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Formulation and evaluation of medicated soap of *Ixora coccinea* root extract for dermal infections

Mohammed Haneefa K.P[1], Shilpa NM[2], Junise V[3], Ajith Chandran[4]
[1, 2, 3,4] *Alshifa College of Pharmacy, Poonthavanam Post, Kerala, India*

Abstract

The main aim of this work was to formulate a medicated soap for dermal infections using the root extract of *Ixora coccinea*. Ethanolic extract of dried roots of *Ixora coccinea* were subjected to preliminary phytochemical evaluation. Soap formulations were prepared using three types of oils: Coconut oil, Olive oil and Peanut oil. The prepared emulgel were evaluated for their physical appearance, pH, % free alkali content, foam height, moisture content, total fatty matter, alcohol insoluble content, antimicrobial activity and stability.It was finally concluded that the formulation F2 with 58.4g of Coconut oil was found to be more promising formulation as it shows better physicochemical characteristics and antimicrobial activity compared to other formulations. Herbal soap of ethanolic extract of *Ixora coccinea* shows significant antimicrobial activity and hence this is a promising candidate in dermal infections.

Keywords: *Ixora coccinea*, medicated soap, foam height, total fatty matter.

INTRODUCTION

Soap is an important surface-active agent and it is chemically the alkaline metal salt of long-chain fatty acids. The most common used fat or oils for production of soap through saponification reactions are animal tallow, coconut oil, palm oil, kernel oil and linseed oil. Similarly potassium and sodium hydroxides are widely used as the caustic alkaline for the purpose. Soap is an anionic surfactant used in conjunction with water for washing and cleaning, which historically comes either in solid bars or in the form of a viscous liquid.

The sodium and potassium salt of higher fatty acids, such as oleic, stearic, lauric, and palmitic acids are called soaps. Sodium salts are called hard soaps and Potassium salts are called soft soap[1,2].

Soap can be produced either by the traditional or modern methods. The traditional method utilized animal or vegetable oil with alkaline source. The modern method is a reaction of vegetable oil with sodium hydroxide (caustic soda), which is derived from chemical process[3,4]. 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 make them useful agent for bathing, laundry, washing, and cleansing of surfaces. 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[5].

Ixora coccinea is an evergreen perennial shrub or tree belonging to family, Rubiaceae. The plant is traditionally found to be useful for many ailments like hepatic disorder, cancer, microbial infection, antioxidant, pain, inflammation etc and has been documented for various medicinal properties. The genus Ixora has been reported to possess different classes of compound mainly aromatic acrid oil, tannin, saponin, carbohydrate, fatty acid, flavonoids like β-sitosterol, and kaempferol[6].

The ethanolic extract of *I.Coccinea* is also reported for its excellent antimicrobial activity [7,8]. The present study was conducted to formulate a medicated soap for dermal infections using the root extract of *Ixora coccinea*. The prepared soap was evaluated for physicochemical as well as for pharmacological activity

MATERIALS AND METHODS

Materials

Coconut oil for soap-making was purchased from the local market. Culture media for the cultivation of bacteria were from Hi-media, Mumbai, India. Clinical pathogens were obtained from local hospitals. All other reagents used were of Analytical Grade.

Plant materials and extract preparation

Ixora coccinea (Family: Rubiaceae) were collected from local areas of Perinthalmanna, Kerala and was authenticated by Dr.A.K. PRADEEP, Asst. Professor, Department of Botany, University of Calicut. The foreign, earthy matter and residual materials were removed carefully from the roots and then cleaned and dried in the shade. It was then powdered to mesh size of # 40. The powder was loaded into the main chamber of the Soxhlet extractor and extracted successively for eight hours using four different solvents with increasing polarity. Viz: - Petroleum ether, Chloroform, Ethyl acetate, and Ethanol. The extracts were filtered and concentrated under reduced pressure and stored at 4- 8^0 C until use.

Preliminary antimicrobial screening of the extracts

All the root extracts of *Ixora coccinea* were subjected to preliminary antimicrobial screening by agar well diffusion method against the organisms *E coli* (MTCC-1698), *S aureus* (MTCC-1143) and *P aeruginosa* (MTCC-2453). The extract which displayed maximum activity was chosen for the formulation of soap.

Formulation of medicated soap

Herbal medicated soap was prepared by cold saponification process. Nine different soap formulations were prepared by using varying amount of oil component as displayed in **Table 1**.

1g of ethanolic extract was dissolved in oil mixture with continuous stirring. The mixture was strained to avoid

foreign particles. The oil extract mixture was poured into the sodium hydroxide solution in a beaker with continuous stirring. 0.1ml of perfume was added and stirred well till the contents were mixed together thoroughly. The melted soap was gently mixed for about 30 minutes and moulded in circular moulds. The soap was allowed to solidify at room temperature and kept under physical observation for any characteristic changes[9].

Evaluation of prepared formulations

The following physicochemical parameters were evaluated to confirm the quality of prepared formulations.

Determination of clarity, colour and odour

Clarity and colour was checked by visual examination against white background, and the odour was smelled.

pH

Digital pH meter was used to determine the pH of all the prepared formulations. All the nine formulations were separately dissolved in 100 ml of distilled water and stored for two hours. The pH was measured using previously calibrated Digital pH meter[10].

Percentage free Alkali content

About 10g of dry soap was weighed and transferred to a beaker containing 150 ml of distilled water. It was boiled under reflux on a water bath for 30 to 40 minutes to dissolve the soap. Cooled and transferred this solution along with the washings to the 250 ml conical flask and made up the volume with distilled water. 10 ml of the soap solution was taken in the titration flask and two drops of phenolphthalein indicator was added. It was then titrated against 0.1M HCl until the solution turn colorles[11].

Foam height

1gm of sample soap was taken and dispersed in 50ml distilled water. It was then transferred into a measuring cylinder, and the volume was made up to 100ml with water. 25 strokes were given and stand till aqueous volume measured up to 100ml and measured the foam height, above the aqueous volume[11].

Moisture content

10g of soap sample was weighed immediately and recorded as "wet weight of sample". This wet sample was dried to a constant weight, at a temperature not exceeding 239º F (115º C) using the suitable drying equipment. The sample was cooled, weighed again and recorded as the "dry weight of sample". The moisture content of the sample was calculated using the following equation[12].

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

Where, %W = Percentage of moisture in the sample, A= Weight of wet sample (grams), and
B = Weight of dry sample (grams).

Total fatty matter

5 g of soap was accurately weighed and transferred into 250 ml beaker. 100 ml of hot water was added to completely dissolve the soap. 40 ml of 0.5 N Nitric acid was added until contents were slightly acidic. The mixture was heated in a water bath until the fatty acids were floating as a layer above the solution. The fatty acids were cooled in ice and separated them. 50 ml of chloroform was added to the remaining solution and transferred it to a separating funnel. Shaken the solution and allowed the solution to separate into two layers. The bottom layer was drained. Added 50 ml of chloroform to the remaining solution in the separating funnel. Separated the fatty acid dissolved in chloroform again as in the previous case and transferred it to the collected fatty matter. The fatty matter was weighed in a pre-weighed china dish. Allowed the contents to evaporate and weighed the residue. From the difference in weight, calculated the percentage of fatty matter in the given soap sample[13].

Alcohol insoluble content

5g of soap sample was dissolved in 50ml hot alcohol. The solution was filtered through a tarred filter paper with 20 ml warm ethanol and dried it at 105ºC for 1 hour. The weight of dried filter paper was taken. Formula % alcohol insoluble matter = Wt. of residue × 100 / Wt. of sample[14].

Antimicrobial Efficacy Studies[15,16,17,18]

The Antimicrobial efficacy studies were carried out to ascertain the biological activity of the optimized formulations by using agar well diffusion method against the organisms *E coli* (MTCC-1698), *S aureus* (MTCC-1143) and *P aeruginosa* (MTCC-2453).

The developed formulations were poured in to separate cups bored into sterile nutrient agar previously seeded with test organisms after allowing diffusion of the solutions for 2 hours, the agar plates were incubated at 37°C for 24hrs. The zone of inhibition (ZOI) measured around each cup and compared.

RESULTS

Preliminary antimicrobial screening of the extracts

All the root extracts of *Ixora coccinea* were subjected to preliminary antimicrobial susceptibility testing by agar well diffusion method against the organisms *E coli* (MTCC-1698), *S aureus* (MTCC-1143) and *P aeruginosa* (MTCC-2453). The ethanolic extract exhibited maximum activity was chosen for the formulation of soap. The observations are recorded in **Table 2**.

Evaluation of physicochemical parameters of soap formulations

The physicochemical parameters were evaluated to confirm the quality of prepared soap formulations. All the nine formulations exhibited good appearance characteristics with dark brown in colour with a smooth homogeneous surface and glossy appearance.

The pH of all prepared formulations, F_1 to F_9 were found to be in the range of 6.6 to 7.6. Other parameters like Percentage free Alkali content, Foam height, Moisture content, Total fatty matter and Alcohol insoluble content were determined; the results are tabulated in **Table.No.3.**

Table 1 Working formula for medicated soap of *Ixora coccinea*

SL NO	INGREDIENTS	F_1	F_2	F_3	$_4$	F_5	F_6	F_7	F_8	F_9
1	*Ixora coccinea* Extract (g)	1	1	1	1	1	1	1	1	1
2	Coconut oil(g)	54.4	58.4	60.4						
	Olive oil(g)	-			54.4	58.4	60.4			
	Peanut oil(g)							54.4	58.4	60.4
3	Sodium hydroxide (g)	6.0	7.3	7.55	6.8	7.3	7.55	6.8	7.3	7.55
4	Flavoring agent (ml)	0. 1	0.1	0.1	0. 1	0. 1	0. 1	0. 1	0. 1	0. 1
5	Water(ml)	19.2	14.7	11.85	19.2	14.7	11.85	19.2	14.7	11.85

Table 2 Preliminary antimicrobial screening of the extracts

Extracts	Zones of inhibition in mm		
	E.coli	***S.aureus***	***P.aeruginosa***
Petroleum ether	8	13	14
Chloroform	11	16	18
Ethyl acetate	14	19	20
Ethanol	18	21	22

Table 3 Physicochemical parameters of soap formulations

Formulations	pH	% free Alkali content	Foam height	Moisture content	Total fatty matter	Alcohol insoluble content
F_1	6.6	0.27	11.4	3.09	77.6	18
F_2	7.1	0.22	14.4	1.37	82.0	16
F_3	6.8	0.27	12.2	3.31	78.4	18
F_4	7.3	0.27	11.6	8.70	72.6	22
F_5	7.5	0.25	12.8	9.41	73.6	22
F_6	6.9	0.26	9.2	3.95	74.4	26
F_7	7.2	0.28	9.4	6.04	76.6	26
F_8	7.6	0.28	10.4	8.46	77.2	28
F_9	6.9	0.27	9.2	8.93	77.2	26

Table 4 Antimicrobial Screening of Soap formulations

Formulations	Diameter of Zone of inhibition of (ZOI) produced by medicated soap of *Ixora coccinea* (mm)		
	E.coli	***S.aureus***	***P.aeruginosa***
F_1	32	34	32
F_2	34	38	36
F_3	32	36	34
F_4	26	32	28
F_5	28	30	30
F_6	31	32	31
F_7	30	34	30
F_8	28	32	32
F_9	30	31	34
Commercial Soap	28	32	31

ANTIMICROBIAL EFFICACY STUDIES

Antimicrobial activity when tested microbiologically by the Cup-Plate technique. Clear zones of inhibition were obtained in all the formulations. The diameter of zone of inhibition produced by formulations against all test microorganisms is given in **Table.No.4**

DISCUSSION AND CONCLUSION

The present work is a novel herbal soap formulation using *Ixora coccinea* (Family: Rubiaceae) alcoholic extract. The roots of *Ixora coccinea* were successfully extracted with Petroleum ether, Chloroform, Ethyl acetate, and Ethanol. The phytochemicals present in the extract were identified by qualitative phytochemical screening. The alcoholic extract contains alkaloids, carbohydrates, tannins, flavonoids, saponins and phenols, and triterpenes. Extractive values indicate the amount of raw drug in the extract or the yield obtained

by using a given solvent. Extractive values of *Ixora coccinea* were determined using Petroleum ether, Chloroform, Ethyl acetate, and Ethanol as solvents. Ethanol soluble extractives were found to be maximum (14.7% w/w). Results revealed that ethanolic extract exhibited maximum antimicrobial activity (zones of

inhibition ranging from 24 to 28 mm) and was chosen for the formulation of soap. Herbal medicated soaps were prepared using ethanolic extract by changing the amount of oil and sodium hydroxide. Nine herbal soap formulations were prepared by cold saponification process.

Soap formulations were evaluated for various physicochemical properties such as appearance, pH, percentage free Alkali content, Foam height, Moisture content, Total fatty matter and Alcohol insoluble content in which they exhibited satisfactory characters.

Total fatty matter is the most important characteristics describing the quality of soap. As per Bureau of Indian Standards (BIS), Grade 1 soaps should have 76 per cent minimum TFM, while Grade 2 and Grade 3 must have 70 per cent and 60 per cent minimum TFM, respectively[19]. The TFM of all prepared formulations were found to be in the range of 72 to 82%. Except F4, F5 and F6 all the other formulations are having TFM greater than 76% and are considered to be Grade 1 soaps.

Antimicrobial activity of soap formulations when tested microbiologically by the Cup-Plate technique, clear zones of inhibition were obtained in all the formulations. The zone of Inhibition for F_2 formulation was found to be 34mm, 38mm and 36mm for *E. coli*, *S aureus* and *P aeruginosa* respectively, which was far better than the zones of inhibition of extract alone. It was finally concluded that the formulation F2 was found to be more promising formulation as it shows better physicochemical characteristics and better antibacterial activity compared to other formulations. Thus, the formulated soaps have shown good antibacterial activities when compared with the commercial soap. Hence these soaps can further be used as a biopharmaceutical product in the treatment for bacterial skin infections as well, along with its usage as normal herbal bath soap.

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