



Formulation and Evaluation of an Antibacterial Herbal Soap from the Fruit Extract of *Aegle marmelos*.

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ABSTRACT

In last five decades, plants have been extensively studied by advanced scientific techniques and are reported for various medicinal properties viz, antibacterial activity, antifungal activity, antidiabetic activity, antioxidant activity etc. Over the last 20 years a large number of plant species have been evaluated for the antibacterial activity. The present study is an attempt to formulate an antibacterial herbal soap from the fruit extract of *Aegle marmelos* by melt and pour method. The dried and processed fruit powder was initially standardized by studying the physicochemical parameters like ash value, alcohol and water-soluble extractive value, foreign organic matter etc. The powder was later extracted with ethanol and the extract was evaluated for the phytochemical constituents. The antibacterial activity of the prepared formulation was tested using agar cup plate method against the organism *Bacillus subtilis* (Gram positive) and *Escherichia coli* (Gram negative). The prepared herbal formulation exhibited a good antibacterial effect. The prepared soap was evaluated for various physicochemical parameters such as pH, foam retention time, foam height etc. for which good results were observed. The easy availability of plant and its antibacterial effect helps manufacturers with cost-effective benefits, easy availability and with minimum or no side effects. The results suggest that formulated herbal soap is suitable for human skin and can be useful to treat skin problems.

Keywords: *Aegle marmelos*, herbal, soap, formulation, evaluation, agar

INTRODUCTION:

India is widely known as the botanical garden of the world since it is the largest producer of medicinal herbs. Medicinal plants act as an indigenous source of new compounds possessing therapeutic value and can also be used in drug development. 80% of the population of developing countries depend on traditional medicines, mostly natural plant products, for their primary health care needs as estimated by WHO. Because of the growing recognition of natural products, the demand for medicinal plants has been increasing all over the world. They have minimal toxicity, are cost effective and pharmacologically active, and provide an easy remedy for many human ailments as compared to the synthetic drugs which are a subject of adulteration and side effects. The alarming increase in the rate of infection by antibiotic-resistant microorganisms has urged scientists to search for compounds which have potential Antibacterial activity. The ability to synthesize compounds by secondary metabolism possessing antibacterial potential makes plants an invaluable source of pharmaceutical and therapeutic products. The effectiveness of plant extracts on microorganism has been studied worldwide (Aberouman A. et. al., 2011).

In last five decades, plants have been extensively studied by advanced scientific techniques and are reported for various medicinal properties viz, anticancer activity, antibacterial activity, antifungal activity, antidiabetic activity, antioxidant activity, hepatoprotective activity, haemolytic activity, larvicidal activity and anti-inflammatory activity etc. (Craig, W. J. et. al., 1999).

Herbal soap is a natural alternative to conventional soap that is often made using botanical herbs and plant-based ingredients. The rising popularity of herbal soap can be attributed to its skin-friendly and environmentally conscious characteristics. By being devoid of harsh chemicals and artificial fragrances, these soaps are particularly well-suited for individuals with sensitive skin. The use of herbs in herbal soap provides various benefits, such as soothing and healing the skin, providing a natural fragrance, and reducing stress and anxiety through aromatherapy. The natural components found in herbal soap possess antibacterial, antifungal, and anti-inflammatory properties, which make them effective in addressing various skin conditions like acne, eczema and psoriasis. Every



herb possesses unique properties which is beneficial for the skin and overall health. With the growing concern over synthetic and chemical-laden products, herbal soap is gaining more popularity as a safe and eco-friendly option for personal care.

Over the last 20 years a large number of plant species have been evaluated for the antibacterial activity. The present study is an attempt to formulate an herbal soap from the fruit extract of *Aegle marmelos* and evaluate it for the antibacterial activity (Mohan V. et. al.,2005).

Aegle marmelos Corr., belongs to the family Rutaceae and is popularly known as Bael tree. Hindu physicians regard the unripe or half ripe fruit as astringent, digestive and stomachic and prescribe it for diarrhoea and dysentery. The tribals in Salem, Dharmapuri, Vellore regions, Tamil Nadu, India offer leaves in the month of July/ August (Shravana) , to God to overcome sterility problem.

Bael (*Aegle marmelos*) has been known to be one of the most important medicinal plants of India since Charak (1500 B.C). More than 100 phytochemical compounds have been isolated from various parts of the plant, namely phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroids, and tannins. These compounds are well known to possess biological and pharmacological activity against various chronic diseases such as cancer and cardiovascular and gastrointestinal disorders. Antioxidant, antiulcer, antidiabetic, anticancer, anti-inflammatory, antibacterial, ant spermatogenic effects have also been reported on various animal models by the crude extracts of this plant. Every part of *Aegle marmelos* plant such as its fruits, stem, bark, and leaves possess medicinal property and is used for treating various eye and skin infections.

Plant Profile:

Bael tree is deciduous, and the crown is compact or dense, with no weeping branches. Sometimes the lower limbs are drooping.

- The tree is tough and widely adaptable to adverse soil and climatic conditions.
- The bael tree can grow up to 10 m or higher with medium or large sizes with numerous branches.
- The fruits mainly occur in the periphery of the canopy.
- The trunk is short and thick with narrow oval shape ends.
- The wood is rigid and slow-growing. The young wood has a central pith.
- Under natural habitats, the trees are smaller and irregular.
- The trees possess short, sturdy, nonspiny, or piercing-spiny branches.
- The straight shaped spines are 3 cm in length when fully grown and originate from the leaves' axis. The tree's bark is flaking, bluish-grey, soft, and contains irregular furrows on the younger branches.

Taxonomical Classification:

Kingdom : Plantae
Sub-kingdom : Tracheobionta
Super division : Spermatophyta.
Division : Magnoliophyta.
Class : Magnoliopsida.
Subclass : Rosidae
Order : Sapindales
Family : Rutaceae

Genus : *Aegle*

Species : *Aegle marmelos* (Kirtikar K.R. et. al., 1999)

Morphological Characteristics:

Fruit:

The fruit typically has a diameter of between 5 and 10 cm (2 and 4 in). It is globose or slightly pear-shaped with a thick, hard rind and does not split upon ripening. The woody shell is smooth and green, grey until it is fully ripe when it turns yellow. Inside are 8 to 15 or 20 sections filled with aromatic orange pulp, each section with 6 to 10 flattened-oblong seeds each about 1 cm long, bearing woolly hairs and each enclosed in a sac of adhesive, transparent mucilage that solidifies on drying. The exact number of seeds varies in different publications. The fruit takes about 11 months to ripen on the tree, reaching maturity in December. It can reach the size of a large grapefruit or pomelo, and some are even larger. The shell is so hard it must be cracked with a hammer or machete. The fibrous yellow pulp is very aromatic. It has been described as tasting of marmalade and smelling of roses. Boning (2006) indicates that the flavour is sweet, aromatic and pleasant, although tangy and slightly astringent in some varieties. It resembles a marmalade made, in part, with citrus and, in part, with tamarind. Numerous hairy seeds are encapsulated in a slimy mucilage.



Figure No. 01: Fruit of *Aegle marmelos*

METHODOLOGY:

Collection & Processing of Plant Material:

Fresh fruits of *Aegle marmelos* plant were collected from the Nanded district in the month of February. Various fruit shapes were observed viz., oval, spherical, oblong and pyriform. Many trees were observed with the fruits having breakage due to irregular patterns of watering. Some of the fruits were having yellow spots and black powder on the peel that may be due to some myopathies.

The fruits were washed thoroughly to remove the dirt in running tap water for 5 min and rinsed with sterile distilled water. The fruit sample was cut into pieces and dried under shade for about 10 days. The dried plant samples were ground well into fine powder in a mixer grinder and sieved to give particle size of 50-150mm.

Study of Physical Characteristics and Standardization of Fruit Powder:

Physical and microscopic characteristics of the powder were studied. Various standardization parameters were evaluated to obtain the quality and purity of the plant fruit, like ash value, acid-insoluble and water soluble as well as sulphated ash value, alcohol and water-soluble extractive value, determination of loss on drying and foreign organic matter etc. (Aliyu A. B. et. al., 2017). The parameters were evaluated as per the methods described in I.P. 1996. The fruit powder was stored in air sealed polythene bags at room temperature before extraction.

Preparation of Fruit Extract:

An extract is a mixture of phytochemicals from any plant which is obtained by extraction of specific parts of the plant. The processed plant material was mixed with 70% ethanol. 50g sample should be soaked in 500 ml of solvent. Total 400g of the fruit powder was macerated with the solvent. Mixtures were kept in the dark for 3 days at room temperature in a sterilized conical flask wrapped with aluminum foil to avoid evaporation and exposure to sunlight was avoided. After three days, slurry was filtered and filtrate was collected and poured in sterile petri-dishes. For few hours, solvent was allowed to evaporate and the dry extract so obtained was used for further testing (Deepan T et. al., 2012).



Figure No. 02: Extraction of Fruit Powder of *Aegle marmelos*

Phytochemical Evaluation of the Extract:

Fruit extract was screened for some secondary metabolites like-saponins, tannins, alkaloids, anthraquinones, phlobatannins, flavonoids, terpenoids, reducing sugar and poly phenols (Olayiwola O. A. et. al., 2013).

Test for Reducing Sugar:

Take 1ml or 1g of plant sample in a test tube and add 10ml deionized water then add few drops of Fehling solution (1ml Fehling solution A and B) and heat at 100°C in a water bath. Brick red precipitate shows a positive result.

Test for Tannins:

Take 2g of aqueous extract in a test tube and add 2 drops of 5% ferric chloride, brown color gives positive result.

Test for Phlobatannins:

Take 2ml plant sample in a test tube and add 10ml deionized water and boil at 100°C with few drops of 1% HCl. Deposition of red precipitation gives positive result.

Test for Saponins:

Saponin content is determined by boiling 1ml plant sample in 10 ml deionized water for 15 min. and after cooling the extract was shaken vigorously to record froth formation.

Test for Terpenoids:

Take 5ml of aqueous extract add 2ml chloroform followed by addition of 3ml conc. sulphuric acid, observe the reddish-brown interface for presence of terpenoids.

Test for Alkaloids:

Take 1ml of aqueous extract in test tubes and add 2-3 drops of Wagner's reagent it gives orange red precipitation.



Test for Flavonoids:

Add few drops of 1% ammonia solution, yellow colour observed, showed presence of flavonoids then after this take ethanolic or aqueous extract and add 10ml DMSO then heat it followed by adding Mg (magnesium chloride), add conc. HCl gives red color to confirmed flavonoids.

Test for Poly Phenols:

Take 2ml ethanolic extract of plant sample and add 1ml Folin-Ciocalteu reagent and 9ml of distilled water between 1-8 min. and add sodium carbonate solution (8ml), vortex to mix, then keep the test tube in dark. Take O.D at 760nm.

Total Phenolic Content:

This procedure was adapted from the Singleton-Rossi method of analysis for total phenols (Vernon L Singleton et al., 1999). Different tubes were prepared with increasing amount of gallic acid solution from 0.2 to 1 ml and distilled water was added to make the volume up to 2 ml. 0.125 ml of Folin-Ciocalteu's reagent was added to all the tubes and incubated for 6 min. 7% Na₂CO₃ (1.25 ml) solution was added to all the tubes and further diluted with deionized water (3 ml). After 90 min. incubation, absorbance of developed color was read at 760 nm A standard graph was made taking a concentration of gallic acid on the X-axis and absorbance of color formed on the Y-axis. From the graph, the total phenolic content was calculated as mg GAE/g of dried plant extract using the equation $T = C \cdot V/M$.

Total Flavonoids Content:

The colorimetric estimation using aluminum chloride was adapted from the procedure reported by Woisky and Salatino in 1998. The stock solution of Quercetin which was used as a standard antioxidant was prepared by dissolving it in 80% ethanol. Different increasing concentrations of this solution were obtained by diluting it with distilled water. Diluted standard solutions and 0.5 ml of each sample were added with 1.5 ml 95% ethanol, 0.1ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and finally 2.8 ml of distilled water. The reaction mixtures were kept for 30-minute incubation at room temperature and the absorbance of the color developed in the reaction mixtures was measured at 415 nm (Woisky, R.G. et. al., 1998). A standard graph was made taking a concentration of quercetin on the X-axis and absorbance of color formed on the Y-axis. The total content was calculated as quercetin equivalent (QE) in mg/g dried weight of the plant extracts using equation ($T = C \cdot V/M$).

Formulation of an Herbal Soap:

An herbal soap can be prepared by following three methods:

- Cold process method
- Hot process method
- Melt and pour method

Out of these methods, the melt and pour method was selected for preparing the herbal soap (R. Margret Chandira et. al., 2022).

Melt and Pour Method:

The melt and pour method is the quickest and easiest way to make soaps. Here, a pre-made melt and pour-soap base and other required ingredients are used for making a natural herbal soap. You do not have to use lye to prepare a soap base.

Requirements:

Natural shea butter pour-soap base, knife, bale fruit extract, burner, glass rod, water bath, beaker, tripod stand, rose water, soap mould.

Procedure:

- 50 g of shea butter soap base was cut into small pieces with the help of a knife.



- A tripod stand was taken, water bath was kept on it, water was added in it. Water was heated.
- After 5-6 min. the beaker was kept in water bath. Then, soap base pieces were added in it over a low flame. Stirring was done continuously with the help of a glass rod (Double Boiler Method).
- After the soap base was melted, 10 g of Bael fruit extract was added in it, keeping the flame of burner low and stirred continuously.
- After 2-3 min. the burner was switched off and the mixture was cooled down.
- A small quantity of rose water was added in to the solution and mixed.
- Finally, the mixture was poured into a soap mould and allowed to set for 24 hours in a cool and dry place.
- After 24 hours, the bar was taken out of the mould and was wrapped in a clean butter paper.

Formula: Following ingredients were used to prepare the herbal soap from the fruit extract:

Table No. 01: Formula for the Herbal Soap

Sr. No.	Ingredients	Quantity	Use
1.	Shea butter soap base	50g	Soap base
2.	Bale extract	10g	Antibacterial
3.	Rose water	q. s.	Fragrance

Evaluation of the Formulated Herbal Soap:

The herbal soap was evaluated for the following parameters (Safal Sharma et. al., 2022):

- **Organoleptic evaluation:** The formulated herbal soap was evaluated for the organoleptic characteristics like colour, odour, appearance etc.
- **Foam retention test:** 25 ml of the 1% soap solution was taken into a 100 ml of graduated measuring cylinder. The cylinder was covered with hand and shaken 10 times. The volume of foam at 1 minute interval for 4 minutes was recorded.
- **pH test:** The pH was determined by using pH paper.
- **Foam height:** Height of the foam produced was measured with a scale.
- **Antibacterial activity:**

Cup plate diffusion method:

- ✓ In a sterile agar petri dish, four holes were made with a sterile cork-borer.
- ✓ In the four holes created, 0.1ml of the phenol solution (Standard Disinfectant), soap solution, bael fruit ethanolic extract solution and 95% ethanol (commonly used disinfectant) were added respectively.
- ✓ The petri dish was inoculated with the bacterial suspension with the help of a sterile inoculating loop.
- ✓ The petri plate was incubated for 24 hours and the zone of inhibition around each hole was noted and diameter of each zone was measured (Dr. A Seetha Devi et. al., 2021).

Determination of Activity Index (AI):

Activity index of the soap solution, extract, phenol and ethanol was calculated using following formula:



$$\text{Activity index} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}}$$

Bacterial culture preparation:

The bacterial cultures of selected strains were maintained on sterile nutrient agar medium (agar 28g in 1000ml distilled water). These micro-organisms were allowed to grow at 35°C-37°C temperature. A fresh inoculum of test microorganism in saline solution was prepared from bacterial stock suspension.

Bacterial Strains:

Bacillus subtilis (Gram positive) and *Escherichia coli* (Gram negative) strains were employed for the test.

OBSERVATIONS AND RESULTS:

Morphological Characteristics of the Fruit:

The collected fruits of the *Aegle marmelos* showed the following morphological characteristics:

Table No. 02: Morphological characteristics of *Aegle marmelos* fruit

Sr. No.	Characteristics	Observation
1.	Size	8 to 9 cm in diameter
2.	Shape	Globose
3.	Colour	Greenish yellow
4.	Odour	Characteristic
5.	Taste	Sweet
6.	Texture	Smooth

Powder Characteristics of the Fruit:

The fruit powder was yellowish-brown in colour with sweet taste. The powder consists of epidermal cells, parenchyma, lysogenous oil cavities, prismatic crystals, sclereids, stone cells, spiral vessels, pitted vessel and seed hairs etc.



Figure No. 03: *Aegle marmelos* fruit powder



Standardization of the Fruit Powder:

Following results were obtained when the fruit powder was evaluated for the physicochemical parameters given below:

Table No. 03: Physicochemical properties of ethanolic extract of the fruit

Sr. No.	Standardization Parameter	Results
1.	% Foreign Organic Matter (w/w)	<2
2.	% Total Ash (w/w)	7.65
3.	% Acid Insoluble Ash (w/w)	0.26
4.	% Water Soluble Ash (w/w)	1.84
5.	Sulphated Ash Value (%)	0.984
6.	Moisture Content (w/w)	1.387
7.	% Extractive Values (w/w)	1.25
8.	Alcohol Soluble	24.76%
9.	Water Soluble	42.71%

All the physicochemical parameters of the dried fruit powder were found to be within standard limits assuring the quality and purity of the fruit.

Study of Physical Characteristics of the Ethanolic Extract:

The dried fruit extract possessed the following characteristics:

Table No. 04: Physical Characteristics of the Ethanolic Extract

Sr. No.	Characteristics	Observation
1.	Colour	Brown
2.	Odour	Characteristic
3.	Consistency	Sticky
4.	% Yield	05% w/w

Phytochemical Evaluation of the Ethanolic Extract:

The phytochemical study of the powder showed the following results:

Table No. 05: Phytochemical study of the Ethanolic Extract

Sr. No.	Phytochemicals	Ethanolic Extract
1.	Reducing sugar	+
2.	Tannins	+
3.	Phlobatannins	-
4.	Saponins	+
5.	Terpenoids	+
6.	Wagner's test for Alkaloids	+
7.	Flavonoids	+
8.	Polyphenols	+
9.	Total polyphenolic content	151.89 mg/g GAE
10.	Total flavonoid contents	81.16 mg/g RE

Physical Characteristics of the Herbal Soap:

The formulated herbal soap possessed following physical characteristics:

Table No. 06: Physical Characteristics of the Herbal Soap

r. No.	Characteristics	Observation
1.	Colour	Brownish Yellow
2.	Odour	Rose
3.	Appearance	Glossy
4.	Weight	60g

Evaluation of the Herbal Soap: The herbal soap prepared showed the following results after the evaluation:

Table No. 07: Evaluation of the Herbal Soap

Sr. No.	Parameter	Observation
1.	Foam retention	4.0 minutes
2.	Foam height	6.0cm
3.	pH	7.00

Antibacterial Study of the Soap: The antibacterial activity by cup plate method showed that phenol (Standard disinfectant) produced the wider zone of inhibition as compared to the soap solution, ethanolic extract and the ethanol (Commonly used disinfectant). The results also showed that the soap solution exhibited a clear and wider zone of inhibition and higher activity index than the ethanolic extract and ethanol confirming the significant antibacterial effect of the soap.



Figure No. 04: Zone of Inhibition of Standard & Samples

1: Standard (Phenol), 2: Soap Solution, 3: Ethanolic Extract, 4: Ethanol

Table No. 08: Antibacterial study of Standard & Samples

Sr. No.	Name of Sample	Bacterial Culture	Quantity of Sample	Zone of Inhibition (mm)	Activity Index
1.	Phenol	10ul	0.1ml	26	1.00
2.	Soap Solution	10ul	0.1ml (2mg/ml)	23	0.88
3.	Ethanolic extract	10ul	0.1ml	21	0.80
4.	Ethanol (95%)	10ul	0.1ml	18	0.69



CONCLUSION:

The dried fruit powder of plant *Aegle marmelos* was extracted using ethanol and evaluated for various phytochemical tests. The extract showed the presence of many phytoconstituents like alkaloids, flavonoids, tannins, saponins, terpenoids etc. The extract also showed presence of flavonoid and phenolic contents. An herbal soap of the extract was prepared satisfactorily by melt and pour method. The prepared soap gave good results when evaluated for the parameters like physical appearance, foam retention, pH, foam height. The soap also showed the antibacterial activity against Gram positive and Gram negative bacteria.

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