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FORMULATION, EVALUATION AND STABILITY STUDY OF FACE WASH CONTAINING POLYHERBAL EXTRACT

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ABSTRACT

Natural remedies are more acceptable in faith that they are safer with less side effects than the synthetic ones. Herbal formulations have rising demand in the world market. Preventive approaches involve removal of oil from the face. Proper cleaning and washing require for this purpose. Various researches have been proved utility of herbal based formulations for cleaning purpose which also removes excess oil. The present work deals with the development and evaluation of herbal face wash containing extract of Neem Leaves (*Azadirachta indica*), Lemon Peel Powder (*Citrus Lemon*), and Liquorice (*Glycyrrbiza Glabra*), Coriander (*Coriandrum Sativam*) distillate using Carbopol 314. Although various topical herbal formulations are available in the market, we propose to make poly herbal formulation using different herbal and synthetic ingredient. Herb was evaluated for various parameters like Qualitative investigation, preliminary phytochemical investigation and Thin layer chromatography of herb and prepared formulation was evaluated for parameter like colour, odour, appearance, consistency, pH, viscosity, feel on application, spreadability, washability, grittiness. The results of quality control parameters were found to be satisfactory.

KEYWORDS: Polymer, TLC, Neem leaves, Lemon Peel Powder, Coriander, Liquorise.

1. INTRODUCTION

The term Cosmeceuticals was first used by Raymond Reed founding member of US Society of Cosmetics Chemist in 1961. He actually used the word to brief the active and science based cosmetics. The above term was further used by Dr. Albert Kligman in the year 1984 to

refer the substances that have both cosmetic and therapeutic benefits. Cosmeceuticals are cosmetic pharmaceutical hybrids intended to enhance health and beauty through ingredients that influence the skin's biological texture and function.

The herbal healing has been mentioned from the ancient era, from Vedas, and even from ancient religious work. Probably it is the oldest medical care system in the world. The herbal healing deals with use of herbs, herbs extracts or natural products for the betterment of health condition. Nowadays in westerns countries medical practitioners also prescribing medicines containing plant extract.[1,2]

The therapeutic use of medicinal plants has gained considerable momentum in the world during the past decade. The overuse of synthetic drugs with impurities results in higher incidence of adverse drug reactions in more advanced communities has motivated mankind to go back to nature for safer remedies.

However, it should be ensured that commercial formulations based on medicinal plants are safe, effective and of standard quality. Today, over the world, there is a great deal of interest in Ayurvedic system of medicine and thus the demand for various commonly used medicinal plants in the production of Ayurvedic medicine is ever increasing.[2]

Although, herbal products are more acceptable with belief that they are safe posses many therapeutic properties and having no or less side effects as comparing to modern chemical entities.[9,10]

Acne may cause long-lasting and detrimental psychosocial and physical effects. It is associated with depression and anxiety, regardless of disease severity, although the psychological effects usually improve with treatment.

Azardirachta indica, Citrus limon, Glycyrrhiza glabra, Coriandrum sativam, a well known traditional medicinal plant used in various indigenous system of medicines, also known as Neem, Lemon, Liquorice and Coriander respectively.[6,9] Face wash should leave skin feeling clean and soft while also looking bright. Some cleansing ingredients may be stronger on the skin; this can be benefit for people who have oilier skin, but a determinant to those with dry skin.

2. MATERIAL AND METHODS

2.1 Collection of Raw Material and Finished Product.

Herbal Face wash contains 4 ingredients viz., aqueous extract of *Azardirachta indica, Citrus limon, glycyrrhiza glabra, Coriandrum sativam* All these plants were collected from Pioneer Pharmacy Degree College, Sayajipura, Vadodara.

2.2 Preparation of herbal extracts.

20 gm of the prepared material macerated with 100ml of water, shaken frequently and allowed to stand for 24 hrs. Thereafter filtered, evaporated the filtrate to dryness and weight was taken.[6,14,15]

2.3 Preparation of Face wash.

Carbopol 334 was swelled in water along with extract and then stirring should be done to mix the carbopol934 to form gel. After swelling of carbomer, glycerine was added. To the above mixture, Sodium lauryl sulphate (SLS) was introduced and gently mixed. Few drops of NaoH and methyl paraben as a preservatives add and kept the beaker aside for 1 day.[6,7,14,15]

2.4 Physicochemical parameters

2.4.1 Moisture content/Loss on drying: Placed 10 gm drug in a tarred evaporating dish and dried at 105ºC for 5 hours till constant weight. Percentage moisture content was calculated on the basis of weight loss and original weight of sample.[25]

2.4.2 Determination of ash

Total ash: Placed 2 g air dried material in a previously ignited tarred crucible (usually platinum or silica). Spread material in even layer and ignited by gradually increasing the heat to 500- 600ºC (480ºC) until it was white, indicating the absence of carbon. Cool in desicator and weigh. If carbon free ash cannot be obtained, the crucible was cooled and residue was moisten with 2 ml of water or a saturated solution of ammonium nitrate. It was dried on a water bath or on a hot plate and ignited to constant weight. Allowed to cool in desicator for 30 minutes, and weigh without delay. The percentage of total ash was calculated with reference to air dried plant material.[25]

Acid insoluble ash: Add 25 ml of hydrochloric acid to ash containing crucible and covered with a watch glass. boiled gently for 5 minutes. The watch glass was rinsed with 5 ml of hot water and added this liquid to the crucible. The insoluble matter was collected on an ash less

filter-paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible. It was dried on a hot plate and ignited to constant weight. The residue was allowed to cool in desicator for 30 minutes, and it was weighed without delay. The percentage of acid insoluble ash was calculated with reference to air-dried plant material.[25]

2.4.3 Determination of extractive matter

Alcohol soluble extractive: Place 4.0 g of air dried powdered material in a glass stoppered conical flask and macerated with 100 ml of the alcohol (ethanol or methanol) in closed flask for 24 hrs. It was shaken frequently for the first 6 hrs and allowed to stand for 18 hours. Then it was filtered rapidly taking care not to lose any solvent and then transferred 25 ml of the filtrate to tarred flat bottomed dish and evaporated to dryness on water bath. It was dried at 105^0C for 6 hours, cooled in a desiccators for 30 minutes and weighed without delay. The percentage of alcohol soluble extractive was calculated with reference to air-dried drug.[25]

Water soluble extractive: Place 4.0 g of air dried powdered material in a glass stoppered conical flask and macerated with 100 ml of the water in closed flask for 24 hrs. It was shaken frequently for the first 6 hrs and allowed to stand for 18 hours. Then it was filtered rapidly taking care not to lose any solvent and then transferred 25 ml of the filtrate to tarred flat bottomed dish and evaporated to dryness on water bath. It was dried at 105^0C for 6 hours, cooled in a desiccators for 30 minutes and weighed without delay. The percentage of water-soluble extractive was calculated with reference to air-dried drug.[25]

2.5 Preliminary phytochemical investigation

The leaves, fruit, peel,and root of *Azardirachta Indica, Coriandrum Sativam, citrus Limon and Glycyrrhiza Glabra respectively* were dried under shade and powdered with mechanical grinder. Dried material was Successively extracted with Water and after complete extraction (24 hr) and resulting powder and semisolid mass obtain. Extracts of *all above four drugs* were subjected to preliminary phytochemical screening for the detection of various plants constituent. The preliminary phytochemical investigations were done by the standard chemical tests.[6,18,19]

Test for alkaloids

The small portion extracts were stored separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal agents, such as Mayer's reagent (cream precipitate) and Dragendorffs reagent (orange brown precipitate).[3,4,19]

Test for carbohydrates and glycosides

Small quantity of extracts were dissolved separately in 5 mL of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the carbohydrates.

Another small portion of extract was hydrolyzed with dilute hydrochloric acid for few hours in a water bath and was subjected to Liebermann-Burchard's, legal and Borntrager's test to detect different glycosides. (Pink to red color indicates presence of glycosides).[4,19]

Test for flavonoids

5 mL of dilute ammonia solution were added to a portion of aqueous filtrate of plant extract followed by addition of concentrated H2SO4.

A yellow coloration observed in extract indicated presence of flavonoids.

Test for steroids 2 mL acetic anhydride was added to 0.5 g extracts with 2mL H2SO4.

The color changed from violet to blue or green in samples indicated presence of steroid.[19,21]

Test for terpenoids (Salkowski test)

Five ml of extracts were mixed in 2 mL of chloroform, and then concentrated H2SO4 (3 mL), was carefully added to form a layer.

A reddish brown coloration formed at the interface indicated presence of terpenoids.[3,4,19]

Test for saponin

About 1 mL of extract were diluted with distilled water to 20 mL and shaken in graduated cylinder for 15 minutes. One cm layer of foam indicated presence of saponin.[3,4]

Test for tannin

When extract were treated with vanillin-hydrochloric acid reagent, pink or red colour was formed due to formation of phloroglucinol.[14,19,21]

Test for protein

Millon's reaction: Millon's reagent (mercuric nitrate in nitric acid containing a trace of nitrous acid) usually yields a white precipitate on addition to a protein solution, which turns red on heating.[19-20]

Test for Steroids (Lieberman's test)

0.5gm of hydro-methanolic plant extract was dissolved in 2ml of acetic anhydride, cooled in ice bath, conc. H_2SO_4 was added. Color changed from violet to blue or green indicates presence of steroid compounds.[14,19,20]

2.6 Preparative thin layer chromatography of water extract of *Azardirachta indica, glycyrrhiza glabra and Coriandrum sativam*.

Ordinarily, microgram quantities of mixture of organic compounds are separated by analytical TLC. It is possible to scale up the quantities to milligram amount (10-50 mg) by using thicker layer (0.5-2.0 mm thickness) of the support material and by the use of larger plates (20 x 20 cm or 20 x 40 cm). Multiple developments also bring about better resolution.

Preparative TLC for the isolation of marker compound from the water extract of *Azardirachta indica, glycyrrhiza glabra and Coriandrum sativam* was perform by using solvent system Toluene: Ethyl acetate: Glacial acetic acid(5: 5:1), Chloroform: Methanol: water (65:35:10), toluene: ethyl acetate (93: 7) respectively.[14,17,18]

Detection

Detection of active constituent by spraying of Vanillin sulphuric acid reagent.

It is composition of solution 1 (1% Ethanolic vanillin), and solution 2 (10% Ethanolic sulphuric acid).

The plate was sprayed with 10 ml (solution 1), followed immediately by (solution2). After heating at 110℃ for 5- 10 min under observation, the plate was evaluated in visual light.[14,17,18]

2.7 Evaluation of quality control parameters for finished product

2.7.1 Description: The general appearance, its visual identity is essential for consumer acceptance, for control of lot-to-lot uniformity and for monitoring trouble free manufacturing. The control of the general appearance of syrup involves the measurement of colour, taste etc.

2.7.2 Spreadability test

The spreadability is expressed in terms of time in seconds taken by two slides slip off from the gel, placed in between the slides, under certain load. The spreadiability is good if the slipping time is less. Two sets of glass slides of standard dimensions were taken. The herbal gel formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 4.7 cm along the slide. A 40gm weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 4.7 cm and separated away from the lower slide under the influence of the weight was noted.[3,20,21]

Formula: $S = M \times LT$

Where, **S**=Spread ability, **M**= Mass in gm (30gm), **L**=Length of the glass (6cm), **T**= Time in sec.

2.7.3 Washability

The washability was determined using formulation already applied on skin and then ease and extent of washing with water were checked manually. Washability was determined by applying gel on hands and exposed to running water.[3,20,21]

2.7.4 Grittiness

Grittiness of the gel was checked for the presence of any gritty particles by applying it on the skin.[3,4,20,21]

2.7.5 Stability study

The stability of the gels was tested using freeze thaw cycling method. The gels were subjected to a temperature of 4°C for 7 days and room temperature for 7 days.[3,20,21]

2.7.6 Measurement of Viscosity: Viscosity of any liquid is measured by comparing it with the viscosity of water. It is measured by Oswald viscometer. At first the viscosity of water is measured. Take water in Oswald viscometer fill up the water and shuck it up to mark A then note the time for water run upto mark A to B. Then do the same for any liquid. Viscosity of water is taken as standard which is 1.Viscosity of any liquid is measured by following formula.[26]

$$\frac{\eta_1}{\eta_2} = \frac{t_1}{t_2}$$

where, η_1 and η_2 are the viscosity of water and liquid respectively while t_1 and t_2 are time taken for reaching water or liquid from mark A to mark B in second. Unit is given in terms of centipoises. Viscosity was measured using the Brook Field Viscometer.

2.7.7 Determination of pH: The pH value of a solution was determined potentiometrically by means of a glass electrode, a reference electrode and a digital pH meter. The pH meter was operated according the manufacturer's instructions. First the apparatus was calibrated using buffer of 4, 9 and 7 pH. One ml syrup was taken in 100 ml demineralised water. The electrodes were immersed in the solution and measured the pH 2.[3,20]

3. RESULT AND DISCUSSION

3.1 Identification and Collection of plants

All the extracts were vacuum dried and in powder form which was used in Herbal Face wash. Colour, odour and taste of all these ingredients are given in Table I.

Table I: Organoleptic characters of ingredients present in Herbal Face Wash.

Sr.No.	Ingredients	Part used	Description		
			Colour	Odour	Taste
1	*Glycyrrhiza glabra*	Root	Brown	Characteristic	Sweet
2	*Coriandrum sativum*	Fruit	Brown	Characteristic	Aromatic
3	*Citrus Lemon*	dried outer part of pericarp	yellow	Characteristic	Bitter
4	*Azarditchta indica*	Leaf	Green	Characteristic	Bitter

Percentage yield and physical characteristics of aqueous extracts of all ingredients used in Herbal Face wash. The results are described in Table no II

Table II: Preliminary phytoprofiles of Aqueous extract of ingredients of Herbal Face wash.

Sr. No.	Extract	State	Colour	Yield(%)
1	*Glycyrrhiza glabra*	solid	Cream	9
2	*Coriandrum sativum*	solid	Brown-black	6
3	*Citrus Lemon*	Semi solid	Brown	9.5
4	*Azarditchta indica*	Soli d	Dark green	11.5

3.2 Qualitative Phytochemical evaluation All the ingredients are subjected for the various phytochemical tests. Results are given in Table III and Fig. 1 to 8.

Table-III: Describes preliminary phytochemical investigation of various extracts of herb.

Chemical constituent	Neem	Coriander	Liquorice	Lemon peel
Alkaloids	+	-	+	+
Carbohydrate	-	+	-	+
Reducing sugar	-	+	-	-
Steroids	+	-	+	+
Glycoside	-	-	+	+
Flavonoids	+	-	+	+
Terpenoids	-	+	-	-
Saponins	-	-	+	-
Proteins	-	+	-	+
Tannins	+	-	+	+

Fig. 1 Mayer's test.

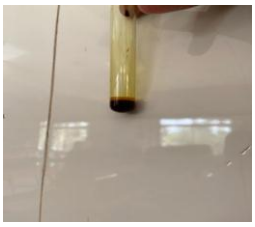

Fig. 2 Molish's test.

A

B

Fig. 3 Presence of flavonoids and Steroids.

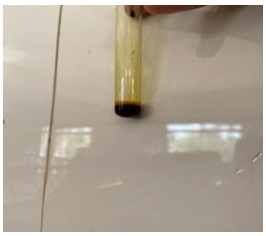

Fig. 4 Presence of terpenoids in Neem.

Fig. 5 Presence of saponins in Liquorice.

Fig. 6 Presence of tannin in Lemon peel and Liquorice

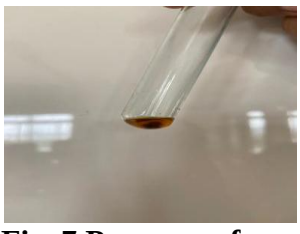

Fig. 7 Presence of protein in Lemon peel, Neem and Coriander

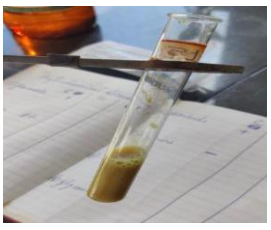

Fig. 8 Lieberman's test

3.3 Evaluation of Quality Control Parameters for Finished Product

Quality control parameters like description, pH, viscosity, specific gravity.

3.3.1 Moisture content and Extractive value

Physicochemical parameters like Moisture contents and Extractive value of ingredients of Herbal Face wash are given in the Table IV.

Table IV: Moisture content and Extractive values present in Herbal Face wash.

Sr. No.	Ingredients	Moisture content (%)	Water soluble Extractive value (%)	Alcohol soluble extractive value (%)
1	*Glycyrrhiza glabra*	5.4	3.50	3.25
2	*Coriandrum sativum*	9	18.2	16.5
3	*Citrus Lemon*	8.2	6.50	0.75
4	*Azarditchta indica*	6.4	21.12	18.32

3.3.2 Ash value

Ash value of all the individual ingredient of Herbal Face wash is given in the Table V.

Table V: Total Ash Content and Acid insoluble Ash.

Herb	Total ash(%)	Acid Insoluble Ash(%)
Azarditchta indica	11.5%	2.37%
Glycyrrhiza glabra	2%	3.64%
Citrus Lemon	12.5%	3.02%
Coriandrum sativum	4%	0.51%

3.3.3 TLC study of Herbal Face wash ingredient

Preparative TLC for the isolation of marker compound from the water extract of *Azardirachta indica, glycyrrhiza glabra and Coriandrum sativam* was perform by using solvent system Toluene : Ethyl acetate : Glacial acetic acid(5: 5:1), Chloroform: Methanol: water (65:35:10), toluene: ethyl acetate (93: 7). Detection of active constituent by spraying of Vanillin sulphuric acid reagent shown in Fig 9. Rf value of given ingredients given in Table VI fulfilling the Herbal Pharmacopoeial limit.

Fig. 9: TLC of Neem, Coriander, Licorice.

Table VI: Rf value of herbs.

Herbs	Rf Value
Neem	0.06
Coriander	0.48
Liquorice	0.11

3.3.4 Formulation of Face wash

The composition of Face wash developed in the laboratory is given in table VII.

Table-VII: Composition of formulation.

S.N.	INGREDIENT	QUANTITY
1	Drug extract	3
2	Carbapol 314	2.5
3	Methyl paraben	1
4	NaoH	0.2
5	Glycerine	2
6	Sodium lauryl sulphate	2.5
7	Peppermint oil	0.5
8	Water	q.s.

3.3.5 Evaluation of Quality Control Parameters for Finished Product

Quality control parameters like description, pH, viscosity, spreadability, washability, grittiness and stability given in Table VIII, IX and X.

Table-VIII: Physical parameters of face wash.

Physical parameters	Inference
Colour	Reddish brown
Odour	Characteristic
Appearance	Translucent
Feel on application	Smooth & slippery
pH	4.40

3.3.7 Evaluation Parameter of formulation

Table-IX: Quality parameters of formulation.

S.N.	Parameter	Observation
1.	Grittiness	Nil
2.	Washability	Washable
3.	Spreadability (gm.cm/sec)	15.66

Table-X: The studies of formulation Stability.

Formulation	pH		Viscosity(cps)	
Face wash	4℃	R.T.	4℃	R.T.
	4.36	4.4	1300	1256

The values shown that Face wash was easily spreadable, washable. No gritty particles were observed in the formulation. On storage of sample at 4℃ and room temperature the appearance of formulation was found to be clear with no significant change in pH and viscosity.

4. CONCLUSION

Skin disease is very common and the need to prevent or treat the disease is in great demand. In the present scenario, people need remedy for skin disease without side effects. Herbal ingredients opened the way to formulate cosmetics without harmful effect, which can impart the required properties to heal the skin disease and the expense will be less when compared with the synthetic products. This study aimed at developing herbal Face wash for anti acne treatment using seed extracts of *Azardirachta indica, Citrus limon, glycyrrhiza glabra, Coriandrum sativam* in Carbopol gel system. Desired formulation of the Face wash was prepared and evaluated for their physicochemical properties, like color, odour, pH, spreadability, viscosity, grittiness. It was concluded that the present research might hopefully bring advancement in the treatment of acnes using herbs as well as in developing herbal formulations for safe and effective management of diseases. Study also concluded that prepared herbal Face Wash possess all essential features of Face wash formulation for topical application.

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