FORMULATION OF HERBAL SHAMPOOS FROM *ASPARAGUS RACEMOSUS, ACACIA CONCIN, SAPINDUS MUKOROSSI*

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ABSTRACT

All shampoos are basically water and detergent mixtures. The main objective of this study was to eliminate harmful materials from shampoo formulation and substitute them with a safe natural product. Formulators must play an active role in educating the consumers about the potential harmful effects of synthetic detergents and other chemical additives present in shampoos. We had taken three plants extract to formulate the herbal shampoo. The taken extracts of plant were *Asparagus racemosus, Acacia concin, Sapindus mukorossi.* Defatted air-dried plants powders were extracted with methanol in soxhlet apparatus set at 60^{0}C for 24 hours. The solvent was evaporated at 50^{0}C using rotary vacuum. The phytochemical screening was done to identify the natural phytochemical in these three plant extracts. The identification of all phytochemicals was finished through TLC. To formulate a clear shampoo base, definite amounts of saponin and salt were added to an aqueous solution containing extracts and juices along with glycerin (1%), methyl paraben (0.05%) and EDTA (0.15%) etc. Formulation was prepared by slightly heating and adding the weighed quantity of herbal ingredients extracts and juices. The pH of the Shampoo was adjusted to 5.5, to retain the acidic mantle of scalp. Synthetic preservatives have sometimes been the cause of adverse effects among consumers. We had used the physico-chemical approach toward preservation and by formulating a self preserving shampoo and it avoided this risk posed by chemical preservatives.

Keywords: Shampoo, formulation, consumer, plants extract, soxhlet apparatus, rotary vacuum, herbal ingredients, synthetic preservatives.

INTRODUCTION

The challenge lies in selecting materials that can be rationally justified as herbal and formulating them into cosmetics whose functionality is comparable with their synthetic counterparts. This is related to hair cleansing and conditioning compositions and methods of making and using thereof. More particularly, the invention relates to hair cleansing and conditioning compositions that incorporate herbal extracts. Herbal extracts are used for a variety of reasons and are chosen based on their particular properties. Shampoo shave primarily been products aimed at cleansing the hair and scalp. Selected ingredients of shampoo that have been popular with the consumer are currently under attack because of potential risks associated with their use. So to provide quality hair care products with focus on purity, effectiveness and safety with ethical method of manufacturing it was planned to develop an herbal shampoo preparation.

MATERIALS AND METHODS

1. Collection of Plant Materials

Plant's powders of *Asparagus racemosus, Acacia concin, Sapindus mukorossi* and *Glycyrrsia glabera* were collected from herbal store of our Institute. The leaves and flowers of *Azadiraachta indic, Bassia malabarica* and *Hibiscus rosasinesis* plants are being collected from the herbal garden. The leaves and flowers were dried at room temperature under a well ventilated shade by distributing them homogeneously. Communution were done by grinding in a mixture and the material was passed through sieve no. 40 to get a uniform powder.[1]

2. Extraction Procedures

Using the soxhlet apparatus and petroleum ether as the solvent, all plants powders were defatted at 45^{0}C for 12 hours. The solvent was evaporated at 50^{0}C using rotary vacuum evaporator (Buchi type).

(a) **Extraction of *Glycyrrsia glabera, Azadiraachta indica, Hibiscus rosasinesis* and *Bassia malabarica*:** Defatted air-dried plants powders were extracted with methanol in soxhlet apparatus set at 60^{0}C for 24 hours. The solvent was evaporated at 50^{0}C using rotary vacuum evaporator to obtain a semisolid extract and stored in a deep freezer at -18^{0}C. The total methanolic extract was suspended in 1:1 ratio of water and methanol for a period of 6 hours on a shaker. Methanol was evaporated at 70^{0}C using rotary vacuum evaporator. This extract was air dried for 12 hours at room temperature and then stored in a deep freezer at -18^{0}C. The extract was then tested for the presence of alkaloids, glycosides, and other phytochemical constituents.[2]

(b) **Extraction and Isolation of saponins from *Asparagus racemosus, Acacia concin* and *Sapindus mukorossi*:** Defatted air-dried plants powders were extracted

with methanol in soxhlet apparatus set at 60^{0}C for 24 hours. The solvent was evaporated at 50^{0}C using rotary vacuum evaporator to obtain a semisolid extract and stored in a deep freezer at -18^{0}C. The total methanolic extract was suspended in 1:1 ratio of water and methanol for a period of 6 hours on a shaker. Methanol was evaporated at 70^{0}C using rotary vacuum evaporator. This extract was air dried for 12 hours at room temperature.

Isolation of saponins was conducted in several stages. First**,** defatted air-dried plants powders were extracted with methanol in soxhlet apparatus set at 60^{0}C for 24 hours, yielding a reddish crude extract. This methanolic extract, after concentration, was dissolved in a minimum amount of distilled water and decant several times with n-butanol. In the final stage, the total saponin present in butanolic extract was precipitated using diethylether and then filtered. The extract was then tested for the presence of saponins.[2]

(c) **Preparation of juices of *Citrus reticulate, Emblica officinalis, Aloe vera*:** Fruits of Orange, fruits of Amla and leaves of Aloe vera were cut to small picas using stainless steel knife. These picas were crushed in electrical juicer. The collected juice strained with a cloth and then filtered using a vacuum pump. The filtered juice was concentrated (1 letter of juice to 250ml) using rotary vacuum evaporator.

3. Tests for Identification of Phytochemical Constituents

(a) Reagents preparation

I. **Dragendorff's reagent:** It is used for detection of alkaloids. Boil 14g of sodium iodide with 5.2g basic bismuth carbonate in 50 ml glacial acetic acid for few minutes. Allow it to stand overnight and filter of the precipitate of sodium acetate crystals. To 40 ml of the red-brown filtrate add 160 ml of ethyl acetate and 1 ml water preserve the stock solution in amber-colored bottle. When needed add 20 ml of acetic acid to 10 ml of this stock solution and make up to 100 ml with water.[3]

II. **Fehling's solution:** It is used for detection of reducing sugars. Dissolve 34.66g of copper sulphate in distilled water and make volume up to 500 ml (solution-A). Dissolve 173g of potassium sodium tartarate and 50g of sodium hydroxide in distilled water and make volume up to 500 ml (solution-B). Mix two solutions in equal volume prior to use.[3]

III. **Ferric chloride (alcoholic):** A 5% w/v solution of ferric chloride in 90% alcohol and used for detection of phenols.[3]

IV. **Hagers reagent:** A standard aqueous solution of picric acid used for detection of alkaloids.[3]

V. **Lead acetate:** A 25% basic lade acetate solution is used for detection of flavonols.[3]

VI. **Mayer's reagent:** It is used for detection of alkaloids. Dissolve 1.36g of mercuric chloride in 60 ml distilled water (A). Dissolve 5g of potassium iodide in 20 ml distilled water (B). Mix (A) and (B) and adjust the volume to 100 ml with distilled water.[3]

VII. **Millon's reagent:** It is used for detecting agent of protein. Dissolve 1g of mercury in 9 ml of fuming nitric acid, keep the mixture well cooled during the reaction. When action is complete, add equal volume of distilled water.[3]

VIII. **Molisch's reagent:** Dissolve 10g of alpha-naphthol in 100 ml of 95% alcohol. It is used for detection of carbohydrates.[3]

IX. **Ninhydrin reagent:** It is used for detection of amino acid. Prepare 0.1% solution in n-butanol.[3]

X. **Wagener's reagent:** It is used for detection of alkaloids. Dissolve 1.27g of iodine and 2g of potassium iodide in 5 ml of distilled water and make up volume to 100 ml with distilled water.[3]

(b) General tests for detection of chemical constituents

I. **Tests for alkaloids:** stir a small portion of the solvent free chloroform, alcoholic and water extract separately with a few drops of dilute HCl and filter. The filtrate may be tested carefully with various alkaloidal reagents such as Mayer's reagent (cream precipitate), Dragendorff's reagent (orange brown precipitate), Hagers reagent (yellow precipitate), and Wagener's reagent (reddish brown precipitate).[4]

II. **Test for carbohydrates:** Dissolve small amount (200mg) of alcoholic and water extract separately in 5 ml of distilled water and filter. The filtrate may be tested carefully with Millon's reagent.[4]

III. **Millon's test:** To 2-3 ml water extract, add few drops of alpha-naphthol solution in alcohol, shake add conc. H_2SO_4 from sides of the test tube. Violet ring is formed at the junction of two liquids.[4]

IV. **Fehling's test:** Mix 1 ml Fehling's A and 1 ml Fehling's B solution, boil for 1 minute. Add equal volume of test solution. Heat in boiling water bath for 5-10 minutes. First a yellow, then brick red precipitation is observed.[4]

V. **Tests for glycosides:** Hydrolyse small portion of the extract with dilute hydrochloric acid for a few hours in water bath and subject the hydrolysate to Liebermann- Burchard's, Legal's and Borntrager's test.[4]

VI. **Liebermann- Burchard's test:** Mix 2 ml extract with 2 ml chloroform. Add 2 ml acetic anhydride and 2 drop conc. H_2SO_4 from the side of test tube. First red, then blue and finally green color appears.[4]

VII. **Legal's test:** To aqueous or alcoholic extract, add 1 ml pyridine and 1 ml sodium nitroprusside. Pink to red color appears.[4]

VIII. **Borntrager's test:** To 3 ml water extract, add dil. H_2SO_4. Boil and filter. To cold filtrate, add equal volume benzene or chloroform. Shake well. Separate the organic solvent, add ammonia. Ammoniacal layer turns pink or red.[4]

IX. **Test for phenolic compounds and tannins:** To 2-3 ml water extract or alcoholic extract, add few drops of following reagent:

a) 5% $Fecl_3$ solution: deep blue black color.
b) Lead acetate solution: white ppt.
c) Gelatin solution: white ppt.
d) Bromine water: decoloration of bromine water.
e) Acetic acid solution: red color solution.
f) Potassium dichromate: red ppt.
g) Dil. iodine solution: transient red color.
h) 1 drop NH_4OH, excess 10% $AgNO_3$ solution. Heat for 20 minutes boiling water bath. White ppt observed then dark silver mirror deposits on wall of test tube.[4]

X. **Tests for proteins and amino acids:** To 2-3 ml water extract or alcoholic extract, add few drops of water and subject the solution to Millon's Biuret and Ninhydrin test.[4]

XI. **Millon's Biuret test:** Mix 2 ml extract with 5 ml Millon's reagent. White precipitation. warm precipitation turns brick red or the precipitation dissolve giving red color solution.[4]

XII. **Ninhydrin test:** Heat 3 ml extract and 3 drops 5% ninhydrin solution in boiling water bath 10 minutes. Purple or bluish color appears.[4]

XIII. **Tests for saponins:** Dilute 1 ml of water extract or alcoholic extract separately with distilled water to 20 ml and shake in a graduated cylinder for 15 minutes. A one centimeter layer of foam indicates saponins.[4]

XIV. **Hemolytic test:** Add extract to one drop of blood placed on glass slide. Hemolytic zone appears.[4]

4. Thin Layer Chromatography of Saponins

Thin-layer chromatography (TLC) was performed using silica gel 60 F_{254} 20 x 20 cm plates, layer thickness 250 µm (Merck KgaA, Darmstadt, Germany).

(a) TLC profile of *Sapindus mukorossi* saponins: Precoated TLC plates were used. Extract was dissolved in 2ml of n-butanol. About 1µl volume of sample solution was applied, and developed with a ethyl acetate-methanol-water (81:11:8). Spots on TLC plates were developed using anisaldehyde/sulfuric acid spray reagent (465 ml of ethanol, 5 ml of acetic acid, 13 ml of p-anisaldehyde, and 13ml of sulfuric acid mixed in order), heated at 110^0C for 10 minutes and visualized under visible light to calculate Rf values.[5]

(b) TLC profile of *Acacia concina* and *Asparagus racemosus* Saponins: Precoated TLC plates were used. 10mg extract were dissolved in 2ml of n-butanol. About 1µl volume of sample solution was applied, and developed with a chloroform-methanol (80:20). Spots on TLC plates were developed using anisaldehyde/sulfuric acid spraying reagent (1:2), plates were heated at 110^0C for 10 minutes and visualized under visible and light to calculate Rf values.[6]

5. Formulation of Herbal Shampoos

To formulate a clear shampoo base, definite amounts of saponin and salt were added to an aqueous solution containing extracts, and juices along with glycerin (1%), methyl paraben (0.05%) and EDTA (0.15%) etc. Formulation was prepared by slightly heating and adding the weighed quantity of herbal ingredients (extracts and juices).

The components and percentage of ingredients used within the final formulations are listed in Table 1. Herbal extracts were diluted with distilled water then glycerin, EDTA, xanthan gum and methyl paraben were added with steering. Juices were mix on mechanical shaker for 20 minutes. Extracts are mix with slow steering on a magnetic stirrer. Then orange oil was added and mixed with slow steering on a magnetic stirrer. Volume made up to 100 ml with distilled water.[7, 8]

RESULTS AND DISCUSSION

1. Selection of Herbs and Plant Materials for Formulation of Shampoos

The diversity of qualities demanded from a good shampoo by today's consumer goes far beyond this general function. Selected ingredients of shampoo that have been popular with the consumer are currently under attack because of potential risks associated with their use. Reasons for selection of plants are given in Table 2.

Table 1: Formulations of herbal shampoos

Compounds	F_1 (%w/v)	F_2 (%w/v)	F_3 (%w/v)
Sapindus mukorossi (extract)	20	-	-
Asparagus racemosus (extract)	-	20	-
Acacia concina (extract)	-	-	20
Azadiraachta indica (extract)	5	5	5
Hibiscus rosasinesis (extract)	5	5	5
Bassia malabarica (extract)	10	10	10
Glycyrrsia glabera (extract)	10	10	10
Emblica officinalis (juice)	10	10	10
Aloe vera (juice)	10	10	10
Citrus reticulate (juice)	10	10	10
Xanthan gum	2	2	2
Glycerin	1	1	1
EDTA	0.15	0.15	0.15
Methyl paraben	0.05	0.05	0.05
Orange oil	q.s.	q.s.	q.s.
Distilled water q.s. to make	100 ml	100 ml	100 ml

Table 2: List of plants selected for formulation of herbal shampoos

Latin Name	Common Name	Reasons for selection
Asparagus racemosus	Asparagus	Saponins rich source, foaming agent.
Acacia concina	Soap pod tree	Remove dandruff, good detergent, promotes hair growth.
Sapindus mukorossi	*Soap Nut Tree*	Foam rich, pleasant aroma, dandruff control, good detergent.
Azadiraachta indica	*Margosa tree*	Antifungal, Antibacterial.
Hibiscus rosasinesis	China rose	Stimulate thicker hair growth and prevent premature graying of hairs, prevents hair loss, used in scalp disorders, recreate pigmentation of hair.
Emblica officinalis	Gooseberry	Source of vitamin 'C' and rejuvenator action, strengthens hair.
Aloe vera	Aloe	Nourishing and conditioning agent.
Citrus reticulate	Orange	Anti-oxidant, anti-microbial, and anti-inflammatory.
Glycyrrsia glabera	Licorice	Anti-inflammatory, removal of skin stains, preservative.
Bassia malabarica	Mahua	Hair growth stimulant, preservative.

2. **Identification Test of Phytochemical Constituents**

The phytochemicals constituents present in crude herbs are summarized in Table 3.

3. Thin Layer Chromatographic Analysis of Saponins

The data of thin layer chromatographic study of saponins is tabulated in Table 4. The qualitative separation of saponins by TLC revealed the presence of 11 spots in *Sapindus mukorossi*, 6 spots in *Acacia concina* and 3 spots in *Asparagus racemosus.* Sharma and Patel, 2009 performed the TLC of saponins and found that Rf value was 0.272 for S***. mukorossi:*** 0.250 for A***. concina and*** 0.285 for A***. racemosus*** due to presence of saponins. TLC profile of this investigation was similar to that reported in literature**.**

Table 3: General observation tests

Plant extract	Alkaloids	Carbohydrates	Glycosides	Proteins & amino acids	phenolic compounds and tannins	Saponins
Asparagus racemosus	+	+	+	+	+	+
Acacia concina	+	+	+	+	+	+
Sapindus mukorossi	-	+	+	+	-	+
Azadiraachta indica	+	+	+	+	-	-
Hibiscus rosasinesis	-	+	+	-	-	-
Emblica officinalis	-	+	-	+	+	-
Aloe vera	-	+	+	-	-	+
Citrus reticulate	-	+	+	+	-	-
Glycyrrsia glabera	-	+	+	-	+	+
Bassia malabarica	+	-	+	-	+	+

+, present; -, absent

Table 4: Qualitative separation of Saponins

S. No.	Colour of spot	Rf values	*S. mukorossi*	*A. concina*	*A. racemosus*
1	Yellow	0.066	-	-	+
2	Brown	0.080	-	+	-
3	Brown	0.150	-	+	-
4	Brown	0.160	+	-	-
5	Brown	0.250	-	+	-
6	Brown	0.272	+	-	-
7	Yellow	0.285	-	-	+
8	Yellow	0.304	+	-	-
9	Yellow	0.400	+	-	-
10	Brown	0.450	-	+	-
11	Yellow	0.464	+	-	-
12	Dark gray	0.512	+	-	-
13	Dark gray	0.584	+	-	-
14	Dark gray	0.666	+	-	-
15	Dark gray	0.728	+	-	-
16	Dark gray	0.768	+	-	-
17	Dark gray	0.810	-	+	-
18	Dark brown	0.824	+	-	-
19	Dark yellow	0.857	-	-	+
20	Dark brown	0.910	-	+	-
+, Present;-, Absent					

CONCLUSION

As seen from the results, it is possible to formulate a completely herbal shampoo that is better than the synthetic ones. The commercial herbal shampoos may contain excessive detergents, which can strip the hair of up to 80% of the oil and thus damage the hair. Using a mild detergent in our shampoo we have ensured that this does not happen. Instead of using cationic conditioners we have used aloe-vera gel and other plant extracts to provide the conditioning effects.

These are not only safer than the chemical conditioning agents, but also greatly reduce the protein loss during combing. The pH of the shampoo was adjusted to 5.5, to retain the acidic mantle of scalp. Synthetic preservatives have sometimes been the cause of adverse effects among consumers. We have used the physico-chemical approach to preservation and by formulating a self preserving shampoo, have avoided this risk posed by chemical preservatives.

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