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FORMULATION OF HERBAL SOAP WITH POTENTIAL ANTIBACTERIAL ACTIVITY

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ABSTRACT: Soap is a salt of fatty acid obtained by treating vegetable oils or animal fats with alkali. In current scenario herbal soap has generated considerable interest and enthusiasm amongst consumers due to ecofriendly nature of the product. The research work deals with the isolation of Curcumin and Embeline with soxhlet extractor from *Curcuma longa* and *Embeliaribes*, respectively. Both phytoconstituents are of therapeutic interest. Hence, these phytoconstituents incorporated to formulate herbal soap. Further these soaps are evaluated for physiochemical properties like appearance, pH test, total fatty matter test, etc. The antimicrobial efficacy of the formulated soaps was checked by agar well diffusion method by using two bacterial strains *Bacillus subtilis* and *E. coli* and one fungal strain *Candida albicans* and thumb impression method. The result of studies showed that Curcumin soap shows better activity than Embeline soap.

KEYWORDS: *Embeliaribes*, *Curcuma longa,* phytoconstituents, *Bacillus subtilis,* Curcumin, Embeline

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INTRODUCTION

Skin is one of the most exposed part of the body which requires protection from the pathogens. To protect the skin from harmful microorganisms and to prevent spreading of many contagious diseases handwashing is an absolutely important precaution. Handwashing is important way to help fight the disease. Harmful bacteria such as E-coli and Salmonella typhi can be carried by people, animal or equipment and transmitted to food. Synthetic hand wash have some adverse effect as follows:-

- Dryness
- Their frequent use can lead to skin irritation
- Also resistance among pathogen

So we formulated and evaluated Antibacterial efficiency herbal hand washes.Aherbal hand soap formulation should give it's effect and at the same time it should not give any unwanted effect rather it should make hand soft and give strong antimicrobial action.

Plant extracts and products have been used for centuries in traditional medicine, cosmetics, natural dyes, and in the treatment of diseases. The main advantage of using natural source is that they are easily available, cheap and harmless compared to chemical products. In present study we formulate herbal soap using plant extracts with potential antibacterial activity and thereby establishing them as a potent antimicrobial agent in the formulation of herbal hand wash.

Soaps are obtained by treating vegetable or animal oils and fats with a strong base, such as sodium hydroxide or potassium hydroxide in an aqueous solution. Fats and oils are composed of triglycerides; three molecules of fatty acids attach to a single molecule of glycerol.The alkaline solution, which is often called lye (although the term "lye soap" refers almost exclusively to soaps made with sodium hydroxide), induces saponification.

Products which are formulated using various permissible cosmetic ingredients to form the base in which one or more herbal ingredients are used to provide defined cosmetic benefits only, shall be called as "Herbal Cosmetics" Herbal soap is one of the herbal cosmetic

Herbal soap has generated considerable interest and enthusiasm amongst the consumers in recent times, due to eco-friendly nature of the product.

There is good scope for setting up herbal soap projects in the country.

While there is no particular entry barrier from the point of view of technology, adequate market thrust is necessary to competitively sell the product in the market.

AIM AND OBJECTIVE

1. Extraction of phytoconstituents from powdered plant material.
2. Formulation and evaluation of herbal soap.
3. To carry out antimicrobial activity of formulation.

MATERIALS AND METHODS

Plant material:

A. Curcumin: Biological source: Curcuma longaFamily: Zingiberaceae
   Collecton: purchased from Patanjali Store, C.B.D. Belapur
B. Embeline: Biological source: *Embeliaribes*Burm.F.Family: Myrsinaceae
   Collection: purchased from SheetalAyurvedic Store, Chembur (East)

Extraction of active constituents:Embeline:

Material: Vidanga poweder-50gm, Solvent: Petroleum ether (60-80)- 400ml

Soxhlet apparatus

Procedure: Weigh about 50gm of vidang powder. Mount it in soxhlet apparatus. Pour about 300ml of petroleum ether.Start the soxhlet and allow the extraction to continue about 2 hrs. After the completion of the process, the solvent was evaporated andether was added for the precipitation of Embeline.

Extraction of active constituents: Curcumin:

Material: Turmeric powder-50gm Solvent: Ethanol-200ml

Soxhlet apparatus

Procedure:Weigh about 50gm of turmeric powder. Mount it in soxhlet apparatus. Pour about 100ml of ethanol.Start the soxhletandallow the extraction to continue. At the end, allow solvent to evaporate and concentrate the extract.

Microbial strains :The bacterial strains used for antibacterial activity were *Bacillus subtilis* and

E.coli.The fungal strain used for antifungal activity was *Candida albicans*.

Control and standards:Streptomycin was used as standard for antibacterial activity while tioconazole was used as standard for antifungal activity.

Formulation:We have tried 4 formulations and selected the following formulation:

Table no.1: Formulation table

Sl. No.	Ingredients	Quantity given (gm)		Activity
		Embeline soap	Curcumine soap	
1.	Castor oil	7.62	7.62	Superfatting agent
2.	Coconut oil	11.42	11.42	To produce lather
3.	Tallow	11.42	11.42	Superfatting agent
4.	Stearic acid	4.57	4.57	Hardening agent
5.	Olive oil	3.05	3.05	To relieve dryness
6.	Alkali (naoh)	5.80	5.80	Saponify oils
7.	Distilled water (for lye solution)	11.58	11.58	To dissolve lye
8.	Glycerin	9.52	9.52	Humectant
9.	Ethanol	19.05	19.05	To make soap transparent and clear
10.	Sugar	9.52	9.52	Increases the lather
11.	Rose water (for sugar solution)	83	83	To dissolve sugar, for Fragrance
12.	Drug	0.0016	0.017	
	Total	100	100	

Procedure: Weigh the oils (Castor oil, Coconut oil, Wool Fat, & Olive Oil) & glycerin into pot & melt. Weigh the Lye (NaOH) & distilled water into 2 separate containers. Add the lye to the water while stirring to create a solution.. Don't have to let it cool down. Pour the lye solution into oils / glycerin &blend to trace. Weigh out stearic acid and 10 extra grams. Melt using a double boiler on the stove. Place pot onto scale & hit tare. Weigh stearic acid into pot. The extra stearic acid that measured out to melt will ensure you don't come up short if any sticks to your container while pouring. Blend again. It will get quite thick because of stearic acid. Weigh denatured alcohol & add to mixture. Stir quickly, breaking up the soap. It will start to dissolve a bit in the alcohol. Scrape the sides of pot to get all of it mixed together. Immediately cover with press & seal the lid. Set to cook. Let the soap cook for 2 hours. During this time the solvents will work on dissolving the soap crystals that form, creating soap. No need to stir. After 2 hours, test soap for clarity.. Create sugar Solution by heating. If sugar doesn't dissolve, add a bit more water. Add the sugar solution to pot &mix, cover & cook for 30 min.- 1 hour .13. You can check the clearness again if you want. Then decide to add more alcohol or sugar solution. Once it done, then add active ingredient. Pour into the moulds and allow it to set for 3-4 hrs.

Physiochemical evaluation test**:**Following test were performed on formulated herbal soap:

1. Appearance:Embeline and curcumin soap were observed for the color, shape and particle size and air entrapment.
2. Primary skin irritation test:For this three human volunteers were selected and prepared soaps were given to them and checked for irritation.
3. Total fatty matter:Accurately weighed 5 gm of soap and transferred into 250 ml beaker. To completely dissolve the soap 100 ml hot water was added.40 ml of 0.5 N HNO3 was added to the mixture until contents were slightly acidic. The mixture was heated over water bath until the fatty acids were floating as a layer above the solution. Then the mixture is cooled suddenly in ice water in

order to solidify the fatty acids and separate them. 50 ml of chloroform was added to the remaining solution and transferred into a separating funnel. The solution is shaken and allowed theto separate into 2 layers and the bottom layer was drained out. 50 ml of chloroform was added to the remaining solution in the separating funnel. The fatty acid dissolved chloroform is again separated as in the previous case and it is transferred to the collected fatty matter

4. pH test:Place water on the surface of the bar of the soap.Rub the water onto the soap until it lathers.Dip the pH strip in the bubbles.Check the pH strip against the pH chart.

5. Foam test:Compare foam forming ability of curcumin and embeline soap. Prepare 0.5% and 1% solution of SLS and 5%, 10%,15% solution of each soap. Take 10 ml of each test solution in test tube, shake vigorously for 30 seconds and keep in stand.Note volume at 0min, 1min, 5min, 15min, 30min and 1hr.

6. Alkali content**:**5gm of soap sample is dissolved in 100ml of hot water. About 40ml of 0.05 HNO_3 is added to make it acidic. The mixture is heated until fatty acids are floating as a layer above the solution. It is cooled under the ice water to solidify the fatty acids. The fatty acids were separated and the aqueous solution was treated with 50ml chloroform to remove the remaining fatty acids. The aqueous solution was measured and 10ml of it was titrated against 0.5N NaOH using methyl orange as indicator and from the titer value the total alkali content was calculated.

7. Skin irritation test on volunteers: soap used for hand washing by volunteers and check for any irritation and itching

Antimicrobial assay:

A. Agar well diffusion method:

Antimicrobial activities of Curcumin soap and Embeline soap were evaluated using well diffusion method on nutrient agar and sabourd dextrose agar for bacteria and fungi, respectively. The inhibition zones were reported in millimeter (mm). *Bacillus subtilis, Staphylococcus aureus* and *E. coli* were used for the antibacterial assay and *Candida albicans* was used for antifungal assay. Nutrient agar plates and sabourddextrose agar plates were inoculated with bacterial and fungal strains, respectively under aseptic conditions. The wells (diameter- 6mm) were formed with the help of borer and filled with 0.05ml of the test samples. The plates were incubated at 37°C for 24 hours. After the incubation period, the diameter of the growth inhibition zones was reported in mm.

B. Thumb impression test:

Thumb impression of the hand exposed to the environment was placed on a sterile nutrient agar plate. Then, the thumb impression of the same hand was placed after washing with formulated embeline soap on the same plate without any overlaps of thumbprints. Same procedure was carried out for formulated curcumine soap with thumb impression of other hand. The pattern of microbial growth on the plates was observed after an incubation period of 24 hours at 37°C.

RESULT AND DISCUSSION:

It was accepted as all the properties of the formulation were satisfactory.

Hence, both the formulations were evaluated for physiochemical properties and antimicrobial activity. Both soap formulations were even stable after six month stability

Table no.2: Physicochemical Evaluation

Sl.No.	TEST	EMBELINE SOAP	CURCUMINE SOAP
1	Appearance		
	Colour	Light yellow	Dark orange
	Shape	Disc shaped	Disc shaped
	Particle size	Uniform	Uniform
	Air entrapment	No	No
2	Primary skin irritation test	No irritation	No irritation
3	Total fatty matter	65%	63%
4	Ph test	8-9	8-9
5	Foam test	Good	Good
6	Alkali content	1.27	1.32

Antimicrobial activity:

1. Agar well diffusion method:

The antibacterial activity of selected formulation against bacterial strains *Bacillus subtilis* and *E.coli*and fungal strain *Candida albicans* was investigated by agar well diffusion method and by using streptomycin and tioconazole as standards for antibacterial and antifungal activity, respectively. The result indicates both curcumine soap and embeline soap shows antibacterial as well as antifungal activity.

Table no.3: Antimicrobial activity by Agar well diffusion method

Sl.No.	Solutions used	Average diameter of zone of inhibition (mm)		
		Bacillus subtilis	***E. coli***	***Candida albcans***
1.	Embeline	14.3	6.6	7.3
2.	Curcumin	5.3	7.3	8.6
3.	Streptomycin	10.18	9.10	NA
4.	Tioconazole	NA	NA	22.10

Fig.a

Fig.b

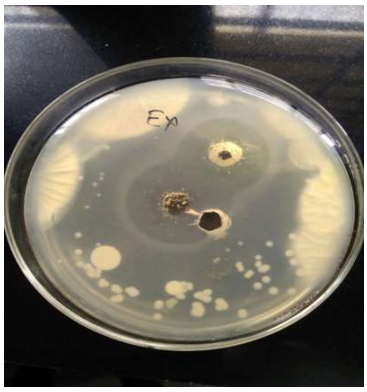

Fig.c

Fig. a: zone of inhibition for *E.coli*, Fig b: zone of inhibition for *Bacillus subtilis*, Fig. c: zone of inhibition for *Candida albicans*

2. Thumb impression method:

The antibacterial activity of selected formulation was studied by thumb impression method. The result indicates that both embeline and curcumin soaps show significant decrease in microbial count after washing hands with individual soap.

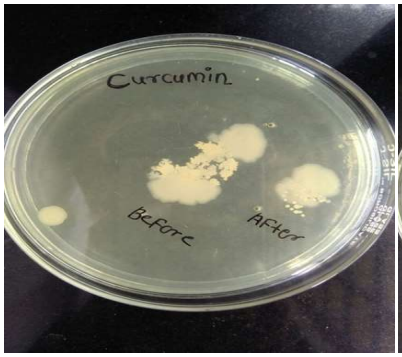

Fig. d

Fig. e

Fig. d: Thumb impression before and after washing hands with curcumin

Fig. e: Thumb impression before and after washing hands with Embeline

CONCLUSION:

Both the formulations do not give any irritancy and itching to skin it was determined by using these soap by some of the volunteers. Furthermore the prepared soap were standardized by evaluating various physic chemical properties such as pH, appearance, odour, total alkali content and foam test etcand Antimicrobial activity exhibit satisfactory effect. WhileCurcumin soap gives more promising physic chemical properties and antimicrobial activity against two microorganisms (*E.coli* and *Candida albicans*) compare toEmbeline soap.

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