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FORMULATION AND EVALUATION OF HERBAL SOAP USING LEAF EXTRACT OF *NYCTANTHES ARBORTRISTIS* AND *MURRAYA KOENIGII*

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ABSTRACT

Bacterial infections are most common in humans. The herbs are known to possess various potentials like antibacterial, anti-inflammatory, and antifungal properties. These can be transformed into various forms, for human use. One such usage is the formulation of herbal soap that is used for treating microbial infections. It also used as a cleaning agent on a daily basis. The present study was aimed to formulate and evaluate herbal soap using *Nyctanthes arbortristis* and *Murraya koenigii* leaves extract, with a focus on phytochemical analysis. For the preparation of herbal soap, herbal leaves extract of *Nyctanthes arbortristis* and *Murraya koenigii* were prepared. It is done by Cold Maceration method using methanol as a solvent. Phytochemical analysis was performed by a simple chemical test. The antibacterial activity of the extract was determined by using Agar well diffusion technique against selected bacterial pathogens. The herbal soap was formulated by adding different ingredients along with herbal extract. The physicochemical characteristics of the herbal soap were evaluated along with the antimicrobial activity against the selected microorganisms. The herbal soap formulations were found to be stable and had good physical characteristics after stability test. The herbal soap also showed significant antimicrobial activity against the tested microorganisms. It was found to be safe for use as it did not cause any skin irritation. Therefore, it can be concluded that *Nyctanthes arbortristis* and *Murraya koenigii* herbal soaps can be a promising natural alternative to synthetic soaps with added antimicrobial activity and skin-friendly properties.

KEYWORDS: *Nyctanthes arbortristis*, *Murraya koenigii*, Herbal soap, Antibacterial activity.

INTRODUCTION

Soap is a sardonic product made by the chemical process of combining fat or natural oil with an alkali such as wood ash or a strong alkaline solution. Usually, potassium hydroxide used for cleaning under controlled conditions.[1] Soap is a special product for washing prepared from natural materials that may contain both plant and animal substances. It includes items such as animal fat, tallow or vegetable oil, castor, olive, or coconut oil. Imaginarily it got its name from Mount Sapo in Rome. The word "Sapo" is the Latin word for soap first appeared in Pliny the Elder's Historia Naturalis. The first soap was made about 2800 B. C. by Babylonians.[2] The skin, or cutaneous membrane covers the outer surface of the body. It is the largest organ in the body in terms of surface area and weight. Skin is the most exposed part of the body to sunlight, environmental pollution, and also to some pathogens. The most common skin disorders are eczema, warts, acne, rashes, psoriasis, allergy, etc. To protect the skin from infectious microorganisms and their spreading, body hygiene plays an important role in the prevention of infectious diseases. Therefore, the use of soap will help to reduce transmission of contagious health related diseases more effectively. Chemically soaps are combinations of fats, oils (of animal or vegetable origin), and Salt. Soaps are generally salts of free fatty acid made via saponification, where alkaline substances react with fatty acids in fats or oils.[3] Other substances are then added to this salt of free fatty acid or soap base, to produce the different types of soaps we have. Medicinal soaps are a simple variation of normal soaps. It contains natural bioactive ingredients which are added into the basic soap medium. It gives a vast variety of biological activities to the final product. Due to the undesirable side effects of synthetic substances, it is preferential to avoid the use of harmful synthetic chemicals from medicinal soap products. In recent years, plant-based natural products have become an attractive alternative. It enhances the important biological characteristics of medicinal soaps. This medicinal soap is used as an alternative to synthetic soap to avoid contagious diseases.

Nyctanthes arbortristis commonly known as Parijataka or Night jasmine. It belongs to the family Oleaceae. It is now considered as a valuable source for several medicines against various diseases. It is also used for the development of some industrial products. Every part of *Nyctanthes arbortristis* is used for medicinal purposes due to its health-benefiting

properties. The present study includes comprehensive information on the chemical constituents, biological activities of important compounds, pharmacological effects, and medicinal applications of Night jasmine.[4] *Murraya koenigii*, popularly known as curry leaf or Kari patta. *Murraya koenigii*, a medicinally important herb has wide therapeutic applications such as in bronchial abnormalities, piles, vomiting, skin infections, etc. The medicinal values have been observed especially for leaf, stem, bark, and oil. The plant has tonic and stomachic properties. The bark and roots of *Murraya koenigii* can be used as a stimulant and to cure eruptions and bites of poisonous animals. The tender green leaves are also having medicinal importance for the cure of dysentery, diarrhoea, and checking to vomit. Leaves and roots are also used as bitter, anthelmintic, analgesic, curing piles, inflammation; itching. They are useful in leucoderma and blood disorders.[5] Based on earlier data this plant has been reported to have anti-oxidative, antimicrobial, anti-ulcer, and cholesterol-reducing activities. Taking into consideration these therapeutic properties present study was designed to formulate and evaluate the herbal soap from the leaf extract of *Nyctanthes arbortristis* and *Murraya koenigii*.

MATERIALS AND METHODS

Plant material:

Nyctanthes arbortristis and *Murraya koenigii* leaves were taken from the botanical garden of Dayanand College, Solapur. The leaves were identified in the Department of Botany, D.B.F. Dayanand College of Arts and Science, Solapur [Maharashtra].

Fig. 1: *Nyctanthes arbortristis.*

Fig. 2: *Murraya koenigii.*

Plant extract Preparation and Phytochemical analysis study:

Preparation of plant extract:

Fresh leaves were washed under the running tap water and dried under shade at room temperature. Dried leaves were powdered in the electronic grinder. The cold extract was prepared by taking the 10 g powder in 50 ml of solvent (100% methanol) and kept at room temperature for 48 hours. Stirring of the solution was done after each 4 to 5 hours. After that solution was filtered using Whatman filter paper. Finally, the filtrate was transferred to rota vapour to evaporate the solvent and to get a solid extract. The extract was kept in a refrigerator at -40^0 C, to be used for further study.[6]

Preliminary phytochemical analysis of both extracts:

A preliminary phytochemical analysis was done to find out the active chemical principle of the particular plant.

Physical characteristics of plant extract:

Physical characteristics of the plant extracts like colour, odour, and consistency were studied.

The percentage yield of plant extract:

The percentage yield of the plant extracts in methanol was determined in terms of the total quantity of powder in grams taken for the preparation of the extract.

Detection tests of plant extracts:

Detection of alkaloids:

50 mg of Solvent-free extract was mixed with a few ml of dilute HCL and filtered. The filtrate was used for various tests as follows.

Wagner's test - To a small aliquot of filtrate in a test tube, a few drops of Wagner's reagent were added. The development of a reddish-brown precipitate indicated a positive test.

Detection of carbohydrates:

Benedict's test - To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated for 2 min in a boiling water bath. A characteristic-coloured filtrate indicated the presence of sugar.

Detection of amino Acids and Proteins:

100 mg extract was dissolved in 10 ml distilled water and filtered through Whatman no.1 filter paper. The filtrate was used to test the presence of proteins and amino acids.

Biuret test - One drop of 2% copper sulphate solution was added to 2 ml of filtrate. To this, 1ml of ethanol was added followed by the addition of excess potassium hydroxide pellets. The development of pink colour in the ethanol layer indicated the presence of proteins.

Detection of saponins:

Foam test - 50 mg of extract was dissolved in 20 ml of distilled water. The suspension was shaken in a graduated cylinder for 15 min. The development of two cm layer of foam indicated the presence of Saponins.

Detection of tannins:

Ferric chloride test - 50 mg of extract was dissolved in 5 ml of distilled water and then a few drops of 5% Ferric chloride were added. The development of dark green colour indicated the presence of tannins.

Detection of flavonoids:

Magnesium and Hydrochloric acid reduction test - 50 mg of the extract was dissolved in 5 ml of alcohol and a few fragments of magnesium ribbon and concentrated hydrochloric acid were added dropwise. The development of pink to crimson colour indicated the presence of flavonoids.

Detection of anthraquinones:

50 mg of extract was dissolved in distilled water. 1 ml dilute ammonia solution was added to 2 ml of extract and shaken vigorously. The development of pink colour in ammonia layer indicated the presence of anthraquinones.

Detection of cardiac glycosides:

Killer kiliani test - 50 mg of the extract was dissolved in distilled water and filtered. Then 1 ml of glacial acetic acid and a drop of Ferric chloride and a drop of concentrated sulfuric acid were added to 2 ml of filtrate. The development of green blue colour to the upper layer and reddish-brown colour at the junction of the two layers indicated the presence of cardiac glycosides.

Detection of fixed oils and fat:

Spot test- A small aliquot of the extract was pressed between two filter papers. The development of oil stains on the paper indicated the presence of fixed oils.[7][8]

Antimicrobial activity:

Test bacterial strains:

In this study, the test microorganisms used for antibacterial sensitivity testing included two Gram-positive bacteria *Staphylococcus aureus* and *Bacillus cereus*, and two Gram-negative bacteria *Escherichia coli*, and *Pseudomonas aeruginosa*. Pure cultures were obtained from Dr. Vaishampayan Memorial Govt. Medical College, Solapur. All strains were maintained in Nutrient agar media at 4°C and activated on Mueller Hinton Agar plates 24 hr prior to any antimicrobial test. The bacterial strains grown in Mueller Hinton Broth (MHB) for 24 hr prior were used for antibacterial assays.

Antibacterial activity of the extracts:

The antibacterial activity of the extract was determined by the agar diffusion method. For this, the fresh culture of *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were suspended in sterile saline to get turbidity of 0.5 McFarland standards. 0.1 ml amount of this suspension was spread aseptically on a sterile Muller Hinton agar medium [Hi media]. Then wells [6 mm diameter] were bored by a sterile cork borer. 0.2 ml amount of each extract [100 mg /ml in 10% DMSO] was added to the wells. It was allowed to diffuse by keeping the plates in freeze for 20 min. 10 % DMSO in one of the wells served as a negative control. Antibiotic, Gentamycin [300mcg, Hi Media] disc was used as a standard positive control. After diffusion of extracts, the plates were incubated at 37 °C for 24 hrs. The diameter of the zone of inhibition was then measured in mm.[9]

Soap preparation:

The soap base was purchased from the local market in Solapur. About 100g of soap base was taken and cut into pieces and melted in a water bath until it turns into a liquid base. To it, 20 ml aqueous extract of *Nyctanthes Arbortristis* was added meanwhile in another 100 g of soap base about 20 ml of aqueous extract of *Murraya koenigii* was added. A few drops of rose oil were also added to both soap preparations. The mixture was poured into a cast and allowed to dry.[10]

Formulation of herbal soap:

Table 1: Formulation of herbal soap.

Ingredients	Concentration
Soap base	100gm
Herbal extract	20%

Steric acid	0.1%
Rose oil	Few drops

Characterization of herbal soap (Evaluation of soap):

Physicochemical evaluation of herbal soap (Organoleptic evaluation):

Organoleptic parameters like colour, odour, and texture were evaluated manually or physically.

Determination of pH

2gm of the finished soap was dissolved in 10ml of distilled water and stirred till the sample dissolved. The pH was determined using a pH meter or pH paper.

Irritation of the skin test:

A patch test of the skin was performed. For this small aliquot of prepared soap was applied to the skin and rubbed for 5 minutes. After 5 minutes it is washed off and observed for signs of irritation, and rashes.

Washing capability:

The herbal soap was put through a formulation test, as well as the simplicity with which it could be washed with water.

Foamability:

Approximately 1.0 gm of herbal soap was taken and dissolved in distilled water (about 50 ml) in a 100 ml graduated measuring cylinder to determine the soap's ability to produce foam. It was shaken for roughly 10 minutes in the measuring cycle. After 10 minutes, the foam height was measured. The mean was calculated after recording the observations for five consecutive experiments.

Foam retention time:

Foam retention time relates to the amount of time that the soap's foam lasts. The foam internal was measured for around 5–10 minutes after repeating the aforesaid process.

Moisture content:

The moisture content was used to measure the percentage of water in the soap by drying it to a consistent weight. Before being dried in a dryer at temperatures ranging from 100 to 1150 degrees Celsius, the soap was weighed and recorded as the "wet weight of the sample." The

sample was refrigerated and weighed to determine the "dry weight of the sample." Moisture content = (22% Initial weight – Final weight / Final weight 100).[11]

Antibacterial activity of prepared soap:

The antibacterial activity of the prepared soaps was determined by the agar diffusion method. For this, the fresh culture of *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa* were suspended in sterile saline to get turbidity of 0.5 McFarland standards. 0.1 ml amount of this suspension was spread aseptically on a sterile Muller Hinton agar medium [Hi media]. Then wells [6 mm diameter] were bored by a sterile cork borer. 0.2 ml amount of soap was added to the wells. It was allowed to diffuse by keeping the plates in freeze for 20 min. After diffusion of extracts, the plates were incubated at 37 °C for 24 hrs. The diameter of the zone of inhibition was then measured in mm.

Stability testing:

Initially, the sample formulation was taken and stored at 37^0 C ambient temperature for 5 months. After 5 months the preliminary stability studies were evaluated for organoleptic characters, pH, and antibacterial activity of prepared soap by agar well diffusion method.[12]

RESULTS AND DISCUSSION

Physical characteristics of leaves extract of *Nyctanthes Arbortristis* and *Murraya koenigii*:

The physical characteristics of the leaf extract of *Nyctanthes arbortristis* and *Murraya koenigii* are mentioned in Table 2. The methanolic extract of both the leaves are dark in colour with aromatic odour, while the consistency of *Murraya koenigii* was Solid oily whereas the consistency of *Nyctanthes arbortristis* was Solid sticky.

Table 2: Physical characteristics of leaf extract.

Name of plant extract	Solvent used	Physical characteristics		
		Colour	Consistency	Odour
Nyctanthes arbortristis	Methanol	Dark green	Solid sticky	Aromatic
Murraya koenigii	Methanol	Dark green	Solid oily	Aromatic

Preliminary phytochemical analysis of methanolic extract of leaves:

The results of preliminary phytochemical analysis of two plant species i.e., *Nyctanthes arbortristis* & *Murraya koenigii* are mentioned in Table 3. The comparative analysis of phytochemicals in *Nyctanthes arbortristis* & *Murraya koenigii* reveals some similarities and

differences in their chemical composition. Both the plants share the presence of alkaloids, carbohydrates, tannin, cardiac glycosides, fatty acid and oil which contribute to their medicinal potential.

Table 3: Preliminary phytochemical analysis.

Sr. No.	Phytochemicals	*Nyctanthes arbortristis*	*Murraya koenigii*
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Saponin	-	-
4	Protein	-	-
5	Amino acids	-	-
6	Anthraquinones	-	-
7	Tannin	+	+
8	Flavonoids	-	-
9	Fatty acid and oil	+	+
10	Cardiac glycosides	+	+

Note: + indicates presence of phytochemicals, - Indicates absence of phytochemicals

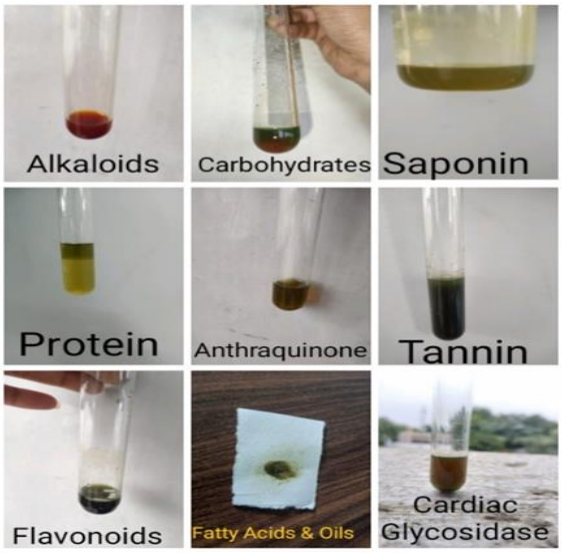


Fig. 3: Phytochemical Test for *Nyctanthes arbortristis*

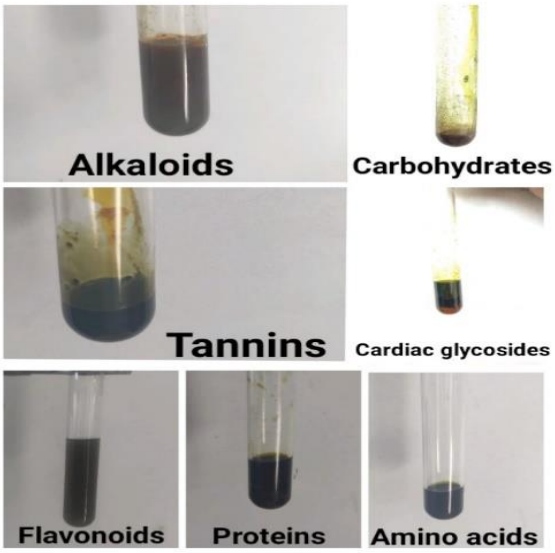


Fig. 4: Phytochemical Test for *Murraya koenigii*

Antibacterial activity of *Nyctanthes arbortristis* & *Murraya koenigii* leaves extract:

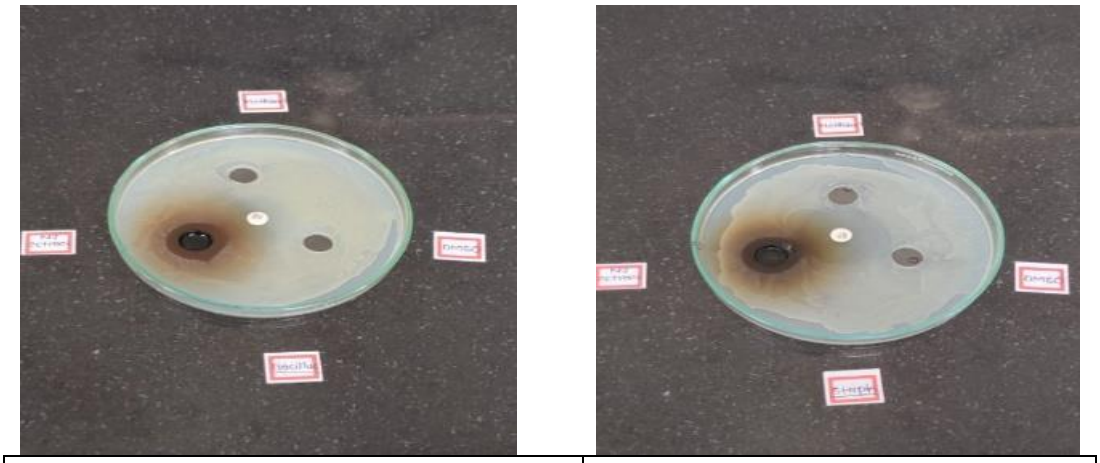
The results of antibacterial activity of *Nyctanthes arbortristis* and *Murraya koenigii* against selected bacterial strains are shown in table 4 and 5. In the present study both the plant extracts effectively inhibited all bacteria tested. Among this *Nyctanthes arbortristis* leaf extract achieved maximum zone of inhibition than *Murraya koenigii*. The differences in antibacterial activity of leaf extracts might be due to the phytochemical components present in the leaf extract.

The results showed that different bacterial species exhibit different sensitivities towards the leaf extract. The maximum activity was exhibited by *Staphylococcus aureus* for both the plant extracts with highest zone of inhibition of 21mm and 32 mm respectively. The antibacterial activity of both the extracts was compared with the standard drug Gentamycin. The results clearly showed that both extracts have shown antibacterial activity equivalent to that of standard antibiotic tested.

Table 4: Antibacterial activity of *Nyctanthes arbortristis* leaf extract.

Sr. No.	Name of organism	Solvent used	Diameter of zone in mm
1	*Staphylococcus aureus*	DMSO Methanol Gentamycin Plant extract	-- 15 30 21
2	*Bacillus* species	DMSO Methanol Gentamycin Plant extract	-- 15 22 26

Note: -- , indicates no zone of inhibition was found



Bacillus* against *Nyctanthes arbortristis | ***Staphylococcus* against *Nyctanthes arbortristis***

Fig. 5: Antibacterial activity of *Nyctanthes arbortristis.*

Table 5: Antimicrobial activity of *Murraya koenigii* leaf extract.

Sr. No	Name of organism	Solvent used	Diameter of zone in mm
1	*Staphylococcus aureus*	DMSO Methanol Gentamycin Plant extract	- 15 27 32

2	*Bacillus* spp.	DMSO Methanol Gentamycin Plant extract	- 15 30 25
3	*E. coli*	DMSO Methanol Gentamycin Plant extract	- 15 29 28
4	*Pseudomonas aeruginosa*	DMSO Methanol Gentamycin Plant extract	- 18 29 30

Note: -, indicates no zone of inhibition was found

Staphylococcus* against *Murraya koenigii	***Pseudomonas* against *Murraya koenigii***	***E. Coli* against *Murraya koenigii***

Fig. 6: Antibacterial activity of *Murraya koenigii.*

Physical properties of herbal Soap:

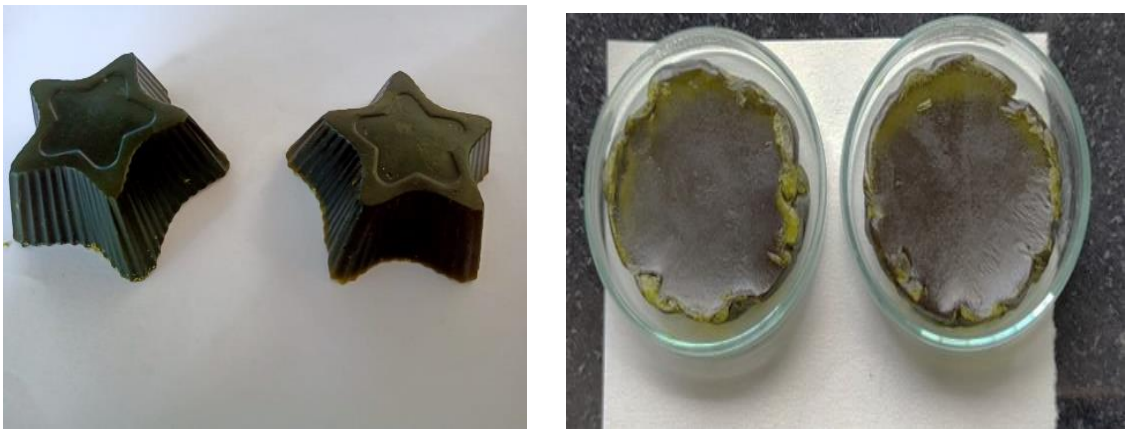
Physical properties of herbal soap can vary depending on the specific recipe and manufacturing process used, here are some common physical properties of both herbal soaps like colour, odour, appearance, irritability, and pH which are referred to in Table 6.

Colour is in green and deep green for *Nyctanthes arbortristis* and *Murraya koenigii* herbal soaps respectively with a pleasant aromatic odour and solid shape, pH is measured with pH paper which is 7 and no irritability is experienced.

Table 6: Physical properties of herbal soap.

Physical parameters	**Herbal Soap**	
	Nyctanthes arbortristis	***Murraya koenigii***
Colour	Green	Dark green
Odour	Aromatic	Aromatic

Appearance	Solid	Solid
Irritability	No irritation	No irritation
pH	7	7



***Nyctanthes arbortristis* soap** ***Murraya koenigii* soap**

Fig. 7: Herbal soap.

Herbal Soap evaluation for various parameters:

Prepared herbal soap was evaluated for various parameters. Both the herbal soap prepared from *Nyctanthes arbortristis* and *Murraya koenigii* has solid consistency and both soaps are washable. The moisture content for *Nyctanthes arbortristis* soap is more than *Murraya koenigii* soap i.e., 4.9% for *Nyctanthes arbortristis* and for *Murraya koenigii* it is 4.7%. The foam height measure was 50mm for *Nyctanthes arbortristis* soap with 210min of retention time & for *Murraya koenigii* soap it was 40mm with 150min of foam retention time. Both the soaps were without any particulate matter.

A few volunteers were selected for the consumer acceptance test and the feedback obtained was very satisfying as the soap has acceptable fragrance, appearance, its foam and without any skin irritation.

Table 7: Herbal Soap evaluation for various parameters.

	Nyctanthes arbortristis	***Murraya koenigii***
Consistency	Solid	Solid
Washability	Washable	Washable
Moisture content (%)	4.9%	4.7%
Foam Height	50mm	40mm
Foam Retention Time	210min	150min
Grittiness	Clear	Clear
Consumer Acceptance test	Acceptable fragrance, appearance, foaming, no skin irritation	

Antibacterial activity of *Nyctanthes arbortristis* and *Murraya koenigii* herbal soap:

The antibacterial activity of soap base and herbal soap made from *Nyctanthes arbortristis* and *Murraya koenigii* was evaluated against *Staphylococcus aureus*, *Bacillus* species, and *Pseudomonas aeruginosa* and *E. coli* using the agar well diffusion method. These are mentioned in Table 8 and Table 9 for *Nyctanthes arbortristis* and *Murraya koenigii* respectively. The results showed that both the herbal soap of *Nyctanthes arbortristis* and *Murraya koenigii* exhibited significant antibacterial activity against all the tested bacteria. The zone of inhibition (diameter of the clear area around the well) was measured to assess the antibacterial activity, and the following results were obtained.

***Staphylococcus aureus*:** Herbal soap of *Nyctanthes arbortristis* showed a zone of inhibition of 23mm, while the soap base showed a zone of 15 mm. Herbal soap of *Murraya koenigii* showed a zone of inhibition of 17 mm, and soap base, the zone observed was of 15mm.

***Bacillus* species:** The herbal soap of *Nyctanthes arbortristis* showed a zone of inhibition of 27mm, while the soap base showed a zone of inhibition of 20mm. Herbal soap of *Murraya koenigii* showed a zone of inhibition of 21mm and for the soap base, it was 20 mm.

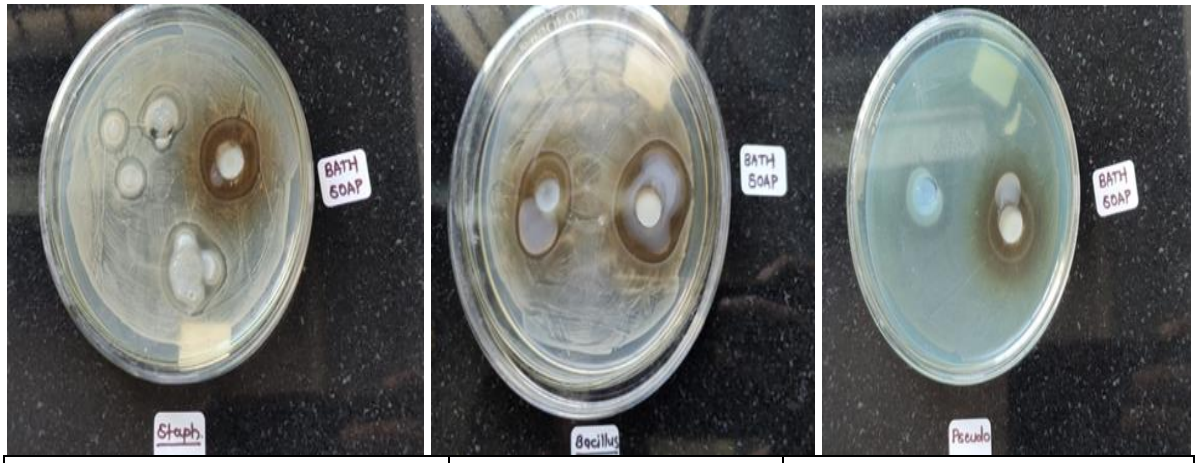
***Pseudomonas aeruginosa*:** The herbal soap of *Nyctanthes arbortristis* showed a zone of inhibition of 23mm, while the soap base showed a zone of inhibition of 16mm. Herbal soap of *Murraya koenigii* showed a zone of inhibition of 20mm and for the soap base, it was 16mm.

***E. coli*:** Herbal soap of *Murraya koenigii* showed a zone of inhibition of 17 mm and it remained the same for the soap base also.

These results suggest that *Nyctanthes arbortristis* and *Murraya koenigii* herbal soap have potential antibacterial activity against *Staphylococcus aureus*, *Bacillus* species, *Pseudomonas aeruginosa* and *E. coli* which are common pathogenic bacteria that can cause a range of infections in humans.

Table 8: Antibacterial activity of *Nyctanthes arbortristis* herbal soap.

Sr. No.	Name of Organism	Zone of inhibition in mm	
		Soap base	Herbal soap
1	*Staphylococcus aureus*	15	23
2	*Bacillus species*	20	27
3	*Pseudomonas aeruginosa*	16	23



Against Staphylococcus aureus	*Against Bacillus*	*Against Pseudomonas aeruginosa*

Fig. 8: Antibacterial activity of *Nyctanthes arbortristis* soap.

Table 9: Antibacterial activity of *Murraya koenigii* herbal soap.

Sr. No.	Name of organism	Zone of inhibition in mm	
		Soap base	Herbal soap
1	*Staphylococcus aureus*	15	17
2	*Bacillus species*	20	21
3	*Pseudomonas aeruginosa*	17	20
4	*E. Coli*	17	17

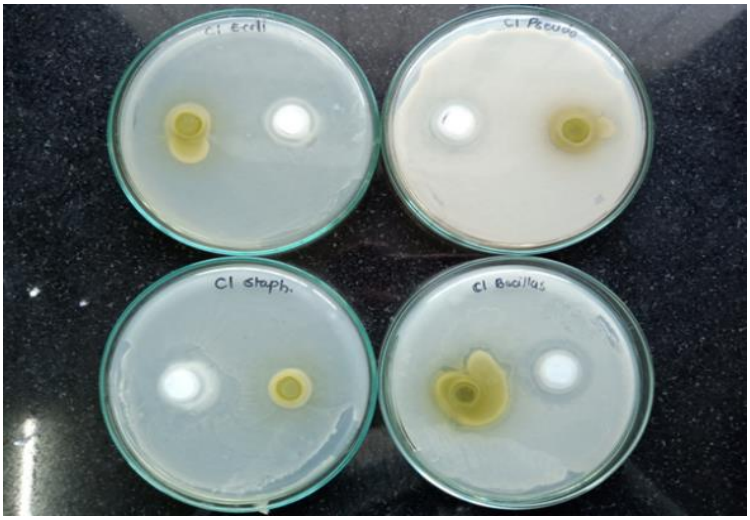


Fig. 9: Antibacterial activity of *Murraya koenigii* soap.

Stability testing of *Nyctanthes arbortristis* herbal soap:

The *Nyctanthes arbortristis* soap was kept at 37^0 C for a duration of 5 months to check its stability. After a duration of 5 months, the soap was evaluated for various tests like pH, appearance, fragrance, irritability, and antibacterial activity. The results obtained were satisfactory as no major changes were observed. The pH was still 7 with good fragrance. The

irritability results were negative and the antibacterial activity was still the same even after the 5 months.

Table 10: Stability tests.

Tests	*Nyctanthes arbortristis* soap
pH	7
Appearance	Solid
Fragrance	Presence of fragrance
Irritability	No irritation

Table 11: Antibacterial activity.

Sr. No.	Name of Organism	Zone of inhibition in mm
1	*Staphylococcus aureus*	23
2	*Bacillus species*	26
3	*Pseudomonas aeruginosa*	23

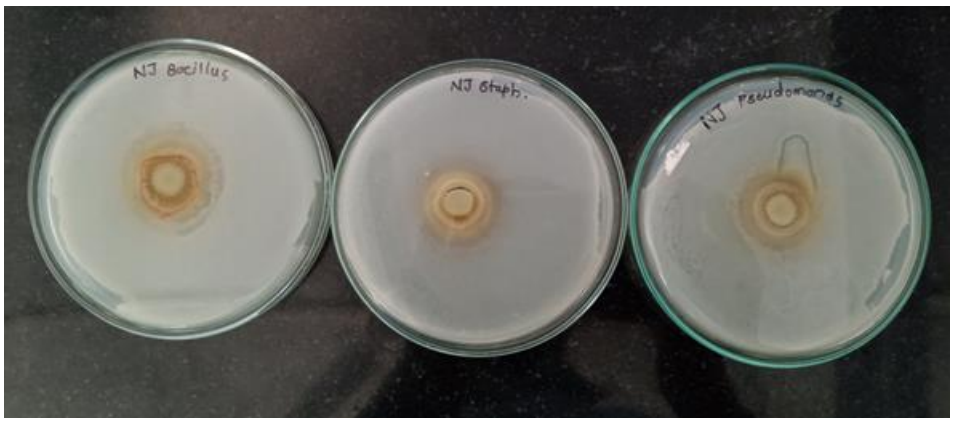


Fig. 10: Antibacterial activity of *Nyctanthes arbortristis* soap.

CONCLUSION

In conclusion, the research work successfully demonstrated the potential of *Nyctanthes arbortristis* and *Murraya koenigii* leaf extracts for developing a herbal soap with potent antibacterial properties. The phytochemical analysis confirmed the presence of bioactive compounds in the plant extracts, which likely contributed to the observed antibacterial activity. The formulated herbal soap showed promising antibacterial efficacy, validating the use of these plant extracts in herbal soap preparation. Moreover, the stability testing results provided assurance regarding the shelf-life and quality of the *Nyctanthes arbortristis* soap

over the specified period. Overall, this study opens up new possibilities for utilizing these plant extracts in natural product development and offers potential benefits in the field of herbal skincare and personal hygiene products.

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