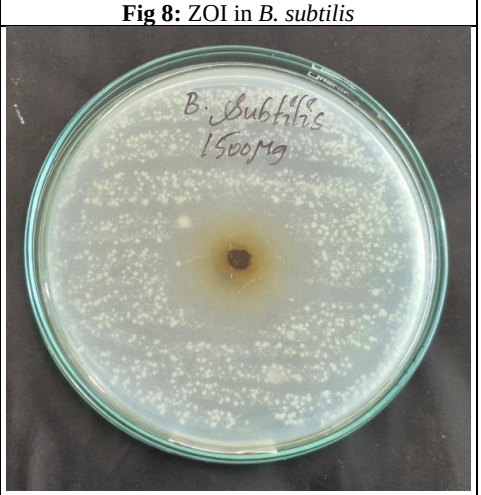


Fig 10: ZOI in *B. subtilis*

Fig 11: ZOI of *E. coli*

Fig 12: ZOI of *E. coli*

Fig 13: ZOI of *E. coli*

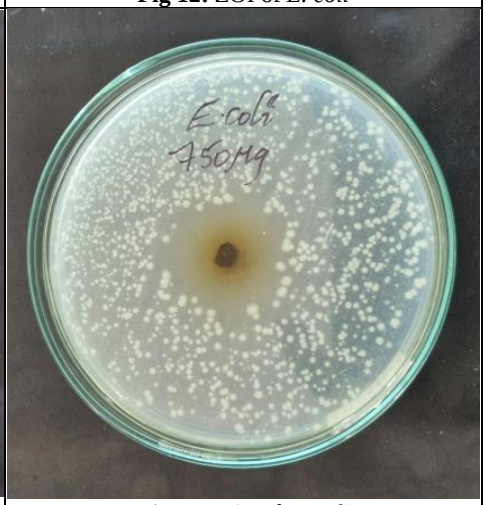


Fig 14: ZOI of *E. coli*

Fig 15: ZOI of *E. coli*

Table 4: Zone of Inhibition

Microorganism	100 µg	250 µg	500 µg	750 µg	1000 µg	1500 µg
E. coli	1.5 mm	1.6 mm	1.7 mm	1.8 mm	2.0 mm	2.5 mm
B. subtilis	1.33 mm	1.5 mm	1.6 mm	1.9 mm	2.2 mm	2.4 mm

Anti-fungal Activity

Sabouraud Dextrose Agar (SDA) is used for isolating and cultivating both pathogenic and non-pathogenic fungi. It has a pH of 5.6, which favors fungal growth.SDA contains peptones (source of amino acids and nitrogen), dextrose (energy source), and agar (solidifying agent).

Procedure:

1. Dissolve 65 g of SDA in 1 L of purified water.
2. Heat and boil with stirring for 1 minute to fully dissolve.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C and pour into Petri plates or slants.
5. Streak specimen on the medium using a sterile inoculating loop to obtain isolated colonies.
6. Incubate plates at 25-30°C in an inverted position with high humidity.
7. Allow plates to incubate at 28°C for 48-72 hours to observe fungal growth and antifungal activity.

Table 5: Sabouraud Dextrose medium formulation

Ingredient	Quantity
Peptone	10 g
Dextrose	40 g
Agar	20 g
Distilled Water	1000 ml

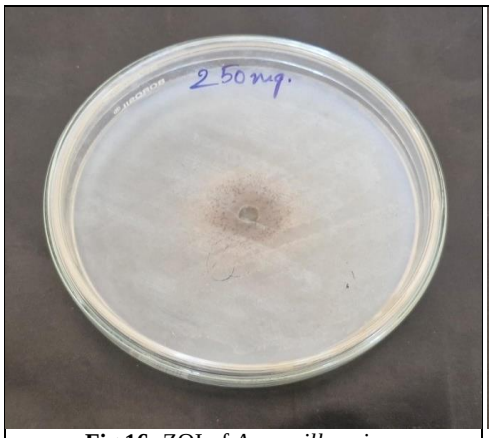


Fig 16: ZOI of *Aspergillus niger*

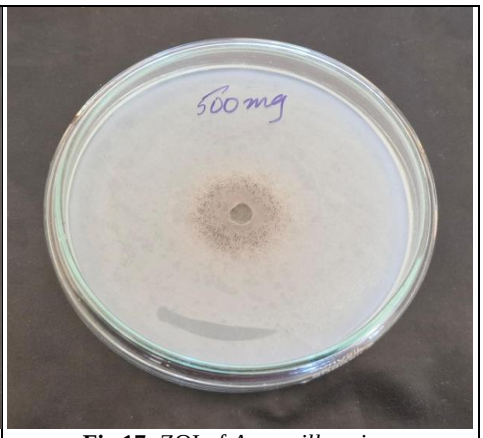


Fig 17: ZOI of *Aspergillus niger*

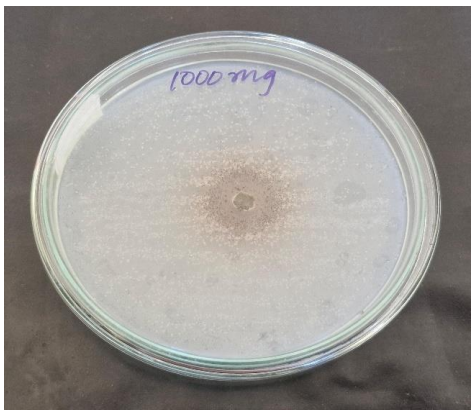


Fig 18: ZOI of *Aspergillus niger*

Fig 19: *ZOI of Aspergillus niger*

Preparation of Herbal Hand Wash Tablets

Table 6: Table of ingredients

Ingredients	Quantity
Crinum asiaticum extract	300 mg
Sodium lauryl sulfate (SLS)	4 mg
Glycerin	2 ml
Ethylenediaminetetraacetic acid (EDTA)	1 gm
Isopropyl Alcohol	2 ml
Sodium benzoate	2 gm
Citric acid	10 gm
Sodium Bicarbonate	28 gm
Starch	4 gm
Peppermint oil	q.s

Procedure For Preparation of Herbal Hand Wash Tablet:

The preparation of the Herbal Handwash Tablet was carried out by the Wet Granulation method:

Firstly, collect all the ingredients that are essential to make the herbal handwash tablet. Antibacterial and Antifungal properties. Take the *Crinum asiaticum* extract by Soxhlet extraction using ethanol. Weigh the entire ingredients accurately and mix them well in a motar and pestle. Add *Crinum asiaticum* extract and mix together with all the ingredients. After mixing all the ingredients, form a damp mass with starch. Pass the damp mass through a sieve to create granules of the desired size.

Dry the granules to remove excess moisture. After drying, the granules are screened again to ensure a uniform size distribution. Take the powder to form the tablet of the required weight in the tablet compression machine.

Fig 20: Herbal Handwash Tablet

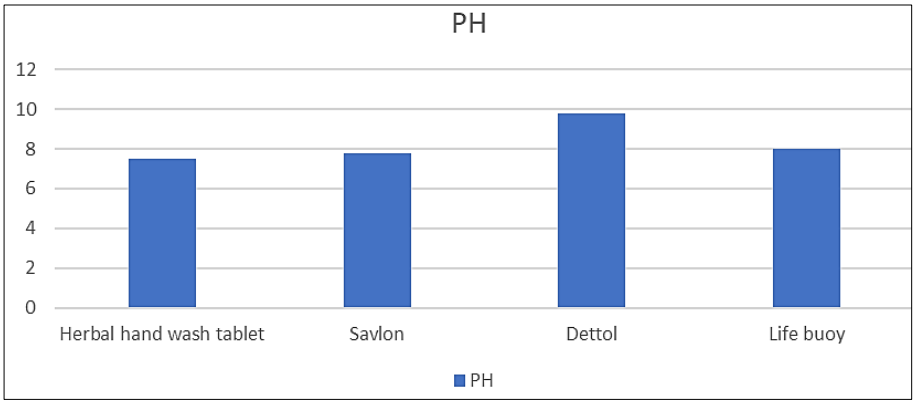
Evaluation Parameters included

1. **Organoleptic Assessment**: Measuring texture, colour, and odour through visual and tactile perception.
2. **Appearance and Homogeneity:** Visual inspection and grittiness check between fingertips.

3. **PH and Viscosity:** Measured using a digital pH meter and Ostwald viscometer.
4. **Foam Retention:** Measured in a cylinder at 1-minute intervals.
5. **Foam Height:** Tested by dissolving the sample in water and distributing it into test tubes with varying concentrations of 10 ml. The test tubes were then shaken for 15 seconds. The test tube is then left to stand for five minutes. Thereafter, the height of the foam was measured.
6. **Skin Irritation Test:** To check the skin's irritability, the herbal handwash tablet was applied over the skin and was allowed to remain for 30 minutes. After the 30 minutes had passed, the skin was checked for itching, rashes, or redness using both sensory and visual methods.
7. **Anti-Microbial Activity**: According to the standard literature-reported protocol, were used to test the anti-microbial activity of hand wash using the agar plate method. For the purpose of evaluating the antibacterial activity, two sterile Petri plates were used. Agar solution containing nutrients was placed on the plates, and solidification was permitted. The subculture was solidified, then introduced into the nutrient agar using the Pour Plate Method and inoculated for 24 hours. Two cavities were created in it using the well diffusion Method after it had been injected for 24 hours. Marketed herbal hand wash is placed in the first cavity, and formulated hand wash is placed in the second. It was ensured that the sample was positioned at the cavity level. To assess the activity, the plates were put in an incubator at 37°C. After an incubation period of 48 hours, the plates were checked to see if the Zone of Inhibition (ZOI) had formed. The antibacterial activity of the formulation is evaluated from the Zone of Inhibition (ZOI). The diameter of the zone of inhibition was measured to assess the effectiveness of the formula

Results

The antimicrobial efficacy of the herbal hand wash formulation was evaluated against *Bacillus subtilis* and *Escherichia coli*. Zones of inhibition (ZOI) were concentration-dependent, with higher concentrations showing larger zones.



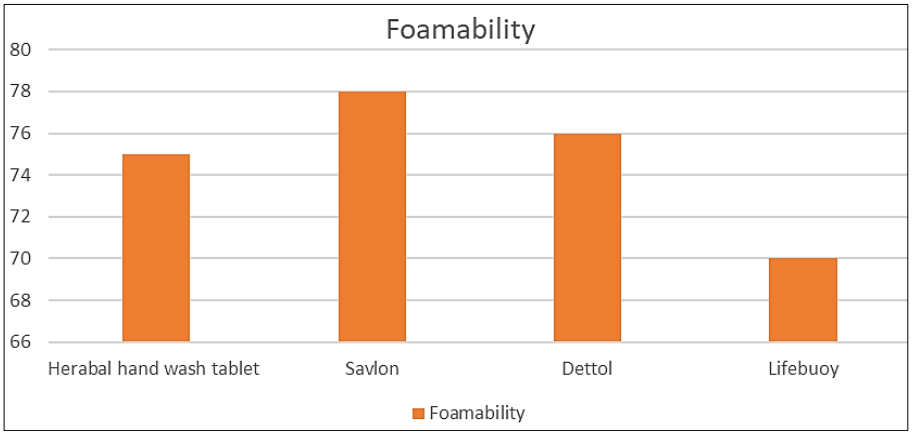


Fig 21: ZOI of Dettol in *B. subtilis*

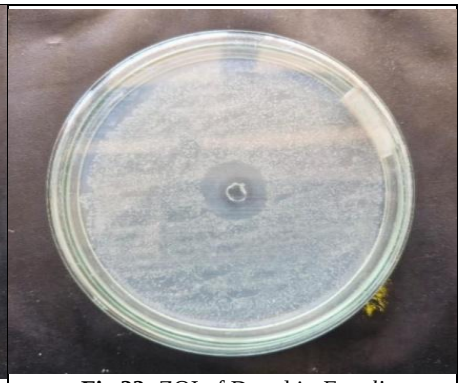


Fig 22: ZOI of Dettol in *E. coli*

Fig 23: ZOI of *Aspergillus niger*

Discussion

The anti-microbial efficacy of the herbal hand wash formulation was evaluated, and the results revealed that it had significantly more anti-microbial activity. Formulated Herbal Hand wash tablet evaluation tests were carried out and compared to the marketed hand wash (Dettol, Savlon, Lifebuoy). Formulated herbal hand wash tablet was evaluated visually for its color i.e. moss green. Odour was found by smelling the product, i.e. aromatic and characteristic. The pH & foamability of formulated Herbal hand wash tablet was compared to marketed hand wash, and it was found to be. While zone of inhibition of formulated herbal hand wash tablet was found to be Anti-microbial activity of the formulated herbal hand wash tablet was compared with marketed hand wash. It was observed that the formulated herbal hand wash tablet has good antimicrobial activity when compared to the marketed Herbal Hand wash

Conclusion

The present study concludes that the *Crinum asiaticum* plant extracts were screened for phytochemicals and antimicrobial activity, and the results showed the existence of bioactive substances with strong antimicrobial effects. Phytochemical screening confirmed the presence of bioactive compounds, such as Glycosides, flavonoids, alkaloids, and tannins, which contribute to the antimicrobial activity. According to the study, these extracts can successfully stop the growth of microorganisms, which makes them a suitable natural substitute that is safe for the environment, efficient, and possibly good for skin health should be developed by comparing with other marketed products. The conclusion for Hand Wash Tablets emphasizes its essential role in maintaining hygiene and preventing the spread of germs. The Handwashing tablets with effectively removes dirt, bacteria, and viruses, reducing the risk of infections and diseases. Using a quality & natural hand wash Tablet, herbal-based, ensures cleanliness while also caring for the skin. With growing awareness of hygiene, hand wash products continue to evolve, offering antibacterial, moisturizing, and eco-friendly options. Ultimately, proper Handwashing tablets is a simple yet powerful habit that promotes health and well-being for individuals and communities allowing for the provision of safe and healthy living through germ-free hand techniques.

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Author Contributions

Conceptualization: Kumbha Prathyusha; Methodology: Poli Sai Padma Priya; Investigation: Sannidhanam Venkata Shanmukha Santosh; Writing - Original Draft: Koppula Jaya Nissi; Writing - Review & Editing: M. Harshitha Sai.

Competing Interests

The authors declare no competing interests.

Ethics Approval

Not applicable. This study did not involve human or animal subjects.

AI Tool Usage Declaration

The authors used AI-assisted editing tools (ChatGPT, OpenAI) only for language refinement and formatting assistance. No AI tools were used for data generation or analysis.

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