

# Formulation of Herbal Soap against Acne Causing Bacteria

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Submission Date: 07-10-2021; Revision Date: 15-11-2021; Accepted Date: 03-12-2021

## ABSTRACT

The use of herbs on skin disorders has been done for thousands of years. Herbal remedies, including those for many kind of disorder especially skin disorders, are currently gaining popularity among patients. In Asia, especially in south east Asian countries, herbal treatments that have been used from ages are now being studied scientifically. In India, records of Ayurvedic medicine, a method of medicine with natural roots date back to about 3000 BC. The system of Ayurvedic medicine combines physiological and holistic principles. *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Staphylococcus aureus* in the follicular canal and sebum production are the reason for leading to Acne vulgaris. Inflammation in acne is caused by *Staphylococcus aureus*. In the present study, aqueous extract of herbs like Guava leaves, Turmeric, Aloe Vera, Soap nuts and Rose petals were used to formulate a herbal soap to combat acne. The preliminary phytochemical analysis of the aqueous extract of these herbs showed the presence of various secondary metabolites such as saponins, phenols, tannins, terpenoids, glycosides and quinones. *In vitro* antibacterial activity was performed against *Staphylococcus aureus* and *E. coli* using agar well diffusion method. The zone of inhibition with guava leaves was observed to be 17mm, while 11 mm was observed with turmeric for *Staphylococcus aureus*. While with *E. coli*, Guava leaves showed a zone size of 9mm and no zones were observed with Turmeric and Aloe vera extracts. Although, Aloe vera is known for its antibacterial activity, in our study Aloe vera did not exhibit any antibacterial activity.

**Key words:** Herbal soaps, Antibacterial activity, *S. aureus*, Guava leaves, Turmeric.

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## INTRODUCTION

The major organ in the body is the human skin, which is the outer layer of the body which is the first line of defense. Skin contains many specialized cells and structures. Acne vulgaris also known as acne is a chronic skin disease involving blockage or inflammation in the pilosebaceous gland that occurs when hair follicles are clogged with dead skin cells and oil. Worldwide, this has been the most common dermatological conditions affecting approximately about 650 million people. These

excess oil and dead sticky cells become trapped in the pore, creating a blockage called comedo (blackhead). If the follicle ruptures, the comedo might turn into a more severe outbreak. Acne vulgaris has been associated with *Staphylococcus aureus* and *Propionibacterium acnes*. Substantial *in vitro* research shows that *S. aureus* may have a pathophysiological involvement in acne vulgaris. In other research, both *S. epidermidis* and *P. acnes* were identified as bacteria likely to cause acne vulgaris.<sup>[1]</sup>

Natural plant-based medications are increasing in prominence due to a number of benefits, including minimal adverse reactions improved patient endurance, lower cost, and acceptance owing to a historical use. Aside from providing reasonable means for the treatment of many diseases that are intractable and incurable in other systems of medicine, herbal remedies give a reasonable method for the cure of several ailments which are difficult and incurable in other medicinal systems. As

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DOI: 10.5530/ajbls.2021.10.80

a result, a variety of herbs are being studied for the therapy of skin conditions varying from itchiness to skin cancer.

## METHODOLOGY

### Sample collection

Herbal formulation was made using the following herbs like Guava leaves, Turmeric, Aloe Vera, Soap nuts and Rose petals.

### Preparation of Guava leaves Extract

1 g of dried guava leaf was extracted with 50 ml of boiling water at 100°C for 20 min. The obtained guava leaf extract was filtered using a vacuum pump with Whatman filter paper and evaporated using rotary evaporator.<sup>[2]</sup>

### Preparation of Turmeric Extract

12 g of turmeric was dissolved in 60 ml of distilled water and was kept in rotary shaker for about 10 hr and 30 min. This extract was concentrated using rotary evaporator and the extract was stored at 4°C in a refrigerator.<sup>[3]</sup>

### Preparation of Aloe Vera Extract

The Aloe Vera leaves were freshly collected and were cleaned individually by distilled water. The spikes and the margins were removed before slicing the leaves and the cortex was carefully separated from the parenchyma using knife. Filets were washed thoroughly with distilled water to remove the exudate from the surfaces. The fresh filets were then stored in a refrigerator.

### Preparation of Soap Nut Extract

About 50 g of soap nut powder was taken and was boiled with 200 ml of distilled water with continuous stirring and was filtered. The filtrate was then placed in the hot air oven at 60°C for drying and was used for further analysis.<sup>[4]</sup>

### Soap making using soap nuts

Whole soap nuts were taken and were crushed. Required amount of water was added and was boiled with continuous stirring for 30 min. The extract was allowed to cool and was strained off and stored in a bottle. The extract appeared light brown in colour due to the presence of saponins.

### Preparation of Rose water Extract

The rose petals were freshly picked and they were boiled in distilled water. It was boiled until the petals have lost their entire colour and the water has gained pink colour. The water was strained and stored in a container.

### Soap making using Rose petals

The Rose flowers were freshly picked from the flower bed. Fresh rose petals were detached, spread in a thin layer and were shade dried for four to five days at the room temperature (25°- 35°C). The dried material was crushed and ground and stored in a tightly closed container.

### Qualitative Phytochemical analysis

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in the aqueous extracts of Guava leaves, Turmeric, Aloe Vera, Soap nuts and Rose petals as per the standard methods.<sup>[5]</sup>

### Antibacterial activity

The extracts were freshly prepared in sterile distilled water to a final concentration of 100 µg/ml respectively for agar disc diffusion test.<sup>[6]</sup> Isolated bacterial species of *Staphylococcus aureus* and *E. coli* were obtained from the Department of Microbiology, JBAS College for Women, Chennai. The antibacterial activity of the extracts of guava leaves, turmeric and *Aloe vera* was carried out using the agar disc diffusion method.

### Preparation of Inoculum

Cultures for experiments was prepared by transforming a loopful of bacterial cell from the stock cultures to test tube containing Muller-Hinton broth and was incubated for 2-6 hr at 37°C to match the turbidity with 0.5 Mac Farland standard.

### Agar Well Diffusion Method

Antimicrobial activity was evaluated by using agar well diffusion method. The inoculum was spread evenly on the Muller-Hinton agar's (MHA) surface using a sterile cotton swab and was allowed to dry for 10-15 min. Wells of 6mm were cut with the help of sterile well cutter. 100µl of different samples were added in the cut wells of MHA and the plate was incubated at 37°C for 24hr. After incubation, a clear zone around the well determines the antibacterial activity of the herbs. Diameter of the zones of inhibition was measured in millimeters.

### Double Boiling Method for Soap formulation

The double boiler was placed on the hot plate. The soap bases were cut into cubes, and were filled up to 75% volume in container. 1-2% of water was added to compensate the soap base. The top was covered and allowed to melt with stirring lightly in between. The melting temperature was around 75-80°C. Once the base completely melted, different extracts were added

which included- 10gm of Aloe Vera gel, 20 gm of Guava leaf extract, 10ml of Soap nut extract and 5gm of Rose pellet extract. It was then poured into a mould. It was allowed to cool at room temperature and then demoulded.

## RESULTS

### Qualitative Phytochemical analysis

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in the aqueous extracts of Guava leaves, Turmeric, Aloe Vera, Soap nuts and Rose petals as per the standard methods.

S. No.	Secondary metabolite	Test	Guava Leaves	Turmeric	Alovera	Rose Petals	Soapnut
1	Phenols and tannins	Ferric chloride test	+	-	+	+	+
2	Alkaloids	Wagner's test	++	+	-	-	+
3	Saponins	Foam test	+	-	+	+	++
4	Flavonoids	Sodium hydroxide test	+	+	+	+	-
5	Terpenoids	Salkowski test	+	+	+	+	+
6	Cardiac glycoside	Keller killani test	+	+	+	+	+
7	Test for quinones	Action of ammonia	+	+	+	+	+
8	Test for proteins	Biuret test	+	+	+	-	+
9	Test for steroids	Libermann Burchard test	-	-	+	-	-

The secondary metabolites were found in abundance in Guava leaves which showed the presence of phenols, tannins, alkaloids, saponins, flavonoids, terpenoids, cardiac glycoside, quinones and proteins. This supported the antibacterial activity of Guava leaves when compared to other herbs used in this study. Other herbs also showed considerable amounts of secondary metabolites when tested.

### Antibacterial Activity

Antimicrobial activity of any substance is defined as its ability to either kill bacteria or inhibit the growth of bacteria. Antimicrobial activity is significant with

respect to the human body in preventing diseases and skin infections. The antibacterial activity of the aqueous extract of components namely guava leaves, turmeric and aloe vera were compared using agar well diffusion method against *Staphylococcus aureus* and *E. coli*. The zone of inhibition with guava leaves was observed to be 17mm, while 11mm was observed with turmeric for *Staphylococcus aureus*. While with *E. coli*, Guava leaves showed a zone size of 9mm and no zones were observed with Turmeric and Aloe Vera extracts. Although, Aloe Vera is known for its antibacterial activity, in our study Aloe Vera did not exhibit any antibacterial activity (Table 1).

The results of this study indicate that the aqueous extract of two different components namely guava leaves and turmeric possesses anti-bacterial activity. The turmeric showed less inhibition when compared to guava leaves.

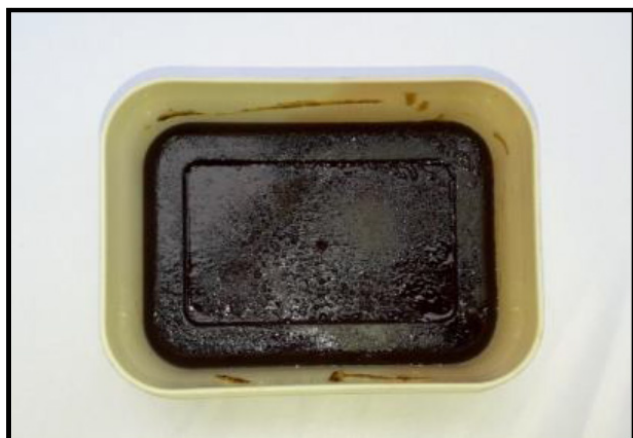
### Formulation of Soap

Soaps act as emulsifiers or surfactants, softening the horny layer of the epidermis and acts as a germicide by enhancing the permeability of microbial envelope thereby disrupting the integrity of microbial cells. Antimicrobial activity of soaps makes them useful agent for bathing, laundry, washing, and cleansing of surfaces.<sup>[7,8]</sup> The cleansing and germicidal properties of the soapy-plants are comparable to those of the standard soaps, which are salts of higher fatty acids. Crude preparations of soapy plants are able to soften the skin epidermis, enhance greater penetration and cleansing of sores and acne and thereby promote rapid healing and resolution of blemishes.

**Table 1: Antibacterial activity of the herbs.**

Herbs	<i>Staphylococcus aureus</i>	<i>E.coli</i>
Guava leaves	17mm	9mm
Turmeric	11mm	No zone
Aloe vera	No zone	No zone





## DISCUSSION

Acne is a common skin condition, caused by changes in the pilosebaceous units, skin structures consisting of hair follicle and its associated sebaceous glands via androgen stimulation. Basically, acne is a disease whose initial pathology is the microscopic microcomedo. The microcomedo may involve into visible open comedones (Blackheads) or closed comedones (Whiteheads). Inflammatory papules, pustules and nodules may develop in due course of time.<sup>[9]</sup> The severity of acne varies greatly among individuals and a genetic background plays an important role. It is estimated that as many as 90% of all teenagers are affected by acne, with virtually 100% of teenagers experiencing some degree of comedones formation.<sup>[10]</sup>

Herbs and their products have been highly esteemed source of medicine throughout the human history. Herbs are widely used today indicating that these are a growing part of modern high-tech medicine. Some of the herbs and herbal treatment which have their role in the management of acne and other skin related disorders are compiled here. Number of herbs with a history of use in traditional cultures has entered the growing cosmeceutical market. An herbal formulation which contains many herbal extracts and have negligible adverse effects compared with modern medicine, are commonly indicated for moderate and severe forms of acne. Some of the herbs that possess promising role against *Acne vulgaris* includes: *Tephrosia purpurea*, *Saw palmetto*, *Rubia cordifolia*, *Viola tricolor*, *Aloe vera*, *Asparagus officinalis*, *Calendula officinalis*, *Betula alba*, *Simmondsia chinensis*, *Vitex agnus-castus*, *Coriandrum sativum*, *Lavandula angustifolia*, *Citrus aurantium*, *Citrus limon*, *Rheum officinale*, *Urtica dioica*, *Curcuma longa*, *Saponaria officinalis*, *Juglans nigra*, *Azadirachta indica*, *Melaleuca alternifolia*, *Populus candicans*, *Arctium lappa*, *Amaranthus hypochondriacus*, *Allium sativum*.<sup>[11]</sup>

In the present study, herbs like Guava leaves, Turmeric, Aloe Vera, Soap nuts and Rose petals were used to formulate herbal soap to combat acne. The preliminary phytochemical analysis of the aqueous extract of the Guava leaves, Turmeric, Aloe vera, Rose and Soap nut revealed the presence of various phytochemicals such as saponins, phenols, tannins, terpenoids, glycosides and quinones which was found to be in agreement with Kokate *et al.*, 2003<sup>[12]</sup> and Harborne, 1998.<sup>[13]</sup>

The family Myrtaceae includes the genus *Psidium*, which is considered to have originated in tropical South America. The leaves of Guava are 2 to 6 inches long and 1 to 2 inches wide, appear dull-green with stiff but coriaceous with pronounced veins and aromatic when crushed.<sup>[14]</sup> Guava leaf has bioactive components which can fight against pathogens, manage blood glucose levels, and can also help in weight loss. The leaves of Guava leaves contain essential oils which are rich in cineol, tannins, triterpenes, flavonoids, resin, eugenol, malic acid, and mineral salts.<sup>[15-17]</sup>

Turmeric is the processed rhizome of *Curcuma longa*. South Asian women have traditionally used turmeric roots for skin care. Scientists have used inventive technique to create a colourless derivative, tetrahydro curcuminoids, which has similar health advantages as yellow turmeric extract. The UV protectant, protein integrity support, tyrosinase inhibitory and antioxidant properties of tetrahydro curcuminoids would work together in an anti-acne formulation to provide multifaceted benefits.<sup>[18,19]</sup> A famous homemade treatment utilized among people is using a mixture of turmeric and coconut oil, which is applied to the afflicted region as well as to surrounding area and when left overnight the size of the acne is reduced. Because of its beneficial benefits on the skin, it is a major component in various cosmetic goods such as creams, soaps, and cleansers.

Aloe Vera is a robust, short-stemmed shrub with water-storing leaves. It is broadly utilized in the cosmetic, pharmaceuticals, and food sectors, and its worldwide annual market price is expected to be \$13 billion.<sup>[20]</sup> Aloe Vera has dense, pointy, succulent green leaves which can grow to be 12-19 inches (30-50 cm) long. Every leaf contains a sticky substance which retains water, causing the leaves to be dense. The “gel” people connect with Aloe Vera products is this slimy, water-filled tissue. This hydrogel includes majority of the plant’s beneficial components, such as vitamins, minerals, amino acids, and antioxidants. The aloe shrub includes a variety of natural phytochemicals with wellness properties, including anthraquinones, vitamins, minerals, polysaccharides,

sterols, amino acids, saponins, salicylic acids, and others.<sup>[21,22]</sup>

*Sapindus mukorossi* is a deciduous tree in the sapindaceae family which grows in the higher parts of the Indo-Gangetic plains, Shivaliks, and sub-Himalayan pathways at elevations ranging from 200m to 1500m. It is among the main significant plants in Asia's tropical and subtropical areas, and is also referred as soap-nut tree. The fruit is valued for the saponins (10.1%) present in the pericarp constitutes up to 56.5% of the drupe known for inhibiting tumour cell growth.<sup>[23]</sup> Much of this plant's pharmacological properties have recently been investigated, including antibacterial,<sup>[24]</sup> cytotoxic,<sup>[25]</sup> molluscicidal,<sup>[26]</sup> insecticidal,<sup>[27]</sup> and fungicidal properties.<sup>[23]</sup> One of the most talked about activities of this plant is the contraceptive of the saponins extracted from the pericarp of the fruit.<sup>[28]</sup> *Sapindus mukorossi* is widely recognised for its ancient therapeutic properties.<sup>[29]</sup> The occurrence of alkaloids, phytosterols, phenolic compounds, tannins, flavonoids, and saponins were detected in the phytochemical analysis from ethanol plant extracts.

Rosaceae is widely cultivated ornamental plant. The flower petal of this plant is reported to have blood purifying properties.<sup>[30]</sup> In ancient medical books several therapeutic effects of this plant, such as treatment of abdominal and chest pain are reported.<sup>[31]</sup> Its flowers are reported to have astringent, analgesic, anti-inflammatory, antidepressant, antibacterial, diuretic and anti-HIV activity and used in folk medicine as a mild laxative.<sup>[32]</sup> Rose flower oil and rose flower extracts have been studied for a number of health beneficial effects including antibacterial activity, antioxidant activity, anti-infective and anti-inflammatory properties and relaxant effect on tracheal chains (Rusanov *et al.*, 2009).<sup>[33]</sup> The qualitative phytochemical analysis of aqueous and methanol extracts revealed the presence of carbohydrates, proteins, glycosides, alkaloids, tannins, phenols and saponins.

The antibacterial activity of the guava leaves showed higher level of inhibition when compared to turmeric whereas Aloe vera showed no zone of inhibition against *Staphylococcus aureus* and *E. coli*. Soap was successfully prepared using Guava leaves, Turmeric, Aloe vera, Soap nut and Rose powder. Soap nut was used for frothing agent and rose powder was used for fragrance.

## CONCLUSION

Herbal therapy does have the ability to heal a wide number of skin ailments. Around 80% of population in India depends on traditional health medicine and

use different plant-based products for curing problems associated with skin. Compared with the conventional allopathic drugs, ayurvedic medicines have relatively low cost and can be of great benefit to the Indian people especially the poor people. Herbal medicines are a rich source of active ingredients and can be safer and cost-effective skin infection treatment ranging from rashes to dreadful skin cancer. Our study showed that the aqueous extracts of Guava leaves were found to be very effective in killing *Staphylococcus aureus* and *E. coli*. Turmeric extracts could only inhibit the growth of *Staphylococcus aureus* and did not have any effect on *E. coli*. Aloe Vera a well-known plant for its antibacterial activity did not show any inhibition with both the bacteria tested.

## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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**Cite this article:** Anjum A, Aruna G, Priya KM, Mubeena KF, Sreenithi R, Rafiq S, Shahina SKJ. Formulation of Herbal Soap against Acne Causing Bacteria. Asian J Biol Life Sci. 2021;10(3):608-13.