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FORMULATION AND EVALUATION OF POLYHERBAL EXTRACT FOR SKIN HYPERPIGMENTATION AS GEL ADVANCED DELIVERY SYSTEMS

Maged Alwan Noman[1], Mahmoud Mahyoob Alburyhi[1]*, Abdalwali Ahmed Saif[1] and Tawfeek A. A. Yahya[2]

[1]Professor Dr. of Pharmaceutics and Industrial Pharmacy, Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Sana'a University, Sana'a, Yemen.

[2]Professor Dr. of Medicinal Chemistry and Drug Design, Department of Medicinal Chemistry, Faculty of Pharmacy, Sana'a University, Sana'a, Yemen.

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***Corresponding Author**
Dr. Mahmoud Mahyoob Alburyhi
Professor Dr. of Pharmaceutics and Industrial Pharmacy, Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Sana'a University, Sana'a, Yemen.

ABSTRACT

Hyperpigmentation is a skin disorder that causes the skin to darken in small patches of certain area or it may cover a large area of the body. Because chemical skin lightening agents may cause adverse effects. The present study focuses on formulation of whitening poly-herbal gel comprising extracts of four medicinal plants include (Licorice, Curcuma longa, Pomegranate peels, and Citrus reticulate Blanco peels), which having significant skin whitening potential for the management of hyperpigmentation problem. These plants have been reported as a good anti-tyrosinase, anti-microbial, antioxidant, anti-inflammatory and prevents skin aging. The extraction of Licorice, Curcuma longa, Pomegranate peels, and Citrus reticulata blanco peels were done, and different gel formulation containing 2%, 5% and 10% extract were prepared. Gel was evaluated for physical appearance, consistency, wash ability, homogeneity, pH, spreadability, viscosity, centrifugation test and skin irritation test. Such poly-herbal gel showed good clarity, with yellowish color, translucent appearance, smooth, no grittiness, good consistency, easily remove, good homogeneities, pH, spreadability, viscosity, no phase separation and no skin irritation when applied on the skin for a period of 24 hours. Formulation containing 10% extract appears to be the best formulation followed by 5% then 2%, in case of overall evaluation,

such as spread-ability, viscosity, and non-irritancy. These results indicate that the prepared poly-herbal gel formulation has significant potential for skin lightening and treating hyperpigmentation effectively and safely.

KEYWORDS: Hyperpigmentation, Poly-herbal, Melanogenesis, Natural Skin Lightening.

INTRODUCTION

Hyperpigmentation is a skin disorder that causes the skin to darken in small patches or it may cover a large area of the body. This skin disorder is caused by the excessive production of melanin by melanocytes in the skin epidermis.[1] It can also be caused by active melanocytes proliferating, thus increasing the number of melanocytes. This overproduction can be caused by excessive sun exposure, hormonal changes during pregnancy or the use oral contraceptives, certain medications, as well as endocrine diseases.[1,2] Melanin is a pigment that is formed by melanogenesis, and it is responsible for skin pigmentation.

Mechanisms involved in skin pigmentation include melanocyte homeostasis, microphthalmia -associated transcription factor **(**MITF-M)-mediated control of melanogenesis, melanin synthesis by tyrosine and other melanogenic enzymes, and melanosome transfer.[3] Three enzymes, namely tyrosinase, tyrosinase-related protein TRP-1, and TRP-2, directly regulate melanogenesis process.[4] Tyrosinase is a crucial enzyme in the melanogenesis process. It catalyzes the transformation of L-tyrosine into L-dopa and then dopachrome, which undergoes a series of processes before spontaneously polymerizing into melanin[4,5] solar lentigines, melasma, freckles, post-inflammatory hyperpigmentation and any other dark mark on the skin are examples of hyperpigmentation.[6]

Finding an alternative skin-lightening agents from natural sources such as plant extracts, are belief to be safer to use as compared with synthetic chemical agents.[7] In the recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries, as herbal medicines have been reported to be safe with minimal side effects especially when compared with synthetic drugs.[8] Melanogenesis is the physiological process of producing melanin, the light-absorbing pigment that is responsible for human skin and hair coloration, together with three other biochromes.[9]

In fact, this pigment's biosynthesis plays a crucial role in skin protection by shielding it from sunlight damage (UV radiation absorption) and ion accumulation, as well as by trapping reactive oxygen species (ROS).[10,11]

Natural tyrosinase inhibitors

Glycyrrhiza glabra extracts

These extracts are known for their antioxidant activity, mainly due to glycyrrhizin, triterpene saponins, and flavonoids. They exhibit skin whitening, anti-aging, anti-acne, and photoprotective effects. Hydrophobic part of the root extract can reduce tyrosinase activity in melanocyte cultures and inhibit UVB induction.[12]

Curcuma longa (Turmeric)

Curcumin, dimethyl curcumin, and bis dimethyl curcumin in Curcuma longa have tyrosinase inhibitory activity, with curcumin showing the highest inhibition. Curcuminoids inhibit L-dopa oxidation, thereby reducing tyrosinase activity. Partially purified Curcuma longa (PPC) inhibits tyrosinase protein levels and suppresses α-MSH-stimulated cells by activating ERK or PI3K/AKT signaling pathways.[13-15]

Pomegranate fruit peel

Phenolic compounds like punicalagin, ellagic acid, gallic acid, caffeic acid, protocatechuic acid, and p-coumaric acid in pomegranate peel exhibit potent antibacterial, antioxidant, and anti-tyrosinase activities.[16-22]

Citrus reticulata (Mandarin orange) peel

This peel is a rich source of antioxidants and bioactive phenolic compounds, including hesperidin, naringin, tangeritin, and rutin, accounting for nearly 86% of the total phenolics extracted. The peel is confirmed a rich source of antioxidants and Vitamin C.[23-28]

In API development process, a detailed characterization of the API and other formulation components is usually carried out during the preformulation stage. Formulation scientist from his experience and knowledge have to significantly in the preformulation study stage and is an important factor in the ADDS (Advanced Drug Delivery Systems) product development process.[29-82]

Objective of study

Formulation and evaluation of a whitening poly-herbal gel comprising extracts of Licorice, Curcuma longa, Pomegranate peels, and Citrus reticulata blanco peels in various concentration that having significant skin whitening potential for the management of hyperpigmentation problem**.**

MATERIALS AND METHODS

Materials

Glycyrrhiza glabra and Curcuma longa L, were procured from the local sources. Pomegranate peels and citrus reticulata blanco peels were collected in January 2024 from the local market at Sana'a city, Yemen., Carbopol 940, propylene glycol 400, methyl paraben, triethanolamine and other excipients were procured from the local sources.

Equipment

Vacuum enhanced filtration (vivohome. China), Rotary evaporator (biobased, china), Electronic thermostatic drying oven (Jiangsu, China), Ph meter (Changzhou Xiangtan, China), Ndj-4s viscometer (grinder (lejieyin, China), Centrifugal (Osterode, Germany).

Preparation of extract

For glycyrrhiza glabra: *It was* powdered using a Silver Crest grinder and sifted with a No. 40 mesh. An amount of 250 g of Glycyrrhiza glabra powder was macerated in 1.25 L of 75% ethanol[83,84] for 7 days. The mixtures were shaken for 10 minutes each day for 7 days. The liquid extract was separated from the solids by vacuum-enhanced filtration through Whatman No. 1 filter paper. The filtrate was then subjected to a rotary evaporator at 40°C under vacuum to remove ethanol.[85,86] The extracts were dried in an oven at 40°C, collected, and stored in the dark under refrigerated conditions.

***For curcuma longa l.*:** It was powdered using a Silver Crest grinder and sifted with a No. 40 mesh. An amount of 250 g of Curcuma longa L. powder was macerated in 1.25 L of 75% ethanol[87] for 7 days. The mixtures were shaken for 10 minutes each day for 7 days. The liquid extract was separated from the solids by vacuum-enhanced filtration through Whatman No. 1 filter paper. The filtrate was then subjected to a rotary evaporator at 40°C under vacuum to remove ethanol. The extracts were dried in an oven at 40°C, collected, and stored in the dark under refrigerated conditions.

***For pomegranate fruits*:** It was washed thoroughly under water to remove dust particles. Peels were separated from the fruits and dried under shade. The dried peels were powdered using a Silver Crest grinder and sifted with a No. 40 mesh. An amount of 250 g of pomegranate peel powder was macerated in 1.25 L of 75% ethanol[88] for 7 days. The mixtures were shaken for 10 minutes each day for 7 days. The liquid extract was separated from the solids by vacuum-enhanced filtration through Whatman No. 1 filter paper. The filtrate was then subjected to a rotary evaporator at 40°C under vacuum to remove ethanol. The extracts were dried in an oven at 40°C, collected, and stored in the dark under refrigerated conditions.

For citrus reticulata blanco peels: It was washed thoroughly under water to remove dust particles. Peels were separated from the fruits and dried under shade. The dried peels were powdered using a Silver Crest grinder and sifted with a No. 40 mesh. An amount of 250 g of Citrus reticulata Blanco peel powder was macerated in 1.25 L of 75% ethanol[89] for 7 days. The mixtures were shaken for 10 minutes each day for 7 days. The liquid extract was separated from the solids by vacuum-enhanced filtration through Whatman No. 1 filter paper. The filtrate was then subjected to a rotary evaporator at 40°C under vacuum to remove ethanol. The extracts were dried in an oven at 40°C, collected, and stored in the dark under refrigerated conditions.

Phytochemical screening

Test for alkaloids

Wagner's test

A small portion of the solvent free extract were stirred separately with 5ml of 1.5% v/v of hydrochloric acid and filtered. The filtrate was tested with various test reagents for the presence of alkaloids.[90] Wagner's test: 2-3ml extract with few drops of Wagner's reagent gave reddish brown precipitate.

Test for flavonoids (Alkaline)

Shimoda test one ml of 1% ammonia solution was added to 2 ml of each extract. Appearance of Yellow colour indicates the presence of flavonoids.[91]

Test for glycosides bontrager's

To 3 ml extract, add dil. H2So4. boil and filter, to cold filtrate add 3ml of chloroform. Shake well. Separate the organic solvent. Add ammonia, layer turns pink or red.[92]

Test for saponins

Foam test

We Shake the small amount of extract with water. Persistent foam observed.[92]

Test for tannins

Ferric chloride

One drops of ferric chloride solution were added to 1 ml of each extract. Brownish green or blue black colour solution indicates the presence of tannins.[92]

Test for phenolic ferric chloride test

One ml of 1% ferric chloride solution was added to the extract. Blue or green colour Indicates the presence of phenolics.[91]

Test for steroids

Salkowski test

Two ml of acetic acid anhydride was added to 0.5 ml of each extract with 2 ml of H2SO4. Bluish green colour indicates the presence of steroids.[91]

Method of preparation of gel

The topical gels were prepared contained extract of Licorice, Curcuma longa Pomegranate peels and Citrus reticulata blanco peels with 2%,5%,10% concentration, using Carbopol 940, propylene glycol-400, ethanol, methylparaben, triethanolamine and quantity sufficient of distilled water to prepare 100g. Water required for these formulations was divided into two parts as shown in Table 1. An accurate amount of extracts were separately dissolved in 30 ml of water and calculated quantity of propylene glycol-400 and ethanol were added.[93] As shown in Table 2, carbapol-940 was dissolved in 70 ml and to this solution methylparaben and were added.[94]

Both solutions were mixed in a beaker and triethanolamine was added dropwise to adjust pH (6-7) and to obtain required consistency. It was then stirred by using propeller for 1 hours at 1000 rpm. After stirring, the prepared gel appeared to be homogeneous and devoid of any bubbles. The prepared gel was kept at room temperature for 24 hours.[95]

Formulation of polyherbal gel with different concentration of herbal extract

Table 1: First Step for Formulation of Poly-Herbal Gel.

Ingredient	F1	F2	F3	Role of Ingredients
Licorice extract	0.6%	1.6%	3.26%	Active Ingredient.
Curcuma longa extract	0.2%	0.2%	0.2%	Active ingredient.
Pomegranate peels extract	0.6%	1.6%	3.26%	Active Ingredient.
Citrus reticulata blanco peels extract	0.6%	1.6%	3.26%	Active Ingredient.
Propylene glycol-400	4%	4%	4%	Humectant
Ethanol	3%	3%	3%	Solvent
Distilled water	20ml	20ml	20ml	Vehicle

Table 1: Second Step for Formulation of Poly-Herbal Gel.

Ingredient	F1	F2	F3	Role of ingredients
Carbapol-940	1.5 %	1.5%	1.5%	Gelling agent
Distilled Water	70ml	70ml	70ml	Vehicle
Methylparaben	O. 2%	0.2%	0.2%	Preservative
Triethanolamine	1.5%	1.5%	1.5%	Stabilizer/Neutralize & Thicker Agent

Evaluation of formulation gel

Physical appearance

Colour odor, and consistency were checked visually[96], while grittiness checked by applying it on the skin.

Consistency

It was tested manually.[96]

Washability

The extent of washing with water was checked manually.[96]

Homogeneity

All formulations were tested for homogeneity visually, for appearance of any aggregates after the gels have been set in to the container.[97]

pH Determination

The pH of herbal gel formulations was determined by using digital pH meter. 1gm of gel was dispersed in 10ml of distilled water and keep aside for two hours. The measurement of pH of formulation was carried out three times and the average values are reported.[98]

Spreadability

It indicates the extent of the area to which gel readily spreads on application to the skin or affected part. The therapeutic potency also depends upon spreading value and the spreadability was calculated by using the following formula, **S=M×L/T.** Where, S-spreadability; M- weight tied to the upper slide (20 g); L-length of the glass (6.5 cm); T-time in sec.[99]

Viscosity

Viscosity was determined by using Brookfield viscometer. Formulated gels were tested for their rheological behaviors by using spindle no 3, the measurement was made over range of speed from 6rpm to 60rpm. Each reading was taken after equilibrium of the sample at the end of two minutes.

Centrifugation test

For centrifugation test, 10g of gel was added in a tapered test tube. In centrifugation, formulation was circulated for 15 min at 3000 rpm at room temperature.[100]

Skin irritation test

Test for irritation was performed on human volunteers. For each gel formulated, five volunteers were selected, and portion of gel was applied on the forearms. The volunteers were observed for lesions or irritation.[101]

RESULTS AND DISCUSSION

The plant was collected, dried, powdered and extracted with ethanol 75%. Three different batches of formulation were prepared using ethanolic extract of Licorice, Curcuma longa, Pomegranate peels and Citrus reticulata blanco peels. F1, F2, F3 were formulated using different concentration (2%, 5% and 10%) of ethanolic extract of Licorice, Curcuma longa, Pomegranate peels and Citrus reticulata blanco peels were used to formulate polyherbal gel using carpool 940, propylene glycol-400, ethanol, methylparaben, triethanolamine.

Preliminary phytochemical analysis was carried for ethanolic extract of licorice, curcuma longa, pomegranate peels and citrus reticulata blanco peels, for alkaloids, flavonoids, glycosides, saponins, tanning, phenolic and steroids as shown in Table 3.

The formulated gel formulations were evaluated visually for colour, homogeneity, consistency and phase separation as shown in Table 4.

The prepared formulations were characterized for physical appearance, consistency, washability, homogeneity, pH, spreadability, viscosity, centrifugation test, skin irritation test as shown in Table 5.

The effect of all formulation 2%, 5%, 10% and control after before and after 24hr of application on forearms of volunteers, give non-irritating effect as shown in Figure 1 and 2.

Table 3: Phytochemical screening of licorice, Curcuma longa, Pomegranate Peels and Citrus reticulata blanco peels extract.

Bioactive compounds	Chemical Tests	Licorice extract	Curcuma longa extract	Pomegranate peels extract	Citrus reticulata blanco peels extract
Alkaloids	Wagner's test	-	+	+	-
Flavonoids	Alkaline reagent	+	+	+	+
Glycosides	Borntrager's test	+	-	-	+
Saponins	Foam test	+	-	+	-
Tannins	Ferric chloride test	+	+	-	+
Phenolic	Ferric chloride test	+	+	+	+
Steroids	Salkowski test	-	+	+	+

Table 4: Physical characteristics of different polyherbal formulation.

Formulation	Colour	Consistency	Texture	Grittiness
F1	Yellowish	Semi-solid	Smooth	No
F2	Yellowish	Semi-solid	Smooth	No
F3	Yellowish brown	Semi-solid	Smooth	No

Table 2: Washability, pH, Homogeneity, Spreadability, Viscosity, Centrifugation Test and Skin Irritation of Different Polyherbal Formulation.

F No.	Wash-ability	pH	Homogeneity	Spread-ability (gm.cm/sec)	Viscosity cps	Centrifugation Test	Skin Irritation
F1	Good	6.64	Good	15	8512	No Separation	None
F2	Good	6.8	Good	17	8502	No Separation	None
F3	Good	6.76	Good	20	8495	No Separation	None

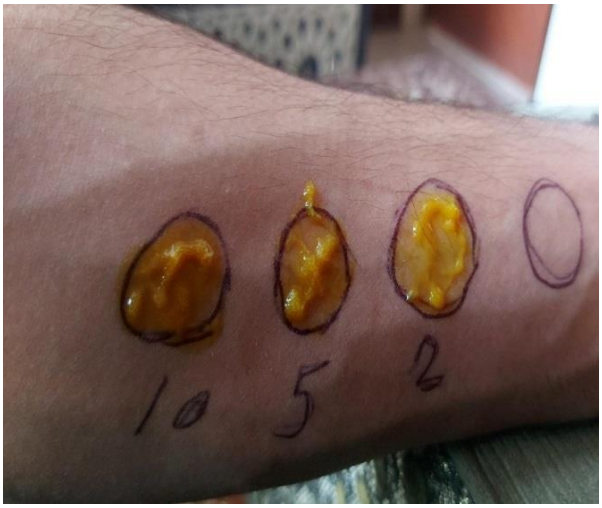

Fig. 1: Application of Polyherbal Formulation as 2%, 5%, 10% and Control (base) at 0 hr. on Forearms of Volunteers.

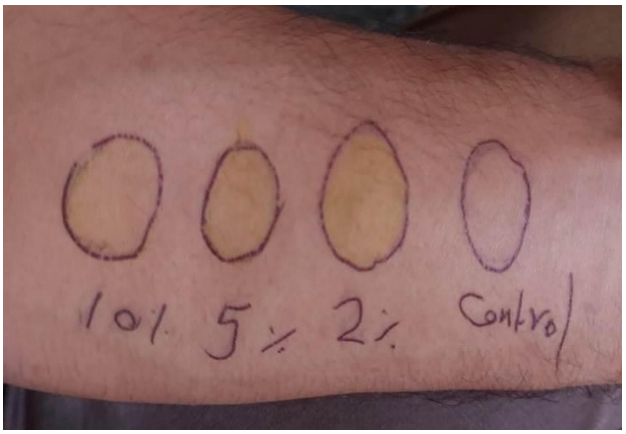

Fig. 2: Effect of All Formulation 2%, 5%, 10% and Control after 24hr of Application on Forearms of Volunteers.

Hyperpigmentation is a skin disorder that occurs widely in the human population in which darkened patches or spots appear on the skin due to excessive sun exposure, hormonal changes in pregnancy or use oral contraceptives. Also, certain medications as well as endocrine diseases are linked to this skin condition. To overcome this problem explores several side effects. Therefore, it needs to focus on the herbal formulation as a topical first-line treatment.[1-8] The formulations were assessed based on physical characteristics, pH, spreadability, viscosity, and skin irritation tests, physical characteristics and pH: All formulations demonstrated good clarity and homogeneity, this indicating successful incorporation of the herbal extracts.

The yellowish translucent appearance was consistent across formulations and suitable for a topical gel. The pH of all formulations was within the range of 5.5 to 6.9, aligning with the

skin's natural pH, which minimizes the risk of irritation and maintains the skin's barrier function.

Spreadability is a crucial parameter as it influences the ease of application and the even distribution of the gel on the skin. The 10% formulation showed the best spreadability, followed by the 5% and 2% formulations. This suggests that higher concentrations of active ingredients may enhance the gel's ability to cover the skin effectively.

Viscosity was found to decrease with the increase concentration of the active ingredients, with the 2% formulation being the most viscous. While higher viscosity can provide a more substantial feel and longer contact time with the skin, it should be balanced to ensure easy application without leaving a heavy residue. All the gel formulations were also evaluated for centrifugation test, the phase separation was not observed.

No skin irritation was observed in any of the formulations during the irritation tests, which highlights the biocompatibility and safety of the chosen herbal extracts for topical use. This is particularly important for formulations intended for prolonged use, such as those targeting skin hyperpigmentation.

Comparative efficacy: The results show that the 10% formulation offers superior spreadability and the highest active ingredients compared to the 2% and 5% formulations. The increased concentration of active ingredients in the 10% gel likely contributes to enhanced efficacy in reducing hyperpigmentation due to a higher presence of bioactive compounds known for their skin-lightening properties. However, the 5% formulation also performed well, offering a balance between ease of spreadability and viscosity, which might be preferable for users who favor a less viscous product. So, the 10% polyherbal gel formulation appears to be the most effective based on the parameters studied, suggesting potential as a promising treatment for hyperpigmentation.

CONCLUSION

It was concluded that, gel composed of extracts from Licorice, Curcuma longa, Pomegranate peels, and Citrus reticulata Blanco peels, show significant promise in addressing skin hyperpigmentation and these extracts possess anti-tyrosinase, antimicrobial, antioxidant, and anti-inflammatory properties, contributing to their effectiveness in managing hyperpigmentation.

The prepared gel exhibited excellent physical properties, including good clarity, consistency, and ease of application, with no adverse skin reactions observed in preliminary tests. These promising results suggest that our polyherbal gel could serve as a safe and effective alternative to chemical skin lightening agents, providing effective and safe management of hyperpigmentation.

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