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FORMULATION DEVELOPMENT AND ANTIMICROBIAL EVALUATION OF POLYHERBAL SOAP

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Abstract

The aim of our study was to develop the polyherbal hygienic soap by using cold process method and evaluate antimicrobial potential by agar well diffusion method. Soap was prepared using coconut oil, castor oil, Neem oil, Mentha oil, rose petals extract and NaOH (lye) and the different extracts were incorporated into the basic saponification reaction. The herbal formulation was prepared then evaluation for the analysis of pH, Moisture content, foaming index, foam retention time, saponification, TFM determination, ethanol soluble matter and antimicrobial activity using different concentration of soap solution comparing with standard. The results demonstrated that pH 6.5–7, moisture content 3.5%, foam index was 16.5, foam retention time 10.0 minutes, Saponification value was 161.287 mg/ml, 72% TFM, ethanol soluble matter was 63.80% for herbal soap. Also the evaluation tests showed that the herbal soap has satisfactory antimicrobial results as compared to standard antibiotic. Moreover, oils used are added to treat various skin infections and for daily usage.

Keywords: Polyherbal Soap, Evaluation, Saponification value, Antimicrobial potential.

Introduction

Herbal medicines, plant products and extracts are imitative for its utilisation since the prehistoric times. Plants comprehending with pharmacological active properties are in utilisation since the existence of mankind as functional foods, medicines, cosmetics, dyes as well as in prevention, cure and treatment of various diseases (Kole *et al.,* 2005; Milovanović *et al.,* 2009; Raskin *et al.,* 2002). The extract produced from roots, stem, leaves, flowers possessing medicinal properties acts as a natural remedy for the disease or ailment. Though with the advent of synthetic medicines, the utilisation of herbal medicines have been recouped. But the standards of the herbal medicines in pursuit of its safety and efficacy have still not matched by the synthetic drugs (Kandasamy *et al.,* 2014). There have been tremendous increases in the use of herbal medicines in the recent times. It has been estimated that in the countries –developed as well as developing, the application of herbal plant extracts in the medicines is about eighty percent of the total world population. It is regarded as one the primary health care measures for the treatment of various ailments especially in the developing countries.(Ekor M. 2014; Thakur *et al.,* 2011). This spectacular rise in the utilisation of the herbal plant and well as it s extracts gave rise to a newer branch of medicinal science referred as 'Herbal Medicinal Products' These may be defined as the plant or part of plant used as a whole or as a extract for the treatment, prevention of disease or ailment to be utilised in the health care management (Mukherjee *et al.,* 2009; Mukherjee, 2015). These herbal medicinal products impart various properties such as anti microbial (Alade and Irobi, 1993), anti-inflammatory ((Dharmasiri *et al.,* 2003), anti-helminthic (Guarrera, 1999), anti diabetic, antioxidant and many more. Thus, herbal medicine imparting various properties are used in various pharmaceutical formulations such as soaps, ointments, gels, creams for the treatment of various skin related disorders (Kareru *et al.,* 2010) as well as for the cosmetic use (Gray and Flatt, 1999). The elevating rise in the pursuit of herbal medicines has caused an increased demand of the larger production of herbal products (Mukherjee, 2015). Herbal medicinal products are in greater demand than the synthetic ones because of many reasons:

- Lesser Side effects
- Better safety and efficacy
- Easily available
- Better compatibility with additives
- Potent therapeutic effect
- Cost-friendly (Saikia *et al*., 2006)
- Greater are for selection
- No requirement of animal testing
- Better compatibility with all types of skin (Joshi and Pawar, 2016).

Skin is the most revealed part of the body which is prone to various foreign particles which may lead to various skin related disorders. Therefore, in order to prevent the skin from various disorders there is a need for the proper cleanliness as well hygiene for the most exposed part of the body and prevent it from pervasive micro-organism spread in the environment thus, preventing various disorders of the skin (Shah *et al.,* 2014).The better and efficaous way to remove all the foreign particles, dirt is the use of Soaps. The utilisation of soap helps in cleansing the skin along with the anti-microbial properties. Various micro-organisms such as *Staphylococcus aureus, Pseudomonas spp., Klebsiella pneumonia* and *Proteus vulgaris* being the causative agents for various skin infections (Darmstadt, 2000; Dryden 2009).

Antimicrobial property in regard to the human body helps in prevention of diseases as well as skin infections. Antibacterial property is defined as the property of inhibiting the growth of bacteria. Soaps are defined as the cleaning

agents–solid, liquid, semisolid powders which help in removing dust, dirt, micro-organisms, stains and for maintaining health and beauty (Ikegbunam *et al.*, 2013). The free fatty acids when reacted with an alkaline base by the process of saponification comprises of a soap. Fatty acids such as lauric acid, palmitic acid, stearic acid help in imparting washing property of soaps (Girgis *et al.,* 2003). The soap industry is estimated to be about 186 billion US Dollars. Also the current trends have elevated to a tremendous] rise in regard of the natural ingredients (Friedman *et al.,* 1996). The origin of the basic soap can be traced back to the Egyptians when an alkaline plant was mixed with animal fats for the generation of crude soaps (Phansteil *et al*., 1998). Herbal soaps have gained importance in the recent years which include the extracts of various plant extracts incorporated in to the basic soap reaction. It is reported that herbal antimicrobial soaps have been reported to have about 60-80% of the property to inhibit the growth of micro-organisms (Solanki *et al.,* 2011). Production natural as well as handmade soaps have been a total artistry work involving various factors such as skill, ingredients, creativity and thoughts tend to produce a quality soap (Friedman and Wolf, 1996). Another factor affecting the quality of soaps are:

- Ability of lather producing
- Colour of the soap
- Fragrance of the soap
- Moisturizing ability
- Compatibility of the skin
- Storage Stability (Berneck˙e, 2013; Burke, 2005).

Herbal soaps can be defined as fatty acids in combination with alkali salts being derived from vegetable or plant origin containing natural fragrances or organic ingredients. The method of preparation is by two processes – hot process and cold process which involves the presence of base such as potassium hydroxide and sodium hydroxide along with the fatty acids to form soap (Berneck˙e, 2013). Cold process is usually preferable process by the artisans. The quality of the soaps are dependent on various factors such as type of alkali used, its hardness, foam height, solubility etc. (Burke 2005; Vivian *et al.,* 2014). Various types of oils are used depending upon the properties they impart such as : Olive oil, Castor oil, Sunflower oil, Palm oil, Rice bran oil, soybean oil etc (Friedman *et al.,* 1996). Additives include anti-oxidants which help in suppression of the oxidation of fatty acids in the herbal soap bar. For instance: Rosemary extracts, tomatoes, fruits etc. Fragrance and coloring agent are also included (Burke, 2005). The aim of this study is to formulate an antimicrobial herbal soap containing various extracts incorporated into one.

Castor oil is obtained from castor plant whose biological source is *Ricinus communis L.* belonging to family Euphorbiaceae (Saxena *et al*., 1991). Basically, castor oil is a non volatile, almost yellow (pale in color). In addition castor oil is non volatile in nature. About 40-50% oil is present in the castor seeds which are subjected to extraction by various methods (Abitogun *et al.,* 2009). These methods include: Mechanical method and solvent Extraction. The former process the seeds are subjected to crushing along with its introduction to a steam vessel for lowering of the moisture content present in the seeds. These are then place in hydraulic press mechanical for the extraction of oil. While the latter method is employed in association with the later as mechanical process extract out about only 40% of the total oil present in the seeds. Solvent extraction method is carried out in Soxhlet apparatus along with solvents such as petroleum ethers, heptanes and hexane (Kazeem *et al.,* 2014). Chemically castor oil consists of about 90% of the toal–ricinoleic acid which provides its vast application in the industry such as chemical industry, about three percent oleic acid and about four percent linoleic acid (Patel *et al.,* 2016; Dunford, 2012). Castor imparts lathering property during the formulation of herbal soap. In addition it provides moisturizing, softening and conditioning effect to the soap. It is often referred to as super fat soap (Kazeem *et al.,* 2014).

Neem oil obtained from Neem tree which is botanically called as *Azadirachta indica,* belonging to the family–Meliaceae which is indigenously found in almost all parts of India, Pakistan and other neighboring countries. On a characteristic basics Neem oil is generally yellowish-greenish-brownish in liquid state. The oil consist of an acrid taste and somewhat obnoxious odour. This results because of latency of sulphur as a volatile element (Mongkholkhajornsilp *et al*., 2005; (Dasa Rao and Seshadri, 1941). Extraction methods for extracting out the oil inside the Neem seeds include: Mechanical method, method of extraction using a solvent and SFE- Super critical fluid method (Liauw *et al.,* 2008; Khanam *et al.,* 2017). Neem oil is said to possess anti bacterial properties and is used in treatment of pain as well as inflammation (Subapriya *et al*., 2005). In addition to it, in a herbal soap formulation along with bactericidal action it helps in treatment of various skin diseases such as ringworm, scabies etc (Heukelbach and Feldmeier, 2006; Khan and Wassilew, 1987; Ameh *et al.,* 2013).

Mentha oil botanically from the family Lamiaceae is obtained from the flowring leaves of *Mentha piperita*. This commonly called Peppermint is found in Europe and its cultivation is in almost all regions of the world (Wikipedia, 2011; Saller, 2004). Mentha as an essential oil consists of methyl esters, alcohols, ocimene, terpenes, thujone, acetaldehyde, menthone, iso-menthone, sabinene as its chemical composition (Badal *et al.,* 2011; Barbaljo *et al.,* 2011). This essential oil is said to have potent antibacterial activity against organism such as *E. coli* and it provides greater antioxidant property in the soap formulation (Tsai *et al.,* 2017). Mentha oil as volatile oil help in relaxation of mind as well as help in ameliorate the mood (Paula, 2000).

Materials and Methods

Collection of Materials/Ingredients

Neem oil, Coconut oil, Mentha oil and Castor oil were purchased from the local market. Sodium hydroxide and Distilled water was obtained from Chitkara University, Punjab. Following table (Table1) represent the list of ingredients used for the formulation of Polyherbal soap**.**

Herbal soap making process

The reaction between an alkali (like Sodium Hydroxide) and any neutral fatty acid is the basic saponification reaction. In this reaction neutral fatty acid used was coconut oil, castor oil and alkali used sodium hydroxide (lye).

a). Weighed amount of coconut oil, castor oil, neem oil and mentha oil were poured in a beaker.

b). Weighed separately rose petal extract in a beaker separately and mixed well in distilled water.

c). In another beaker, prepared the basic saponification reaction by adding 6g of NaOH in 10 ml of distilled water.

d). Added the extract solution in the solution of lye and mixed well with the help of magnetic stirrer.

e). Then mix extract and lye was then poured over coconut oil involving basic soap formation with continuous stirring on a magnetic stirrer without heating involving the cold process of soap formation.

f). The soap mixture was then allowed to solidify and kept at room temperature. Sindhu and Kaur, 2018; Hassan *et al.,* 2015).

Evaluation Parameters

pH of the Polyherbal soap: 10% of soap solution was prepared by dissolving 10 gms of soap in distilled water in a volumetric flask of 100 ml. For the determination of pH, pH meter was used. Electrode was introduced into the solution an the pH was noted down (Dalen and Mamza, 2009; Warra *et al.,* 2010).

Colour and clarity characterization: The soap was visualized against a white background for the determination of its color and to see the clarity of the formulated Polyherbal soap (Sindhu *et al.,* 2018).

Foam forming ability : For the determination of the Polyherbal soap for its ability to form foam about 1.0 gm of soap was taken and was dissolved in distilled water (about 50ml) in a 100 ml graduated measuring cylinder. It the measuring cylinder was then shaken for about 2-3 minutes and it was allowed to stand for about 10 min. Foam height was measured after 10 minutes. Record the observation for three consecutive experiment and the mean was taken (Hassan *et al.,* 2015; Isah, (2006).

Retention time of foam : Foam retention time refers to the time for which the foam produced by the soap retains. The above procedure was repeated and the foam interval was measured for about 5-10 minutes (Hassan *et al.,* 2015).

Saponification value determination : The amount of Potassium Hydroxide in milligrams which is required for the complete saponification of fat or oil of 1 gm. In either words it is defined as the mean of molecular weight of fatty acid which is present in oil or fat (Schumtterer *et al.,* 1983). For the determination of saponification value about 2 gm of the soap sample was taken in a conical flask and 0.5M KOH solution was added to it. This mixture was heated to abut 55 degree Celsius along with stirring continuously on a hot water bath. Then the temperature was further increased 100 degree Celsius and boiling was continued for about 1 hour Titration was performed with phenophtlein was an indicator and 0.5M HCl. The end point observed is pink color disappearance. Saponification is calculated as:

$$\text{Saponification Value}: \frac{\text{Avg Volume of KOH} \times 28.056}{\text{Weight of oil (g)}}$$

Determination of moisture content: Determination of moisture content refers to the amount of moisture which is present in the soap sample. Lesser the moisture content more is the stability of the formulation. TO determine the moisture content about 5-6g of soap sample was weighed on a tarred china dish or crucible using analytical weighing balance. It should kept in mind the china dish used should be dried and free from moisture and dried completely. The crucible contain the sample is then heated form about 2 hour at a temperature of 101 degree Celsius (AOC, 1997). Moisture content was analyzed as:

$$\text{Moisture Content} = \frac{\text{Difference in weight} \times 100}{\text{Initial weight}}$$

Determination of TFM (total fatty matter):

The procedure for the analysis of total fatty matter present in the soap sample is carried out by the reaction of the soap with an acid in association of hot water. In this procedure approximately 10g of the soap sample was taken and dissolved in 150 of water (distilled). It was dissolved by heating. Then this soap solution was treated with 20% sulphuric acid and heated till the solution gets cleared. Fatty acids would be observed at the surface or the film which were then solidified by the addition of 7 gms of bees wax and again heated. Cake formation takes place and it was removed and weighed.

$$\% \text{ Total Fatty Matter} = (A - X)/W \times 100$$

where, X= weight of wax

A= weight of wax+ oil

W= weight of soap (Debnath *et al.,* 2011)

Ethanol soluble matter determination : Ethanol soluble matter will be contain anhydrous molecules of soap in addition to the inorganic salts. It is determined by dissolving the soap sample in ethanol and heating it. Initial and weight are subtracted from one another.

Antimicrobial testing of the given sample : The given sample of the soap was tested for its antimicrobial properties. By bore diffusion method. The micro-organism used were *E. coli.* In this method soap solution was prepared by dissolving 1 g of soap in distilled water. Various concentrations were produced such as 5, 10, 20, 50 mg/mL, The antibiotic used is Ciprofloxacin -5µg. The plates were then kept for incubation for about 24 hours at a temperature of 37 ºC. Calculated the zone of inhibition.

Result and Discussion

The Polyherbal soap results of various evaluation parameters are shown in the table 2. The table depicts that the pH of the herbal formation was 6.5-7 which was optimum for its utilization on the skin. Higher as well as lower skin pH refers to the harmful effects on the skin (Mak-Menshah *et al.,* 2011). The foaming index of the given herbal formulation was found to be 16.5 while the foam retention time was found to be 10 minutes. This means the lather producing ability of the soap was satisfactory and stable. The total fatty mater determination was 72%. The quality of soap is represented by the total fatty matter. If the total fatty matter is lower, then it is not optimum for the dry skin. Greater the fatty matter more it helps in moisturizing the skin (Ara *et al.,* 1990). The saponification value was found to be 161.287 mg/ml, and the percentage of moisture content in the herbal soap was evaluated to be 3.5%. Greater the moisture, more will the deterioration of the sample. The ethanol soluble

matter was found to 63.80% as shown in table 2. In addition, the antimicrobial testing was successfully performed as shown in the table 3 with successful inhibition of the micro-organism *E. coli and S. aureus.*

Conclusion

The evaluation parameters carried for standardizing the herbal soap by color determination, pH, TFM, ethanol soluble content, Saponification value were carried out. This led to an outcome of the formulation of stable Polyherbal soap possessing potent antimicrobial activity against various micro-organisms such as *E. coli and S. aureus.* In addition this formulation was found to be used for daily use and did not cause any skin irritation. The blends of various oils in this soap formulation helped in providing specific activity to the formulation possessing potent medicinal properties (Ameh *et al.,* 2013).

Table 1 : Formulation Ingredients of Polyherbal Soap

Sr. No.	Ingredients	Quantity (%)
1.	Coconut oil	40
2.	Castor oil	35
3.	Neem oil	5
4.	Mentha oil	2
5.	Rose petals extract	0.5
6.	NaOH	2.5
7.	Distilled water	q.s

Table 2 : Results of various evaluation parameter

S. No.	EVALUATION PARAMETER	READINGS
1	pH	6.5-7
2	% Total fatty matter	72%
3	% Moisture content	3.5%
4	Saponfication Value	161.28g/ml
5	Ethanol soluble matter	63.80%
6	Foam Index	16.5
7	Foam retention time	10.0 minutes

Table 3 : Antimicrobial property

Sr. No.	Herbal Soap Solution %	MIC (mm) *E. coli*	MIC(mm) *S. aureus*
1.	25	12	14
2.	50	16	20
3.	75	21	29
4.	Antibiotic (Ciprolfoxacin 5µg)	34	40

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