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Pharmaceutical Standardization and Evaluation of Herbomineral Cream (ALG-06) for Leucoderma

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ABSTRACT

The aim of this study was to formulate and evaluate the herbomineral cream ALG-06 which is used in the treatment of Leucoderma. In this formulation we had used mineral drugs (red oxide and purified Sulphur) along with specific combination of four common medicinal herbs (*i.e. Azadiracta indica* A *Juss. Psoralea corylifolia L., Punica granatum L., Trigonella foenum-graecum L.*) in the extract form. The quality of the cream (ALG-06) was evaluated by organoleptic features (color, odor and appearance) and on other hand its chemical and physical characteristics such as pH,viscosity, solubility in different solvents along with bio burden and irritancy test on the skin was also performed. Cream was observed in the accelerated stability condition along with 40 °C / 75 % relative humidity and at room temperature (RT) for a period of 6 months. Phytochemical screening of herbomineral extract and standardization were carried out by TLC fingerprints of extract with two different solvent systems.

INTRODUCTION

Leucoderma is the hypopigmentation disorder in which melanocytes in the skin are destroyed. The precise cause of Leucoderma is complex and not fully understood but distribution of lesions varies from one ease to another. The worldwide population having Leucoderma is considered to be between 1% and 2% between both sexes and all races equally; however, it is more noticeable in people with dark skin. Patients of leucoderma can experience emotional stress particularly if the condition develops in the visible areas of the body [1].

In growing age the use of herbal drugs play an important role in health care because the outcomes of adverse reaction of the synthetic drugs (corticosteroid or methoxsalen, psoralen etc.) and other type of treatments in this regard are also exist such as application of arsenic, charcoal and coal tar and patient are facing their deleterious effects [2,3]. Moreover, protective measures have also been taken for hiddening of white patches such as camouflage and self-tanning agents therefore people have started looking towards the ancient healing system like Unani, Ayurvedic and Chinese etc. because of its great potential, their safety and efficacy. Some herbal remedies are used currently in the treatment of Leucoderma such as *Glycyrrihiza glabra* L., *Salvia miltiorrhizae* L., *Piper nigrum* L. *and Psoralea corylifolia* L. are frequently utilized not only as a monoherbal but also in poly herbal preparations without establishing their clinical findings which are still lacking except some of them. This herbomineral formulation was prepared with the combination of four common herbs along with minerals named ALG-06. In order to achieve maximum cure by devoiding hazardous effects of many treatments which are previously used to cure Leucoderma, following standardized herbs and minerals are used in the formulation of ALG-06 cream [4].

Trigonella foenum graecum L. (Fabaceae) is also known as

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fenugreek. A robust, erect, aromatic annual herb reaching up to 60 cm in height and cultivated throughout the country. The seeds used in many traditional systems as anti-diabetics, antibacterial, GIT stimulant and used as good nutritional supplement and condiment too. The seed is bitter in taste and possess antipyretic, anthelmintic, antileprotic, antibronchitic, carminative and aphrodisiac properties. The main constituents are flavonoids, polysaccharides, saponins, alkaloids, fixed oils and fibrous materials [5].

Azadiracta indica A Juss. (Meliaceae) is the one of the most ancient and popular plant grown abundantly which is used in unani and ayurvedic medicines and is particularly prescribed for various skin diseases due to its powerful antibacterial, antifungal and antiseptic properties. It is hardy, fast-growing evergreen tree with a straight trunk, long spreading branches and moderately thick, rough, longitudinally fissured bark. Mature trees attain a height of 7-20m with a spread of 5-10m. The main active constituents of Azadiracta are liminoid (nimbidin, nimboline), terpenoids, flavonoids, glycosides, proteins and carbohydrates. All parts of the plant (seed, fruit, flower and bark) are used in many medicinal preparations [6].

Psoralea corylifolia L. (Fabaceae) is the herbaceous plant mainly distributed in all provinces of Pakistan also found in Bengal, India, Srilanka, and China. An erect annual herb up to 90cm high stems and is covered with white hairs with simple and alternative leaves. Seeds are black ovoid somewhat reniform shape with bitter taste. Due to existence of Psoralen and furancoumarin are able to promote new pigment when exposed to sunlight are richly found in seeds therefore it is frequently used in various skin diseases such as eczema, psoriasis, leprosy especially in Leucoderma. It is also containing essential oil, coumarin, flavonoids and terpenoids [7].

Punica granatum L. (Punicaceae) is a typical fruit found from many subtropical and topical regions including almost all Mediterranean countries. A small, dense tree or shrubs, 5-10m high, with white slender branches often tipped with a spine. The fruits are large leathery skinned, globules berries with yellowish red external surface or rind containing numerous seeds and a juicy red pulp. The fruit is highly valued mainly due to its exceptional and unique sensory and nutritional properties. The seed and juice are considered as a blood tonic due to having rich iron for strengthening hematopoietic, cardiac and pulmonary systems and also produce positive effect on GIT. It has shown the promising antioxidant, anti-inflammatory, neuroprotective, anticancer, antiglycemic activities. The most therapeutically beneficial constituents along with the iron are ellagic acid, ellagitannins, punicic acid, fatty acids, sterols, vitamins B1, B2, B12, Vit B3, C and variety of minerals, carbohydrates and amino acids [8,9].

Sulphur, a pale yellow nonmetallic element antimicrobial action occurring widely in nature in several free and combined allotropic forms. In pharmaceutical topical preparations it is used not only in many skin disorders but also beneficial in healing wound and deep injuries [10].

Red ochre has been medicinally used since the ancient times. It is composed mainly of iron oxide and used in the treatment of various skin diseases like pruritus, scabies, urticaria and burns [11].

Material and Methods

Instrumentation

Grinding machine (West point automatic series TSK 333 France), Rotary evaporator (Eyela Japan), Lyophilizer (freeze dryer model FDI, Eyela, Tokyo Rikakekai co. Ltd Japan), pH meter (730 WTW-Germany), Viscometer (Dv-II+Pro Brookfield-USA), Electronic balance (XT220A China), Spectrophotometer (Lambda-35 Perkin Elmer USA), Climatic chamber (KBF 720 Switzerland).

Chemicals

Cetostearyl alcohol, Liquid paraffin, propyl paraben, methyl paraben and others chemicals used were of analytical grade.

Collection of plant materials

The seeds of three plants (*Psoralea corylifolia* L., *Punica granatum* L., *Trigonella foenumgraecum* L.) and two minerals (red oxide and purified sulphur) were purchased from Herbal Market Sadder Karachi except *Azadiracta Indica A Juss.* (Neem) seeds were collected from trees grown in Karachi University campus in April 2009 and these materials were authenticated by Prof. Dr.Ghazala H.Rizwani in the Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi. A voucher specimens No.034, 035, 036, 037, 038, 039 were deposited at the herbal museum of Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi. Pakistan.

Extraction of herbs

Each herbs used in the formulation were washed, shade dried and coarsely powdered in grinding machine separately along with minerals then mixed together (550 gm) by soaking in 1000 ml of 80 % methanol (Merck) and kept in a room temperature till exhaustion. The combined extract was evaporated to dryness on rotary evaporator followed by lyphollization to obtain as semisolid reddish brown extract (83 gm) which was used for the solubility test, phytochemical analysis and formulation of cream.

Solubility test of extract

Behavior of solvent with crude drug extract exhibited the chemical nature of drugs. The solubility of HME extract was determined by addition of deionized water, liquid paraffin, ethanol, dimethyl sulf oxide, hexane, ethyl acetate and chloroform. (Table 1).

Phytochemical Analysis

Phytochemical analysis of the extract was carried out by using different chemical reagents for the determination of the existence of the different natural chemical class of constituents such as cerium ammonium sulphate reagent, iodine vapors and UV elimination specially for detecting all classes of compound while in broad research perspective individual classes of compounds i.e. alkaloids, triterpenes, saponins, tannins,

flavonoids and glycoside were identified by Dragendroff's and Mayer's test ,Libermann test ,Lead acetate and $FeCl_3$, Molish test, shinoda test and Frothing test respectively [12] (Table 2).

Preparation of TLC

TLC is a qualitative method of analysis which is used for determining the nature and amount of active substance present in crude drug or drug mixture. In this regard we have taken two solvent systems such as chloroform-methanol-water (8:1:1) and hexane-ethyl acetate (9:1) and extract was reconstituted with MeOH and placed on TLC plate(thickness 20x20cm 60 F 254 Merck) which is utilized as stationary phase. Developed chromatogram was dried and sprayed with iodine vapors then exposed under UV radiation at 254 and 366 λ max spot appeared on chromatogram were marked. Finally the chromatogram was sprayed with cerium ammonium sulphate reagent then observed various colored spots after heating at 110 °C for 10-20 min on chromatogram as a result of each detecting treatments followed by the determination of Rf value of spot in each reagent accordingly. This method can be utilized as a standard finger print for evaluating the quality and reproducibility of the sample.

Formulation of ALG-06 cream

Antileukoderma ALG-06 cream was prepared in two phases.
In phase I cetostearyl alcohol was melted on water bath at 75 °C along with white petrolatum to get oil portion while In phase II aqueous part was prepared by dissolving required quantity of methyl paraben and propyl paraben in boiling water at 70 °C then added sodium lauryl sulfate in warm water by continuously stirring until it completely dissolved. Thereafter Phase I and phase II were combined together by mixing continuously in a homogenizer to get smooth and creamy base followed by addition of standardized HME extract (Table 4).

Evaluation of ALG-06 cream

Accelerated stability test

The effective, stable and prompt actions are the characteristics in good cream formulation criteria. Capability of medicinal products in a specific formulation is needed proper appearance with perfect stability. It should be in a particular container to remain within its physical, chemical, microbiology, therapeutics and toxicological specification. To achieve this standard stability testing of the ALG-06 cream was performed and observed under different accelerated condition for 6 months according to the ICH guidelines. 50g ALG-06 cream was kept at ambient temperature RT and 50g of cream at 40 °C / 75 % RH in a stability chamber and noticed the cream color, odor, appearance, bio burden, pH and viscosity on 0, 1st, 2nd, 3rd and 6th months. The organoleptic evaluation was carried out by color, odor and appearance etc. Physical Properties of cream was evaluated by the following features.

pH: A 100g of cream (ALG-06) was used to determine its pH.
Viscosity: 100g of sample cream was used to determine the viscosity (cp) with spindle RV5 measured at 1 rpm.

Bio burden test

For determining the contamination in our formulation we had performed bio burden test in this regard aerobic bacterial count by SDA, aerobic fungal count by TSA and Coliform count by using MacConkey agar was performed [13].

Skin Irritancy test

Skin irritancy test for formulation was also checked the allergenicity of ALG-06 cream then the cream was applied on right hand about 1sq.cm and time was noted. Irritancy, edema and erythema were checked up to 24hours, 48hrs and 72hrs interval of time respectively [14].

RESULTS

Formulation of ALG-06 cream was also subjected to various analytical parameters (i.e. Preliminary screening, chromogenic testing and chromatography). First we checked the solubility of extract with different solvents, which were shown in the Table 1, which is important to make reconstitutions of herbs and minerals to prepare standard formulation. The combined HME extract has been soluble in almost all organic solvents such as ethanol, DMSO, chloroform, hexane and ethyl acetate while partially soluble in the liquid paraffin. Alkaloids, tannin,

Table 1: Solubility testing of herbomineral extract [4] in different solvents.

Solvent	Solubility	Inference
Deionized Water	Not soluble	Clear solution
Liquid paraffin	Partially soluble	Dense solution
Ethanol	Soluble	Dark brown
Dimethyl sulf oxide	Freely soluble	Thick dark brown
Chloroform	Soluble	Dark maroon
Ethyl acetate	Soluble	Dark brown
Hexane	Soluble	Dark brown

Table 2: SExistence of naturally occurring compounds in extract

S. No	Phytochemical agents	Test	Results
1	Alkaloids	Dragendroff's test, Mayer's test	+ + +
2	Carbohydrates	Molish test	+ + +
3	Glycosides	Frothing test	+ +
4	Flavonoids	Shinoda test	+
5	Triterpenes	Libermann's test	+ +
6	Tannins	Ferric chloride test, Lead acetate test	+ + +
7	Sterols	Salwaski's test,	+ +

+ + +Intense, + + Moderate, +Slight

glycosides, flavonoids, carbohydrates, triterpenes and sterols were found in the combined extract as a result of phytochemical screening (Table 2). On TLC chromatogram in combined extract we detected with varying relative front Rf values showing the different class of natural compounds, in order to confirm these compounds by spraying with specific chromogenic reagents (Table 3). Different coloration and varying distance and intensities were observed when spray with 5% cerium ammonium sulphate reagent on chromatogram, while prior to this observation photoactive conjugated chromophores were observed under UV light at λ max 254nm and iodine vapors spray for the determination of total number and type of components present in combined extract HME.

The formulation was appeared in a shiny clear and smooth form and showed no phase separation. Evaluation of ALG-06 cream was done by different physical parameters such as pH, viscosity, and bio burden and skin irritation test (Table 6). The

Table 3: Chromatographic separation of methanol soluble HME extract with two different solvent systems

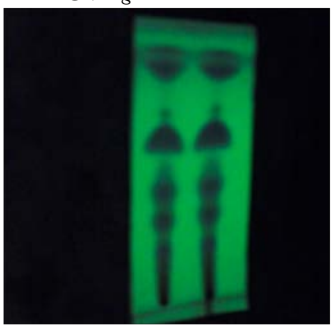
Mobile phase	At 254nm		Iodine vapours		CAS reagent		
	No. of spot	Rf.	No. of spot	Rf.	No. of spot	Rf.	Color
Chloroform: methanol: water (8:1:1)	04	0.79 0.55 0.44 0.35	04	0.79 0.55 0.44 0.35	07	0.97 0.91 0.83 0.79 0.44 0.36 0.25	Black Brown Bright yellow Bright orange Orange Black Yellowish black
Hexane: ethyl acetate (9:1)	06	0.87 0.72 0.58 0.41 0.32 0.21	06	0.87 0.72 0.58 0.41 0.32 0.21	06	0.85 0.65 0.61 0.32 0.21 0.15	Dark orange Black Yellowish green Brown Bright orange Black

pH and viscosity of cream was 5.0 and 47600cp.Stability is the imperative parameter for the evaluation of any formulation. It was found that the cream would be stable at Room temperature or ambient condition and no degradation occurred regarding color, odor, appearance, pH, viscosity and bio burden test but only slight degradation was observed in the color and viscosity at 40°C/Relative humidity 75% was monitored in the chamber which was not affected the efficacy of the cream (Table 7). This also reflects that a drug is compatible with all excipients (Table 4).

Discussion and Conclusion

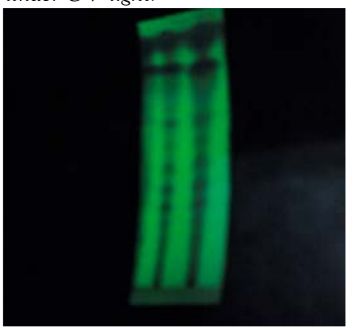
Detection of phytochemical agents present in the formulation showed its medicinal significance due to containing of steroids, flavonoids and tannins as major components. In leucoderma melanocytes are disappeared from the affected area due to antioxidant potential. Flavonoids and steroids which act over there as radical scavengers which protect cells against the damaging effect of reactive oxygen species [15]. They are effective against viral infections, allergic reactions and anti-inflammatory and platelets aggregation activities. Addition of minerals i.e. sulphur and red ochre causing powerful antimicrobial action in the formulation. Moreover,it is also protective against sunburn when its use along with Psoralea corylifolia like herb in ALG-06. Occurence of natural steroidal compounds has least side effects as compare to synthetics ones. Moreover in combinations of phytochemical agents of plants also has a synergistic effect for the reduction of irritation and producing good therapeutics

Figure 1: TLC fingerprint of methanolic soluble HME extract under UV light.



Hexane: ethyl acetate (9:1)

Figure 2: TLC fingerprint of methanolic soluble HME extract under UV light.

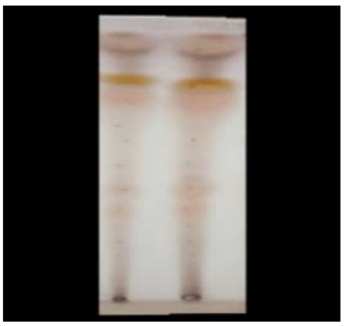


Chloroform: methanol: water (8:1:1)

Figure 3: TLC finger print of methanolic HME extract on silica gel spray with cerium ammonium sulphate.

Hexane: ethyl acetate (9:1)

Figure 4: TLC finger print of methanolic HME extract on silica gel spray with cerium ammonium sulphate.



Chloroform: methanol: water (8:1:1)

Table 4: Formulation of (ALG-06) cream

Ingredients	Percentages
Methyl paraben	0.25%
Propyl paraben	0.15%
Sodium lauryl sulphate	0.6%
Propylene glycol	12%
Ceto stearyl alcohol	25%
White petrolatum	25%
HME extract	5%
Purified water q.s	100gm

Table 5: Organoleptic Properties of ALG-06 cream

Parameters	Cream
Color	Light brown
Odor	Herbal smell
Appearance	Smooth shiny

Figure 5: TSA test of ALG-06 cream

Figure 6: MacConkey Test of ALG-06 cream

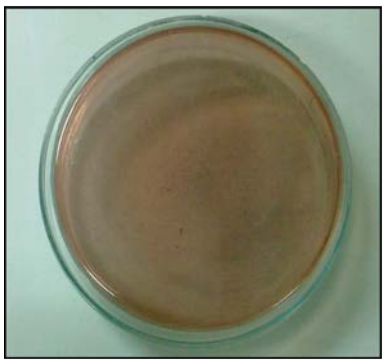


Table 6: Physical properties of ALG-06cream

Parameters	Value
pH	5.0
Viscosity	47600cp
Skin irritancy test 24hrs 48hrs 72hrs	No irritation persist
Bio burden test SDA agar TSA agar MacConkey agar	Nil

Table 7: Stability studies of ALG-06 Cream

Temperature	Ambient RT					Accelerated Stability Condition (40°C / 75% RH)				
Months	0M	1st M	2nd M	3rd M	6th M	0M	1st M	2nd M	3rd M	6th M
Color	~	~	~	~	~	~	-+	-+	-+	-+
Odor	~	~	~	~	~	~	~	~	~	~
Appearance	~	~	~	~	~	-	~	~	~	~
pH	5.0	4.8	5.1	4.9	4.9	5.0	4.6	4.3	3.9	4.1
Viscosity	47600	47590	47470	47600	47688	47600	43555	41000	41200	41222
Bio burden test										
TSA agar	~	~	~	~	~	~	~	~	~	~
SDA agar	~	~	~	~	~	~	~	~	~	~
MacConkey agar	~	~	~	~	~	~	~	~	~	~

~ no change -+ slight change ++ degradation

response without any ill effects. A unique combination of common herbs and minerals in this formulation with valuable medicinal, edible and cosmetics agents along with minerals, vitamins, natural steroids and flavonoids made the cream ALG-06 more efficacious, stable and safe formulation when undergone stability studies (pH, viscosity and bio burden test) to cure leucoderma.

These research studies were performed according to the WHO [16] guidelines which are necessary to standardize any herbal product followed by validation through pharmacopeial standard before the clinical trials, to acquire the maximum efficacy and safety of the formulation.

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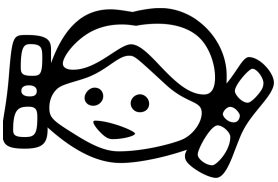

I am Ambreen Huma a PhD student in the Department of Pharmacognosy,Faculty of Pharmacy,University of Karachi. I am doing research under supervision of Profressor Dr.Ghazala H. Rizwani,Dean faculty of Pharmacy,University of Karachi.The main objective of this research project is to formulate such a preparation in which we use herbal combination with some minerals for the treatments of vitiligo patients. This hypopigmented disease is very common now adays due to many reasons and many treatments are currently available to curing this condition.This formulation have a unique combination of edible and medicinal herbs and commonly used minerals with special technologically extracted, reconstitution and making in a dosage form with control stability studies protocol following the USP standard which definetly proved as good remedy in vitiligo.

