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FORMULATION, DEVELOPMENT AND CHARACTERISATION OF ANTI-MICROBIAL HERBAL SOAP USING *Moringa olifera* AND *Albizia amara* LEAVES EXTRACT

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ABSRTACT

Objective: The objective of this study is to investigate the presence of phytochemicals, and create and assess antimicrobial herbal soap a variety of bioactive herbal plant extracts with different dermatological and ethnic significance in ayurveda, including *Moringa oleifera*, *Albizia Amara*, Coconut oil, Jasmine oil, *Aloe vera barbadensis*, Piper betel, green tea, olive oil.

Methods: All plant components' extracts were obtained using various, effective extraction techniques. After that, the extract was combined with lye and fatty oil to make a cold-process soap.

Results: The prepared soap was compared to soap that was sold. The prepared soap was found to be good in appearance, color and odor. pH, % free alkali content, foamability, moisture content, alcohol insoluble matter,

and total fatty matter were found to be 8.53, 0.28, 6.8cm, 6.2cm, 5.6cm,1.8gm, and 11.45,61% respectively. Studies on antioxidant activity and antibacterial activity were conducted, indicating that produced soap is a powerful source of both antioxidants and antibacterial agents.

Conclusion: Based on the study's findings, it can be said that antimicrobial herbal soap can be made utilizing the cold process method, considering several factors such skin condition and herbal potentials and activity. There are several alignments and associated defects in various polyherbal or chemical-based formulations, which can make a significant difference in the field of herbal cosmetics.

Keyword: Herbal Soap, Antimicrobial, Antioxidant, *Moringa oleifera, Albizia Amara.*

1. INTRODUCTION

Traditional medicine has been using plants with therapeutic qualities for ages. The extract obtained from the leaves, stem and roots of various medicinal plants have been employed as a natural remedy in curing various ailments and diseases. Even though many of the plant-based products have been replaced by synthetic chemicals, the safety and efficacy of Ayurvedic products has set standards.[1] The active constituents responsible for such medicinal values are employed topically as creams, soaps, oils and ointments for treating skin related ailments like acne, wounds, eczemas, and ring-worms, as an anti-microbial agent and for cosmetic purposes.[2]

In order to avoid skin problems, it is important to keep the body's largest organ (and one that is constantly exposed to the elements) clean and free of any microbes that could be floating about outside. Skincare herbal formulations that fight fungi, bacteria and microbes may be made from any number of plant components, including the stem, leaves, roots, bark, flower, and fruit.[3] When these medications are meant to be applied topically, they are frequently made as creams, lotions, gels, soaps, and ointments. One of the most popular formulations for skincare and the treatment of skin ailments is herbal soap.[4]

A natural soap is prepared without a non-natural surfactant, with addition of functional ingredient from natural substance, such as essential oil or plant extract. In most cases, soaps are manufactured using the melt-and-pour process, the hot press method, or the cold press method.[5] In the manufacture of soap, various oils such as coconut oil, palm kernel oil, olive oil, castor oil, sunflower oil, rice bran oil, and soybean oil are among the oils that are used. The quality of the soap may be determined by the kind of oil that was used, the kind of alkali that was used, the soap's hardness, the height of its froth, the amount of moisture it contained, and the total fatty matter.[6]

Herbal soaps are those that include herbal extracts should have considerable antibacterial, antimicrobial, anti-aging, anti-oxidant, and antiseptic action, promote skin conditioning, have a great foam, have a pleasant aroma, and be soft on the skin. Herbal soaps do not include any man-made additives and chemicals such synthetic fragrances, flavours, fluoride, etc.[7] Herbs as from beauty of nature have been used to treat a wide range of skin problems, from the very moderate to the serious. The use of various herbal medicines for the treatment of skin

infections has been investigated by many traditional medical systems, including the Ayurvedic, Siddha, and Unani systems of medicine.

The aim of the present work is to formulate an herbal soap containing the extracts of *Moringa oleifera*, *Albizia amara,* Betel leaf, and green tea and analysing its physicochemical properties, anti-oxidant activity, and antimicrobial properties.[8]

2. METHODOLOGY

Collection of Plant Material

All plant material like coconut oil, olive oil and jasmine oil were collected from Ayurvedic store and remaining like *Moringa oleifera* leaves, *Albizia Amara* leaves, betel leaves extract were collected in and around Bengaluru. Collected plant parts were washed with tap water.[9]

Ingredients Used in The Formulation

Coconut oil, Olive oil, *Albizia Amara, Moringa Oleifera,* Jasmine oil, Green tea leaf extract, Betel leaf extract, Aloe vera juice.[10]

Extraction

The collected plant parts (leaves) were washed, air dried for 4 days and dried in a hot air oven at 40^0C until constant weight was obtain and powered to a coarse powder, and store in air tight bottles for the studies. A sample 50g of each plant was added to a round beaker containing 150 ml ethanol or distilled water and boil for four hours. Then the extract was filtered and the filtrate was concentrated using a rotary evaporator. Water extract was freeze dried. The extract was stored at 4^0C until used.[11]

Formulation Of Herbal Soap [12]

Formulation of soap containing active plant potentials was carried out using cold saponification method. Soap is a mixture of various naturally occurring fatty acid salts, either in the form of potassium or sodium.10gm of coconut oil and mixed with 5ml of olive oil was taken in the beaker. In separate beaker 7gm of alkali (sodium hydroxide) was dissolve with 30ml of ethanol and distilled water with continuous stirring. The oil filled beaker is then filled with the solution. The resultant mixture was kept at hot plate at low heat with continuous stirring until the smell of oil/fat disappear and lead to formation of homogenous solution. Whatman No.1 filter paper and a Buchner funnel was used to filter the mixture. Filtrate obtained was then added with 5gm of *Moringa oleifera*, 5gm of *Albizia Amara*, 2gm of green tea leaf extract, 2gm of Betel leaf extract and 2ml of Aloe vera juice along with 1gm of stearic acid, 1gm of soft paraffin and Quantity sufficient of water with continuous stirring in a water bath until the extract get dissolve and become homogenous. To it addition of few drops Jasmine oil was added and mixed properly. The homogenous semi-solid mixture form poured into a mould and allow to solidify at room temperature and physical observation was done for any characteristic changes.

Evaluation Of Soap

The following Phytochemical analysis and Physico-chemical parameters were assessed for determining the quality of prepared formulation against marketed herbal soap.[13]

Physical parameters

The colour and clarity of the prepared soap were observed with naked eye keeping it on white background. The odour of the soap was smelled.[14]

pH

A digital pH meter was used to measure the soap's pH after it was made. The prepared formulation was dissolved in 100 ml distilled water and kept for 2 hr. pH measurement of the solution was done using a previously calibrated pH meter.

% free Alkali content

The beaker containing 10g of dried soap was then filled with 150ml of distilled water. The soap was heated at reflux for 30 to 40 minutes on a water bath in order to dissolve it. This solution was cooled, transferred with the washings to the 250ml conical flask, and the capacity was filled with distilled water. Two drops of the phenolphthalein indicator were added to 10ml of the soap solution in the titration flask. The solution was then titrated against 0.1M HCL until it turned colourless.[15]

Foam stability

Same quantity of soap sample and quantity of distilled water along with process was carried out as that of foamability and add the mixture was kept stand still for 30min. After 30min measurement of foam height was done from above the water volume.[16]

Moisture content

A sample of soap weighing 10g was weight right away and noted as "wet weight of the sample". Using the appropriate drying equipment, this wet sample was dried to a constant weight at a temperature not to exceed 115^0C. After cooling, the sample was weight once more to determine its "dry weight." The following equation was used to calculate the sample's moisture content.[17]

$$\%weight = A-B/B \times 100$$

Where; %weight = % of moisture in sample,

A= weight of wet sample (gm),

B = weight of dry sample(gm)

Alcohol insoluble matter

50ml of warm ethanol was introduced to a conical flask containing a 5gm sample of soap in order to dissolve it. Using tarred filter paper and 20ml of warm ethanol, the liquid was filtered and then dried at 1050 C for an hour. The weighted filter paper had dried out.[18]

Determination of total fatty matter (TFM)

The total fatty matter test is carried out by reacting the soap with acid in the presence of hot water and measuring the fatty acids obtained. About 10g of the finished soap was weighed and 150ml distilled water was added and heated. The soap was dissolved in 20ml of 15% sulphuric acid while heating until a clear solution was obtained. Fatty acids on the surface of the resultant solution were solidified by adding 7g of bee wax and reheating. In order to make cake, the setup was allowed to cool. After removing the cake, it was wiped dry and weighed using a formula to determine the total amount of fat.

$$\%TFM=(A-B)/Wx100$$

Where; A= weight of wax+ oil

X= weight of wax

W= weight of soap.

Determination of anti-oxidant activity [19]

Diphenyl Picryl Hydroxyl (DPPH) radical scavenging method

DPPH assay is carried out as per the method of Rajkumari et al.[20] In brief, 80µl of DPPH solution; various concentration of test solution and quantity sufficient to 240 µl with HPLC grade methanol. The different concentrations tested for reference standard are 0.9, 1.8, 3.7, 7.5,15, µg/ml. The different concentrations tested for test samples are, 31.25, 62.5, 125, 250 and500 µg/ml. The reaction mixture is mixed and incubated at 25°C for 15 minutes. The absorbance is measured at 517nm using semi-autoanalyzer. A control reaction is carried out without the test sample.[21]

% Inhibition = (OD of sample – OD of sample) / OD of control *100

Statistical evaluation

Half maximal Inhibitory concentration (IC50) is the concentration of the substance required to inhibit a biological process such as an enzyme, cell, cell receptor or microorganism by half conducted by GraphPad prism.

Anti-microbial activity

Test compounds like soap Sample (25µl), Standard Tetracycline (25µl) E coli were added to the 5mm well on agar plates.The treated plates with E coli were incubated in aerobic chamber at 37°C for 24hrs. The treated plates were observed for zone of inhibition around the wells.[22]

3. RESULTS AND DISCUSSION

All the plant materials Jasmine oil, Coconut oil, Olive oil, were collected from Ayurvedic store and remaining like *Moringa oleifera* leaf, *Albizia amara* leaf, Aloe vera juice, Betel leaf, and green tea were collected in and around Bangaluru. The purpose of used in the antimicrobial herbal soap and its details are mentioned below table no.1

Table 1: Composition of antimicrobial herbal soap formulation (100 gm)

SI. No	Composition	Uses	Quantity
1	*Aloe vera*	Moisturizer, Antiseptic	2ml
2	Coconut oil	Natural fat	10gm
3	Olive oil	Moisturizer, antioxidant	5ml
4	Jasmine oil	Astringent, Antiseptic	q.s
5	Sodium Hydroxide	Oil and fats lather to saponification	7gm
6	Ethanol	Lye	30ml
7	*Moringa oleifera*	Antimicrobial, antifungalal	5gm
8	*Albizia amara*	Antimicrobial, Foaming activity	5gm
9	Betel leaf	Stimulant, Antiseptic	2gm
10	Green tea	Antibacterial, antioxidant	2gm
11	Stearic acid	Hardener	1gm
12	Soft paraffin	Soothing agent	1gm
13	water	vehicle	Q. S

Phytochemical analysis

Phytochemical analysis of *Moringa oleifera* revealed the presence of phytochemicals such as flavonoids, glycoside, terpenoid and tannins and the analysis of the plant extract from *Albizia amara* revealed the presence of phytochemicals such as alkaloids, steroids, terpenoids, phenol, tannins, flavonoid saponins and terpenoids. Phenol is considered as an important phytochemical for health constituents. Plant phenolics, or flavonoids, are the biggest class and are well-known for their potent antioxidant properties.

Physicochemical Evaluation of antimicrobial herbal soap

The physicochemical properties of soap include moisture content, PH, Color, Total fatty matter (TFM), Alkali content which are listed below.

Fig:1 Prepared soap.

Table 2: Evaluation of Formulation for Physical Appearance

SL.NO	FORMULATION	PHYSICAL APPEARANCE
1	Prepared Soap	Brownish, Jasmine like smell
2	Marketed soap	Greenish, Neem like smell

Determination of pH

The pH of the antimicrobial herbal prepared soap was found to be 8.53, which lies between the normal pH range 8-10 as reported by National Agency for Food and Drug Administration and Control (NAFDAC). The obtained pH value is like that marketed soap i.e 7.90.

Fig:2 pH a) Prepared soap b) marketed soap

Foamability

The foaming index of the prepared antimicrobial herbal soap was found to be 5.3cm which is low compared to marketed soap (6cm). Although foam generation has little to do with cleansing ability it is of utmost importance to the consumer and therefore considered as a parameter in evaluating soaps.

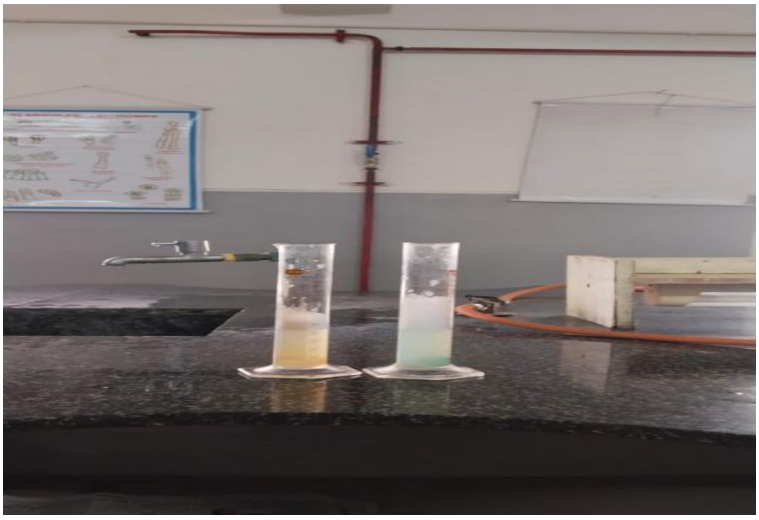

Fig:3 Foam determination: a) Prepared Soap b) Marketed Soap.

Determination of Moisture Content

Moisture content is a parameter that measures the shelf life of a product. High moisture content in the soap could lead to a reaction of excess water with un-saponified fat to give free fatty acid and glycerol in a process called hydrolysis of soap on storage. The moisture content of the prepared soap is 26%, which is high compared to marketed soap (20%). This might be due to the difference in soap preparation methods.

Total fatty matter

Total Fatty Matter (TFM) is how much fat substance the soap has, that is, it is the indication of soap quality. The more it has better the quality of the soap. The Total Fatty Matter of the prepared herbal soap was 60% which is closer to 67% marketed soap.

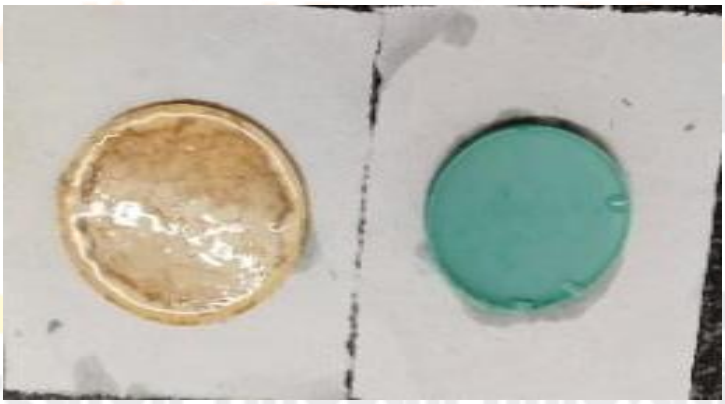

Fig:4 Total fatty matter a) Prepared soap b) marketed soap

Determination of %Free alkali Content

The alkali content of formulation Soap was found to be 0.23% which is lower than 0.25 as shown by marketed Soap. The soap is of higher quality when the value is lower. This suggests that the manufactured soap won't damage skin. The alkaline substances present, helps in neutralizing the body's protective acid mantle that acts as a natural barrier against bacteria and viruses. Healthy skin has a pH 5.4 to 5.9.12 The alkalinity Favors detergency.

Determination of Alcohol insoluble matter

The alcohol insoluble matter measures the amount of non-soap ingredients known as builders or fillers such as sodium silicate, sodium phosphate, sodium carbonate and minor constituents (bleaches, whitening agents, and fluorescing agents) present in the finished product. The alcohol insoluble matter of Prepared soap was found to be 9.22% which is less than marketed soap which is 11. 45%.

Determination of Antioxidant activity

DPPH Analysis

The antioxidant activity test is conducted to find out how much the soap ability in reducing free radical of DPPH. DPPH serves as a free radical compound that will react with antioxidant soap. The ability to capture free radicals is related to the ability of a compound to donate electrons or hydrogen. Any molecule that can donate electrons or hydrogens will react and fade the DPPH color from purple to yellow to form the 1,1-diphenyl-2-picrilhidrazine compound. Ascorbic acid was used as positive control.

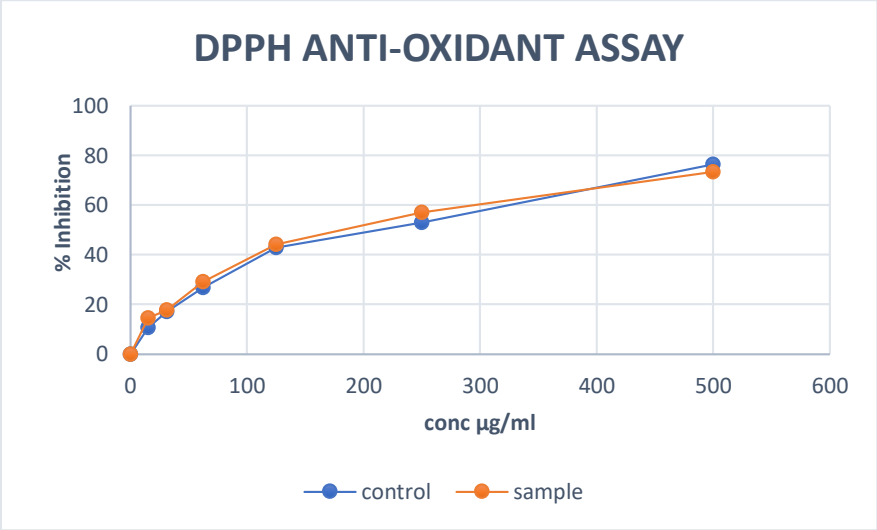

Fig:5 Antioxidant activity of the control and prepared herbal soap

Result (fig:5) showed the percentage inhibition of DPPH radical of the Prepared soap and Control at different concentrations. The IC50 values of the formulated herbal soap and ascorbic acid were found to be 2.049 mg/ml and 0.507 mg/ml respectively. Antioxidant activity is very strong if it has IC50 value less than 0.05mg/ml, strong if IC50 values between 0.05-0.10mg/ml, medium if IC50 values between 0.10-0.15mg/ml and weak if IC50 values between 0.15-0.20mg/ml. The lower the IC50 value, higher is the scavenging activity. The IC50 represents the concentration of the sample extract required to scavenge 50% DPPH radical. Thus, comparing with the IC50 values obtained, the formulated herbal soap seems to have low antioxidant activity. The loss of antioxidants might be caused by the reaction temperature as antioxidants are thermally sensitive. The antioxidant properties are contributed by the presence of polyphenolics, flavonoids, vitamin C, and monophenolics making the identification and quantification of these compounds important. The presence of these phytochemicals in less amount might have also been a reason for low anti-oxidant activity.

Determination of Antimicrobial activity

The antibacterial activity of herbal soap was determined by measuring the diameter of zone of inhibition and result was tabulated in Table 11 & Fig 17 the Sample has shown inhibitory activity against E. coli in well diffusion.

Table:2 Inhibitory activity of test compounds against test organisms

Test Organisms	Test Compounds	Conc.per well	Zone of inhibition (mm)	Figure reference number
E coli	Control	-	-	Figure 1
	Tetracycline (Standard)	25 µg/ml	25	
	Control	250 µg/ml	09	
	Sample	250 µg/ml	13	

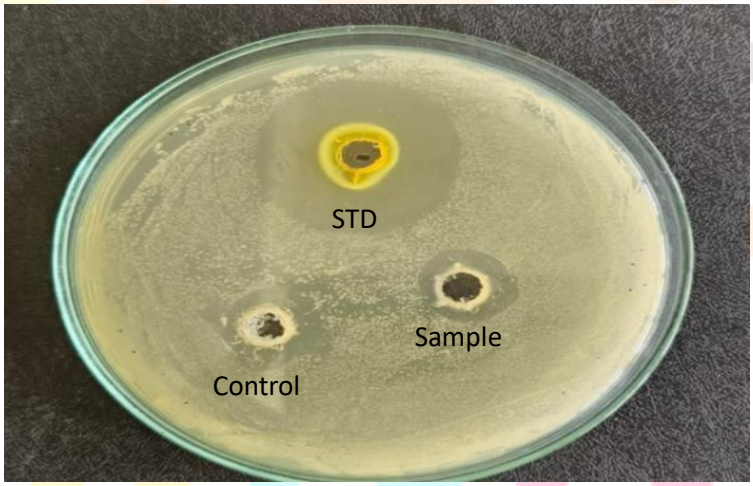

Fig:6 Inhibitory activity of test sample against *E.coli*

Std-Standard (Tetracycline). Control, Sample

4. CONCLUSION

The objective of the study is to develop a stable and functionally effective of Anti-microbial herbal Soap by excluding synthetic chemicals, which are normally incorporated in such formulation larger extent. The Formulation herbal soap is not only safe, but also improves and strengthens the skin and it is proved that soap does not give any irritancy to skin. In conclusion, aqueous extracts of the plant materials show the potential in soap formulation. The Physico-chemical and biological parameters of the prepared soap were studied. The formulation was good in appearance, with pleasant odour and colour. The pH was found to be in the range that

is 8.53. Other parameters like % free alkali content, Foamability, Foam stability, Moisture content, Total fatty matter, and Alcohol insoluble matter was determined which signified the standard values for soap. Biological parameters like Antioxidant and Antibacterial studies were conducted, which indicates the prepared soap to be a potent antioxidant and antibacterial source. The study's findings suggest that the formulation of herbal soap utilizing the cold process method can take into account various factors, including skin type and the potential and activity of the herbs. This sought of herbal formulation can bring a big difference in the field of herbal cosmetics as there are many alignment and related flaws in different polyherbal or chemical-based formulations which can be removed.

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