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Development And Assessment Of Acalypha Indica Soap: Exploring In Vitro Antimicrobial Properties

P. Jegadeeswari[1], R. Prasanth[1], B.N. Jayaprakash[1], G. Asha[1], S. Anbazhagan[2], S.A. Vadivel[3*]

[1]Research student, Surya School of Pharmacy, Villupuram.

[2]Professor, Department of Pharmaceutical Chemistry, Surya School of Pharmacy, Villupuram.

[3]Associate Professor, Department of Pharmaceutics, Surya School of Pharmacy, Villupuram.

[*]**Corresponding Author:**

S.A. Vadivel

Associate Professor, Department of Pharmaceutics, Surya School of Pharmacy,

Villupuram. Email ID: vetrivadivel24@gmail.com

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ABSTRACT

Specifically, the development and evaluation of a herbal soap that contains *Acalypha indica* extract is the focus of this research program., known for its antimicrobial properties. The soap was developed using traditional Cold process method and evaluated for its physicochemical properties, antimicrobial efficacy, and skin benefits. The plant extract was analyzed for saponins, alkaloids, flavonoids, tannins, and steroids. The products were tested for antibacterial activity against *Staphylococcus aureus, Bacillus spp., Escherichia coli*, and *Klebsiella spp*. using the agar well diffusion method. The results suggest that *Acalypha indica*-based soap exhibits significant antimicrobial activity, making it a potentially effective alternative to commercial soaps for maintaining skin hygiene..

Keywords: *Acalypha Indica, Formulation, Herbal Soap, Antibacterial, Antifungal Activity*.

1. INTRODUCTION

When it comes to the human body, the skin is the largest organ, accounting for more than fifteen percent of the total body weight in adults. It is responsible for a number of essential functions. including protection against external aggressors, prevention of excessive water loss, and regulation of body temperature. The skin also acts as the first line of defence against environmental threats such as microorganisms, harmful chemicals, and ultraviolet (UV) radiation.

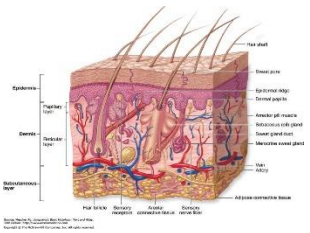


Fig.1.structure of skin

Acalypha indica has therapeutic properties. India, Sri Lanka, Thailand, and Pakistan often have it. Eye infections, respiratory issues, rheumatism, skin disorders, and blood sugar lowering are treated with plant extracts from the leaves, roots, and stems. Several extraction methods are used to extract active compounds from *Acalypha indica*. Heat stress can degrade photochemical components extracted by the Soxhlet extraction process, but it is often successful and accurate..1

Acalypha, a Euphorbiaceae blooming plant, has many medical uses. Most of the 460 species in the flora are located in somewhat temperate climates, except for Europe. This plant is abundant on India's plains and used in traditional medicine. Acalypha indica's many medical characteristics and therapeutic uses, as well as phytochemical study, make it a powerful medicinal plant. 'Kaphgajri' and 'Kaphkaturi' are native names for this occurrence. It is called "Indian Copperleaf" or "Indian Mercury" in English. Hindi calls it "Kuppi," and Sanskrit calls it "Harita manjari."

Many Ayurvedic practitioners use Acalypha indica as a medication. 2. Hepatoprotective, anti-inflammatory, antitussive, antibacterial, antifungal, and wound-healing activities have been found in it. This plant's root, especially, has tonic, astringent, febrifuge, and potent purgative qualities, according to past research. Roots can treat chest, joint, migraine, blood dysentery, and low blood sugar. The number is three. Treating the root can help fever, heart disease, retained excretions, and biliousness. Leaf extracts treat pustules, bug bites, jaundice, hemorrhoids, rheumatism, ulcers, and external skin eruptions. Leaves also heal eczema and ringworm. Leaf juice helps treat scabies and other dermatological issues. The plant extract and decoction treat rheumatism, asthma, and pneumonia.4

Fig.2.*acalypha indica*

2. MATERIAL AND METHODS

Acquisition of samples

The leaves of *Acalypha indica* samples were handpicked from the medicinal garden of Surya school of Pharmacy, Vikravandi, and also in the backyard of the student's houses. Coconut oil, and other additives along with the excipients were purchased locally.

Authentication

The legitimacy of the samples was verified by the Department of Pharmacognosy at the Captain Srinivasa Murthy Central Ayurveda Research Institute, which is located in Arumbakkam, Chennai.

Preparation of extracts

Following the desiccation of the entire *Acalypha indica* plant at room temperature, the plant was crushed to a mesh size of #40 and then stored..[5,6.]

Fig.3.Dried plant leaf

Fig.4.Powdered sample

Preparation of extraction

The powder was then extracted with ethanol and water in separate Soxhlet apparatus. The extracts were concentrated in a rotational evaporator and stored for further use.[7,8.]

Fig.5.Extraction by Soxhlet apparatus

Fig.6. Crude drug separated by Rotatory evaporator

PHYTOCHEMICAL INVESTIGATION

Qualitative Test of Fresh Extract

After that, the additional tests are carried out in order to determine the secondary metabolites that are present in the sample.[9,10]

1: Alkaloids

Alkaloids were identified by using the following tests; Mayer test, Wagner test, Dragendroff's test and Hager's test. On the following procedure were utilized for the above test. For Mayer's Test: Test tube containing, 2ml of extract and 2ml of Mayer's reagent. Then it was mixed thoroughly and the solution was boiled by using water bath, the yellow color precipitate was observed.

2: Carbohydrate

Carbohydrates were identified by using the following test; Molisch' test, benedict test, Fehling's test. The below procedure were utilized for the above test. For Molisch test: test tube containing 2ml of extract and 2ml of Molisch reagent. Then it's mixed thoroughly and the solution was boiled by using water bath, the violet color is observed.

3: Glycoside

The modified Borntrager test requires three milliliters of the sample in a sterile test tube and two milliliters of the reagent to detect glycosides. Mix thoroughly and boil the mixture in a water bath. After boiling, cool the test tube to room temperature. Then, observe the solution for a rose-pink color in the ammoniacal layer. A rose-pink color indicates a positive result for glycosides, while its absence suggests they are not present. Ensure proper reagent preparation and procedure adherence for accurate results.

4. Saponins

Through the use of the froth and foam tests, it is possible to determine whether or not saponins are present. To begin, the froth test is performed by transferring three milliliters of the sample solution into a clean test tube. After completely mixing the solution, add three milliliters of the froth reagent. Keep an eye out for the creation of foam in the solution; the presence of foam is an indication that saponins are present in the sample.

5: Phytosterols

The Salkowski test and the Liebermann-Burchard test are both reliable methods for determining the presence of phytosterols. To get started with Salkowski's test, put 2.5 cc of the extract into a test tube that has been thoroughly cleaned. The extract should be thoroughly mixed after two milliliters of Salkowski's reagent have been added to it. After the amalgamation has been treated to boiling in a water bath, the process is complete. It is important to keep an eye out for the appearance of a golden yellow color, which provides evidence that phytosterols are present in the sample.

6: Phenols

For phenol identification, ferric chloride is used. Start by adding two milliliters of extract to a sterile test tube. When the extract is well mixed, add two milliliters of ferric chloride. Heat the mixture in a water bath until it reaches the appropriate temperature. After the solution boils, let it cool and notice the color change. The extract turns bluish-black when phenols are present.[10]

7: Tannins

The Gelatin test detected tannins. Start by adding two milliliters of extract to a sterile test tube. The solution should be thoroughly stirred after adding gelatin. After heating in a water bath, the mixture should cool. The solution contains tannins if it forms a white precipitate.

8: Flavonoids

The Lead Acetate test detects flavonoids in samples. Start by adding 3.2 ml of extract to a sterile test tube. After adding two milliliters of lead acetate reagent, mix the extract. Slowly heat the solution. Watch for yellow precipitate. This yellow precipitate in the extract indicates flavonoids.

9: Proteins

The identify proteins contains in the fresh extract of sample, perform the Xanthoprotein, Ninhydrin, and Million's tests. For the Xanthoprotein test, add 2 ml of the extract and Xanthoprotein reagent to a test tube, heat gently, and cool; the absence of yellow color suggests no proteins.

10: Amino Acid

To detect proteins, use the Xanthoprotein and Ninhydrin tests. In the Xanthoprotein test, place 2 ml of extract into a test tube, add 2 ml of Xanthoprotein reagent, mix thoroughly, then boil the solution in a water bath and allow it to cool. The absence of yellow color indicates no proteins are present.

11: Terpenoids

To detect terpenoids, carry out the Chloroform test by mixing 2 ml of the sample with 2 ml of chloroform in a test tube. Heat the mixture with sulfuric acid, then allow the chloroform layer to separate. It is possible to determine whether or not the sample contains terpenoids by observing the presence of a grayish tint in the chloroform layer.

12: Steroids

The Extract containing steroids was identified by the following procedure. About 2ml of sample was taken in a test tube, 2ml of chloroform, Additionally, two millilitres of sulfuric acid were introduced into the test tube. Two layers were formed. The chloroform layer exhibited a crimson hue. I observed an additional layer of sulfuric acid with a greenish hue.

13: Anthraquinones

Presence of Anthraquinones was confirmed by using the following procedure, 2ml of sample of extract were taken in the clean test tube, 2ml of methanolic reagent and 10% of ammonium hydroxide were added, the solution was mixed thoroughly. Bright pink color was observed.

FORMULATION OF HERBAL SOAP

Preparation of Soap Base

Ten grams of coconut oil are poured into a 100-milliliter beaker to make cold-process soap. In a water bath, heat the liquid to 40–45 degrees Celsius while churning. A strong consistency will ensue. Weigh the ingredients into a clean beaker before adding distilled water. Add this solution to the coconut oil mixture at 40–45 degrees Celsius until a base consistency is reached. After pouring the mixture into soap molds, let it firm for two hours.

Preparation of Herbal Soap

Avoid mixing liquids with the thermometer during this experiment. Instead of fire-polished glass, use one. You should hold the thermometer with its bulb submerged in a liquid and check the mercury level to acquire an accurate reading. On the analytical scale, weigh a 150-milliliter beaker and add NaOH pellets. Work carefully with sodium hydroxide (NaOH) because it can burn skin and eyes. Hold ice water in the beaker. Continue stirring NaOH pellets and water until a transparent solution forms. Add fat to a 250-milliliter beaker on the balance scale. Next, place the beaker on a low-temperature heating plate and stir occasionally. After heating the fat to 42–50 degrees Celsius, remove it from the hot plate and mix in the lye solution. This is when to add perfume or other chemicals. After placing the emulsion in a plastic cup, let it react. Keep it for two weeks. While this happens, the soap will develop a powdered sodium carbonate layer. As soap dries, leftover sodium hydroxide mixes with airborne carbon dioxide. Once the powdered layer is removed, the soap can be used immediately.[11,12]

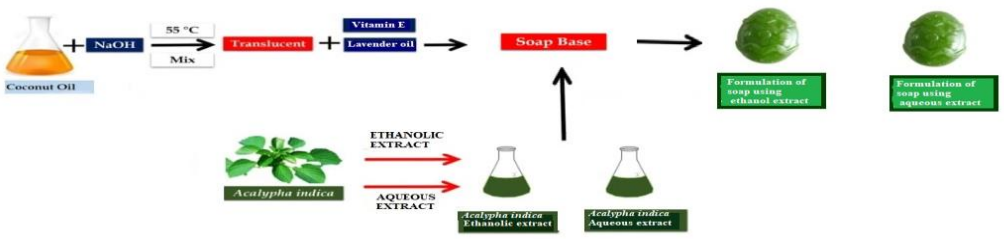


Fig.7.cold process method

EVALUATION OF FORMULATED HERBAL SOAP

A wide variety of physicochemical characteristics were examined in order to determine the level of quality of the soap that was made. Fifteen in total

Determination of clarity, colour, and Odor

The color and clarity of the object were examined using unassisted vision on a white backdrop, and the odor was evaluated using olfactory inspection.[15]

pH

A pH strip was attached to the new soap, and a digital pH meter was used to dissolve one gram of soap in ten milliliters of water to determine its pH. Both approaches were done simultaneously[15]

Determination of percentage free alkali

Reflux in a water bath was used to boil 5 grams of the sample in 50 milliliters of neutralized alcohol for 30 minutes. After cooling, one milliliter of phenolphthalein solution was added. Then, 0.1 N hydrochloric acid was added as quickly as feasible.[15]

Foam height

A 0.5-gram soap sample was dissolved in 25 ml clean water. Next, it was placed in a 100-milliliter measuring cylinder and decreased to 50 milliliters by adding water. Twenty-five strokes were delivered until the aqueous solution reached fifty milliliters. Following this, the height of the foam in relation to the aqueous solution was additionally measured.[15]

Foam retention

A one-percent soap solution was made and 25 milliliters was put to a 100-milliliter measuring cylinder. For five minutes, the cylinder was manually covered and agitated. At one-minute intervals, foam volume was monitored for four minutes.[15]

Alcohol-insoluble matter

Five grams of the chemical that was being investigated were placed inside of a conical flask. The material was entirely dissolved by means of quick agitation after fifty milliliters of warm ethanol were added to the mixture. The specimen did not entirely dissolve in the solution. A mixture of twenty milliliters of warm ethanol was added to the solution after it had been filtered through tared filter paper. Next, there was a drying stage that lasted for one hour at a temperature of 105 degrees Celsius. An evaluation of the dry paper.[15]

Total fatty matter (TFM)

Total fatty matter (TFM) was measured via a reaction between soap and acid in warm water; the generated fatty acids were subsequently investigated. Ten grams of synthetic soap broke down in one hundred fifty milliliters of distilled water when heated. To make the solution clear, twenty milliliters of 15% hydrochloric acid was added while heating. After adding seven grams of beeswax and warming, the solution's surface fatty acids solidify. After that, it hardened into a cake until ready. After retrieving the cake, it was cleaned to remove moisture and weighed to calculate Total Fat Matter (TFM) using the formula.

$$\% \text{ TFM} = \frac{(\text{Weight of the cake} - \text{Weight of the wax}) \text{in g}}{\text{Weight of the soap in g}} \times 100.15$$

Moisture content

Drying soap to a fixed weight determined its moisture content. After measuring and documenting the soap's "wet weight of sample" it was dried at 100–115 degrees Celsius in a dryer. The "dry weight of the sample." was determined after refrigeration. Through the utilization of the formula % Moisture content = (Initial weight - Final weight) / Final weight × 100, the process of determining the moisture content was accomplished.

Cleaning efficiency by thumb impression test

The thumbs of exposed hands were carefully placed on sterile nutritional agar medium with the proper spacing. The impressions of one thumb, cleansed with medicinal soap, and the other with control soap were carefully placed on separate areas of the same nutritious agar medium plate. To avoid thumbprint overlap, this was done. Microbial growth on plates was detected after 24 hours at 37°C incubation.[16]

Irritation of the skin test

Dermatologists assessed the herbal soap's skin-irritation risk. The preparation does not irritate or redden. The scenario was monitored all day.[16]

Washing Capability

The herbal soap's composition and water-rinsing ease were assessed.[16]

Stability test

The term "medication stability" refers to the time between the formulation's manufacturing and packing and the point at which its chemical or biological activity remains at or above a predetermined level of labeled potency until its physical properties have not changed significantly. Any process that creates or evaluates medicine dosage forms must consider the active component's stability to determine acceptance.[16]

***IN-VITRO* ANTIMICROBIAL ACTIVITY**

***In-vitro* Antibacterial Activity**

The antibacterial properties of plant extracts were tested against *Escherichia coli, Klebsiella spp., Bacillus spp.*, and *Staphylococcus aureus* using Mueller Hinton Agar (MHA) plates. Wells containing 50 µL, 75 µL, and 100 µL of extract were created, along with a positive control. To confirm the antibacterial agent's efficacy, the zone of inhibition was measured after 24 hours at 37°C.

***In-vitro* Antifungal Activity**

The antifungal activity was tested against *Aspergillus Niger* using the same MHA plate method. The plant extract was applied in varying concentrations, after 24 hours of incubation, the zone of inhibition showed that the antifungal medication worked.

3. RESULT AND DISCUSSION

The consequence of plant extracts of phytochemical investigation, Formulation of herbal Soap and evaluation of herbal soap, and In-vitro antimicrobial testing including *In-vitro* Anti-Bacterial testing and Anti-Fungal testing.

Table.1: Qualitative analysis of *Acalypha indica* leaf extract

Sl.No.	Phytoconstituents	Result	
		Aqueous Sample	Ethanolic Sample
1	Alkaloids	Positive	Positive
2	Carbohydrates	Positive	Positive
3	Flavonoids	Positive	Positive
4	Saponins	Positive	Positive
5	Tannins	Positive	Negative
6	Phenols	Positive	Positive
7	Steroids	Negative	Positive
8	Terpenoids	Negative	Negative
9	Catechol	Positive	Positive
10	Anthro quinone	Positive	Negative
11	Glycosides	Positive	Positive
12	Protein	Positive	Positive

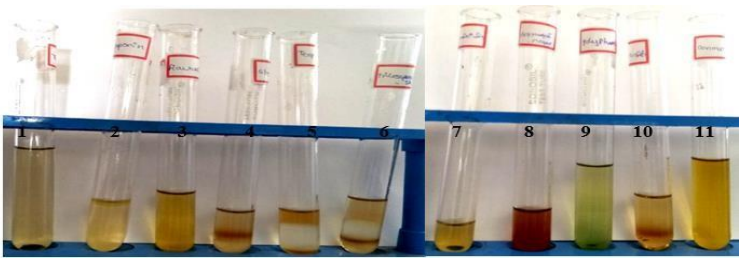
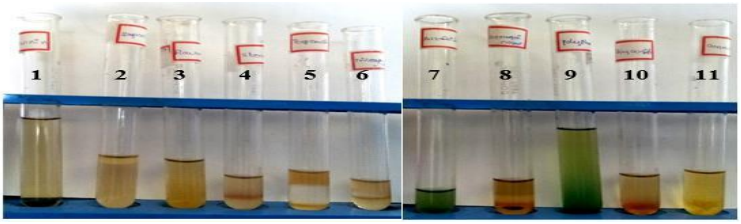


Fig.8.Qualitative analysis of Phytochemicals in Acalypha indica leaves ethanolic extract.



A qualitative examination of the phytochemicals found in the aqueous extract of the leaves of Acalypha indica is shown in Figure 9.

(Fig.8,9: 1.Tannin, 2.Saponin, 3.Flavonoids, 4.Steroids, 5.Terpenoids, 6.Triterpenoids, 7.Alkaloids, 8.Anthraquinone, 9.Polyphenol, 10.Glycoside and 11.Catechol)

Formulation of Herbal Soap

Soap Base:

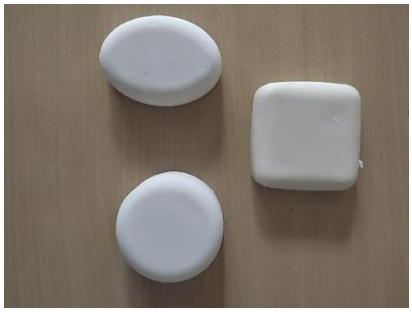


Fig.10.soap base

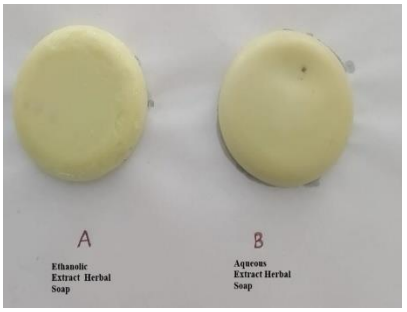


Fig.11.Formulated soap

Table.2: Evaluation Parameters of formulated Herbal Soap

Sl. No.	Parameters	Observation	
		Ethanolic Sample	Aqueous Sample
1	Color	Mild green	Mild green
2	Odor	Pleasant	Pleasant
3	PH	7.23	7.10
4	Percentage of Free Alkali	2.5	2.2
5	Foam height	15cm	16cm
6	Foam Retention	5mins	4mins
7	Total Fatty Matter	65.14	65.18
8	Alcohol Insoluble matter	27	25.9
9	Moisture content	11.8	12.4
10	Cleaning Efficiency	High	High
11	Irritation on skin	No	No
12	Washing Capability	Good	Good

Antibacterial activities:
Gram positive bacteria:

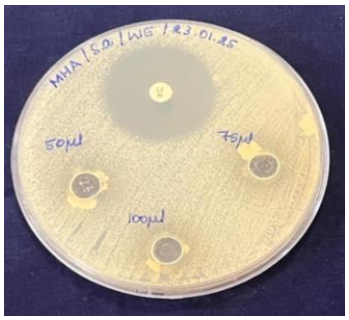


Fig no: 12.*Aureus* Aqueous Extract

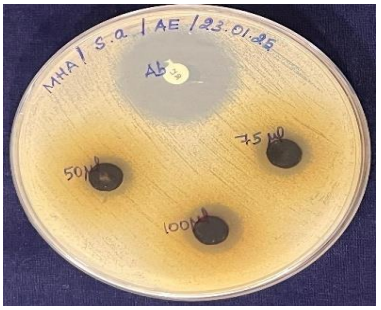


Fig no: 13.*Aureus* Ethanolic Extract

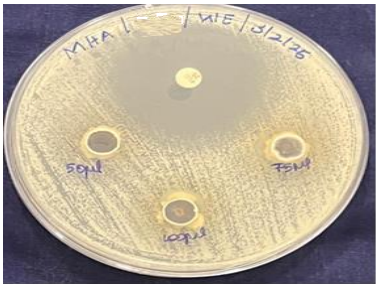


Fig no: 14. *Bacillus* Aqueous Extract

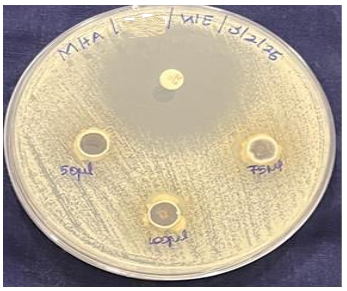


Fig no: 15. *Bacillus* Ethanolic Extract

Table.3: Zone of inhibition for gram positive organism

Organism name (Gram positive bacteria)	**Zone of inhibition**		
	Stdandard (Linezolid)	**Ethanolic extract**	**Aqueous extract**
Staphylococcus aureus	35mm	50µL = 12mm 75 µL = 14mm 100µL = 15mm	50µL = 11mm 75 µL = 13mm 100µL = 15mm
Bacillus spp	33mm	50µL = 13mm 75 µL = 14mm 100µL = 15mm	50µL = 12mm 75 µL = 13mm 100µL = 15mm

Gram Negative bacteria:

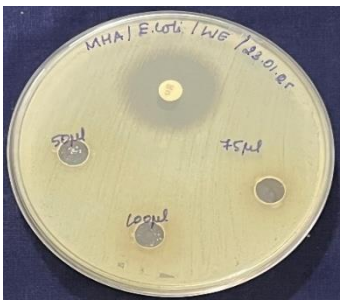


Fig No: 16. *E.Coli* Aqueous Extract

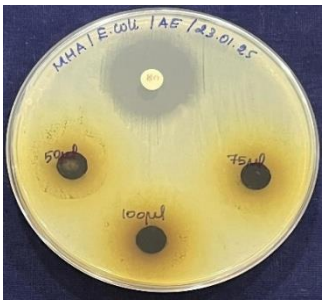


Fig No:17. *E.Coli* Ethanolic Extract

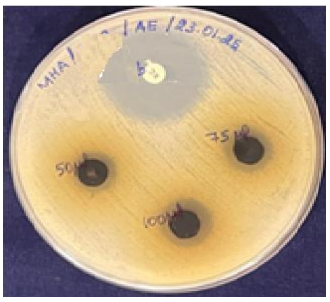


Fig No:18. *Klebsiella Sp* Aqueous Extract

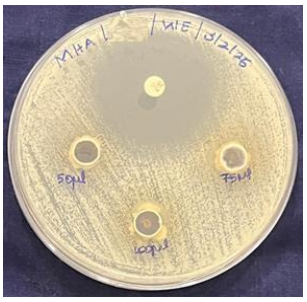


Fig No:19. *Klebsiella Sp*. Ethanolic Extract

Table.4: Zone of inhibition for gram negative organism

Organism name (Gram negative bacteria)	**Zone of inhibition**		
	Stdandard (Chloramphenicol)	**Ethanolic extract**	**Aqueous extract**
Escherichia coli	24mm	50µL = 13mm 75 µL = 15mm 100µL = 16mm	50µL = 12mm 75 µL = 13mm 100µL = 15mm
Bacillus Subtilis	22mm	50µL = 13mm 75 µL = 15mm 100µL = 16mm	50µL = 13mm 75 µL = 14mm 100µL = 15mm

Antifungal activities:

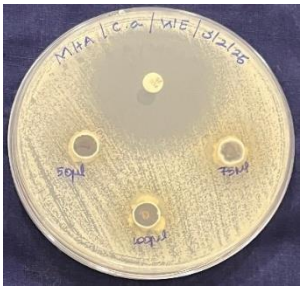


Fig. No:20 *A. Niger* Aqueous Extract

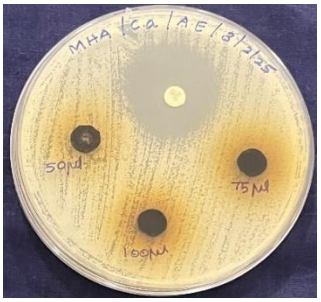


Fig. No:21 *A. Niger* Ethanolic Extract

Table.5: Zone of inhibition for Aspergillus niger

Organism name	**Zone of inhibition**		
	Stdandard (Fluconazole)	**Ethanolic extract**	**Aqueous extract**
Aspergillus niger	32mm	50µL = 13mm 75 µL = 15mm 100µL = 16mm	50µL = 14mm 75 µL = 15mm 100µL = 16mm

4. CONCLUSION

This study produced and tested herbal soaps prepared from Acalypha indica ethanolic (F1) and aqueous (F2) extracts using the cold method. Alkaloids, tannins, saponins, flavonoids, terpenoids, phenols, glycosides, anthraquinones, steroids, proteins, and amino acids were found in phytochemical analysis. These ingredients boost the soaps' medicinal efficacy. The physicochemical criteria were met, making the herbal soaps appropriate for topical usage. In vitro antibacterial activities of produced soaps were assessed using the agar well diffusion method. F1 was an ethanolic extract that inhibited Staphylococcus aureus and Bacillus subtilis. Inhibition zones of 13mm, 15mm, and 16mm were delivered at 50µL, 75µL, and 100µL dosages.

The aqueous extract (F2) showed inhibition zones of 12mm, 13mm, and 15mm for the same bacterial strains and concentrations. For Gram-negative bacteria (Escherichia coli and Klebsiella spp.), the inhibition zones for ethanolic extract were 12mm, 14mm, and 15mm, whereas the aqueous extract showed inhibition zones of 11mm, 13mm, and 15mm. Additionally, both extracts exhibited antifungal activity against Aspergillus niger, with inhibition zones of 13mm, 15mm, and 16mm across all tested concentrations. These results indicate that the formulated Acalypha indica-based herbal soap possesses significant antimicrobial properties, with the ethanolic extract showing slightly higher efficacy compared to the aqueous extract. Acalypha indica has been shown to be effective as a natural antibacterial agent utilized in personal care products, as demonstrated by the findings.

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CONFLICT OF INTERESTS

We have No conflict interest.

AUTHOR CONTRIBUTIONS

Equal contribution for all authors.

S.A.Vadivel https://orcid.org/0000-0001-5814-7775

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