Drug development of herbomineral capsule (ALG-06) used for hypopigmentation specially in vitiligo

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Abstract: Many herbomineral preparations in traditional medicines are being used from time immemorial like Kushta Abrak Safaid, Busoor-e-Labinyah etc. as therapeutics remedies in common ailments such type of preparations are known to have additive and pronounced effects to cure any obstinate disease condition. The main objective of this research study is the formulation of herbomineral capsule (ALG-06) to treat such type of condition like hypopigmentation in case of vitiligo. In order to achieve the best quality of this formulation physicochemical analysis i.e. fluorescence test, ash values, extractive values and moisture content of combined powdered drug of herbs and minerals were performed followed by phytopharmaceutical calculation of flow ability of blended powder by means of angle of repose, porosity, bulk and tap density, compressibility and hausner ratio, these properties assisted to estimate the best form of powdered material filled in right size of capsule for the desired strength i.e.500mg. Accelerated stability studies were also performed to establish the efficacy of the formulation. In this regard organoleptic properties (color, odor, appearance and taste), weight variation, disintegration and bio burden of ALG-06 formulation were monitored at 40°C/75% relative humidity (RH) along with a room temperature (RT) for a period of one month.

Keywords: Hypopigmentation or vitiligo, physicochemical, pharmaceutical, herbomineral capsule.

INTRODUCTION

Vitiligo is a depigmentation of skin due to the dysfunction of melanocyte. Actual cause of vitiligo is unknown, but research suggests that it may arise due to disturbance of autoimmune and genetic system while oxidative stress, neural, viral, infectious or digestive distresses are also causes. The incidence worldwide population is considered to be between 1% and 2%. People with vitiligo having persistence pain and senseless patchy skin causing emotional stress, particularly if the condition develops on the visible areas of the body and people who have vitiligo feel ashamed, depressed or concerned about how others will respond otherwise the person over all in healthy condition (Halder, RM *et al*., 2008).

Herbal medicines, a form of complementary and alternatives medicine has been used since ancient times for the treatment of vitiligo and against various diseases because the people are becoming conscious of the potency and side effects of conventional drugs as compare to herbal drugs. In an appropriate uses they are comparatively more effective, less toxic and easily available at affordable prices. Now-a day's combine use of minerals in herbal formulation are very common because of having great potential in the fast healing process which are previously known specially in Unani, Chinese and Ayurveda. Some mono herb such as *piperine* (K.R. Vinod *et al*., 2010), *ficus hispida* or polyherbal remedies such as combination of *Xanthium strumarium, Sophora flavescens, Atractylodes japoenica* and *Arisaema amurense* (Jimi Yoon *et al*., 2011) used currently in the treatment of vitiligo but still more research has been required in this regard in order to achieve maximum cure in minimum length of time. We have tried to make such a unique combination of herbs and minerals named as AlG-06 capsules, in which combination the seeds of four herbs i.e. *Trigonella foenum graecum L. (TFG), Azadirachta Indica A Juss. (AI) Psoralea corylifolia* L. (PC) and *Punica granatum* L. (PG) and two minerals i.e. Red ochre (RO) and *Sulphur* (S) were used. Followed by the phytopharmaceutical evaluation (physicochemical and pharmaceutical) to meet the standard of the herbomineral formulation as safe and efficacious therapeutics agent against hypopigmentation or vitiligo.

MATERIAL AND METHOD

Instrumentation

Physical balance 1Libro AEG-120 Shimadzu (Japan), Grinder West Point automatic series TSK-333 (France), Moisture balance MOC-120H (Shimadzu), Disintegration tester DS-0198 (curio), Climatic chamber KBF 720 (Switzerland), Sieve#40.

Reagent

Alcohol 10%, magnesium stearate, talc, ethyl acetate, chloroform, Hexane. All chemicals are of analytical grade.

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Authentication of materials

The two minerals (red ochre and purified sulphur) and the seeds of three plants (*Psoralea corylifolia L., Punica granatum L., Trigonella foenum-graecum L.)* were purchased from Herbal Market Sadder Karachi except *Azadirachta indica A juss.* seeds were collected from trees grown in Karachi University campus in April 2009 and these materials were authenticated by Prof. Dr. Ghazala H. Rizwani, 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.

Composition of capsule

Combined drug material was prepared for encapsulation in each 500mg capsules with specific proportion or ratio (table 1).

Physicochemical parameters

For the analysis and evaluation of the quality of single dosage form, a number of different pharmacopeia physicochemical tests were performed.

Fluorescence analysis (Rama Swamy Nanna, 2013)

This method can be used as a diagnostic tool for the identification of authentic samples and detecting adulterants in un known samples with the help of specific fluorescent colors. Dried powdered plant materials and two minerals were taken10ml of each solvent such as ethanol, chloroform, ethyl acetate, n-hexane and then color reaction were observed under ordinary and Ultraviolet lights at 254nm and 366nm (table 2).

Alcohol soluble extractive (Manish and Chandra, 2010)

One gm of powdered material was taken in closed conical flask and added 20ml of 80% alcohol and shakes well continuously for 6hr in an electrical shaker and kept till night for maceration and carefully filtered. Filtrate was then evaporated to dryness and kept at 105°C and evaluated. Percentage of alcohol soluble extract was then obtained (table 3).

Water soluble extractive

One gm of powdered material was taken in a closed conical flask and added 20ml of water and shakes well continuously for 6hrs in an electrical shaker and left over night for maceration and filtered off. Filtrate was then evaporated to dryness and kept at 105ºC and weighed. Percentage of water soluble extract was then obtained and calculated.

Ash value

Total ash

Three gm of powdered material was weighed and burnt in a ceramic pot or china dish at a temperature not exceeded 450◦C until free from carbon, cooled and weighed till a constant weight was achieved for three consecutive readings. Percentage of ash was then calculated (table 3).

Acid-Insoluble ash

The total ash was acquired by boiling for 5min with 25ml of dilute HCL; the insoluble matter was washed with hot water and ignites until to constant weight. The percentage of acid-insoluble ash was then obtained (table 3).

Moisture content

One gm of powdered material was kept in a dish of moisture balance and wait for drying at 105°C. The weighed of the dried powder was taken for percentage calculation (table 3).

Pharmaceutical parameters

a. Purification of Sulphur (Ather, 2008)

The internal use of Sulphur is advised only in the combination of milk or in the form of sulphureted butter. The Sulphur is melted with w/w vegetable oil or clarified butter in a stainless steel pot and heated slowly. Stainless steel pot was filled with 2/3 cow's milk. The melted Sulphur is poured into through a clean muslin cloth. When Sulphur is combined with, it is solidified, removed the solidified form and washed with water and dried. This process is repeated thrice.

b. Purification of red ochre

Red ochre was washed thoroughly with clean water and allowed to dry under sunlight.

c. Preparation of AlG-06 granules for encapsulation

After the washing and cleaning of each plant seeds and minerals were oven dried at 35-45°C and coarsely powdered in grinder separately and passed through sieve#40. Properly mixed 50g coarse powder of each seed and two minerals was used for capsule filling and 50g were used for phytopharmaceutical testing.

After the purification of sulphur and red ochre, all the ingredients were mixed according to the appropriate quantity and added 10%alcohol with preservatives (methyl paraben and propyl paraben) and then over dried it at 45ºC followed by addition of magnesium stearate and talc as lubricating agents.

Phyto pharmaceutical parameters

Phyto and mineralo pharmaceutical analysis of combined powder was performed.

Flow characteristics of powder

Flow ability is the basic parameter for the uniform volume filling of powder in the capsule formulation as well as clinical effectiveness of drugs. Flow ability of powder was checked by different methods (Ishaque *et al*., 2011) (table 4).

Table 1: Composition of individual drug used in ALG-06 capsules

S. No.	Name of drug	Part used	Medicinal uses	Constituents	Quantity
1.	PC*L*. (Fabaceae)	Seed	Antipyretic, diuretic, anthelminthic, laxative and for healing wounds, stomachic, stimulant, aphrodisiac, asthma, cough, nephritis, psoriasis, vitiligo, antitumor, antibacterial, and antiviral, hair loss, alopecia areata.	Essential oil, terpenoids, flavonoids, coumarin (Khushboo *et al*., 2010)	150mg
2.	TFG*L*. (Fabaceae)	Seed	Antipyretic, astringent to the bowls, cure leprosy, increase the appetite, aphrodisiac, antidiabetic, Anemia, Asthma, Boils, antioxidant, Bronchitis, cancer, Gallbladder Problems, Heartburn, Inflammation, cholestrol lowering effect. (Fedelic ashish *et al.*, 2009)	Flavonoids, polysaccharides, saponins, alkaloids, fixed oils and fibrous materials.	100mg
3.	PG*L*. (Punicaceae)	Seed	Hypoglycemic, antoartherogenic, anti-diabetics, anti-diarrheal, Helminthic effect.	Ellagic acid, ellagitannins, punicic acid, fatty acids, sterols, vitamins B1, B2, B12, Vit B3, C and variety of minerals, carbohydrates and Amino acid. (sharmin *et al*., 2012)	100mg
4.	AI *A juss*. (Meliaceae)	Seed	Anti-inflammatory, antibacterial, antispermicidal, antifungal, antimalarial, antipyretic, hypoglycemic.	Nimbidin, azadiractin, nimbin, nimbolide, sodium nimbidate, gedmin (Sharma *et al*., 2011)	100mg
5.	Sulphur	Mineral	Antiseptics, desicative, laxatives, hemorhoids, diaphoretic, expectorant, antipyretic and as a blood purifying.	Sulphur is essential of all life. Sulfur is an important part of many enzymes and in antioxidant molecules like glutathione and thior edoxin. Organically bonded sulfur is a component of all proteins, as the amino acids cysteine and methi onine (Greenwood *et al*., 1997)	25mg
6.	Red Ochre	Mineral	Anti-diarrheal, anti-hemorrhagic for internal bleeding, antiseptics, hygroscopic, soothing and vulnerary.	In Urdu it is called geru and in English is kaolin and china clay. It is prepared by purified native hydrated aluminum silicate of variable composition. Pure kaolin contains 70% alumina, 26%silica, iron oxide 4% (Akhtar, 2008)	25mg

Table 2: Fluorescence test of each plants seeds and minerals.

Chemical reagent	Plant A (T)	Plant B (P)	Plant C (A)	Plant (N)	Mineral (S)	Mineral (R)
Dry powder as such						
Ordinary light	Mustard	Black	Reddish	Yellowish brown	Yellow	Dark pink
254nm	Dark brown	Brown	Dark brown	Mustard	Dark yellow	Brown
366nm	Dark brown	Dark brown	Dark brown	Dark brown	Dark green	Dark brown
Powdered treated with ethanol						
Ordinary light	Lemon	Maroon	Yellow	Yellow	Yellow	Light brown
254nm	Dark yellow	Brown	Dark yellow	Greenish yellow	Orange	Light brown
366nm	Dark yellow	Dark brown	Dark yellow	Dark yellow	Purple	Light brown
Powdered treated with Chloroform						
Ordinary light	Mustard	Maroon	Yellow	Yellow	Lemon	Pink
254nm	Dark brown	Maroon	Pale yellow	Golden	Greenish	Purplish
366nm	Dark brown	Dark maroon	Purplish yellow	yellow Dark yellow	yellow Dark yellow	purplish
Powdered treated with hexane						
Ordinary light	Pale Yellow	Maroon	Pale Yellow	Yellow		Cloudy
254nm	Dark yellow	Dark maroon	Purple	Yellow	Light yellow	pink
366nm	Brown	Light maroon	Purple	Dark brown	Purplish purplish	Dark brown Dark brown
Powdered treated with Ethyl acetate	Golden yellow	Orange	Orange yellow	Golden yellow	Lemon yellow	Pinkish Oranges
Ordinary light						
254nm	Greenish yellow	Orange	Brown	Greenish yellow	Greenish yellow	brown
366nm	Dark yellow	Maroon	Dark brown	Dark yellow	yellow	Brown

Angle of repose of powder

For the determination of cohesiveness and non-cohesiveness of the powdered material, the angle of repose of powder of ALG-06 was measured by fixed cone method. For this purpose, take 3 gm. of powdered material and then the calculation of angle of repose was measured by following formula:

$\text{Tan } \theta = h/r$

$\theta = \tan^{-1} h/r$

Where h is height or heap of powder and r is diameter of Petri-dish.

Porosity of powder

To check the porosity of powder, a cylinder was freely filled up to 100 ml (bulk volume of powder). Then the cylinder was tapped for 100 times on smooth surface in order to obtain the pack volume of powder.

Porosity was calculated by formula:

$Є$ = porosity of powder = Vb – Vp / Vb

Where Vb is bulk volume and Vp is packed volume

Bulk density and tapped density

Bulk and tapped densities were determined.

$\rho_{bulk} = m / Vb$

$\rho_{tapped} = m / Vp$

Where m is weight of powdered drug.

Compressibility index and Hausner ratio

The bulk and tapped densities were used to calculate the Carr's compressibility index and the Hausner ratio to provide a measure of the flow properties and compressibility of powders.

$$C.I = \frac{\rho_{tap} - \rho_{bulk}}{\rho_{tap}}$$

$$HR = \frac{\rho_{tap}}{\rho_{bulk}}$$

Where ρ tap is the tap density and ρ bulk is the bulk density.

Accelerated stability studies

Stability studies of formulated oral dosage form was also carried out to establish standards the physical, chemical, microbiological and therapeutics changes occurred in the drug material were determined. To achieve this standard stability testing were performed and observed the capsules under different accelerated conditions for a period of one month according to the ICH guidelines. Two sets of 120 ALG-06 capsules were taken and kept separately at ambient temperature (RT) and at 40°C / 75 % RH in a stability chamber and noticed the color, odor, appearance, bio burden, weight variation and disintegration on 0, 10^{th}, 20^{th} and 30^{th} day of the capsules (table 6).

Physical properties of capsules were evaluated by the following features in accelerated stability studies to determine the quality of formulation.

Uniformity of weight

Ten capsules were weighed individually as well as together in a precision balance and average weight was determined. It was observed that all capsules come under the upper and lower limit of weight variation and was found the average weight of 500mg±7.5%.

Disintegration time

At least six capsules were used to determine the disintegration time of capsules.1000 ml beaker was filled with distilled water (approx. 900ml) and equilibrated to 37±0.5ºC. Six capsules were subjected in to the beaker and time required for the last capsule to disintegrate was recorded.

Bio burden test

For determining the contamination in formulation (ALG-06) bio burden test was also performed on capsules. Aerobic bacterial count by SDA (Sabouraud dextrose agar), aerobic fungal count by TSA (Tryptic soy agar) and Coliform count by using MacConkey agar were tested in this regard (Clontz, 2008).

DISCUSSION

Herbomineral formulation ALG-06 capsules was subjected to the various analytical techniques. Organoleptic evaluation was shown that powder is reddish brown in color with characteristics herbal smell, bitter in taste and the capsules are smooth shiny, light and dark blue in color (table 5).

The main view of this study is to formulate the quality drug, which is compatible, safe and acceptable along with efficacy for the patients of vitiligo. Prior to convert the blended powder into dosage form it was passed through different procedures which were necessary for getting pharmaceutical dosage form such as purification of Sulphur and red ochre is an important step to reduce the toxicity, improve the natural state and strengthen the efficacy of these minerals used in this formulation drug. In physicochemical parameters (fluorescent analysis, ash test, moisture contents and extractive value) were included. The results of fluorescent analysis of the powdered plant materials and minerals used in different chemical reagents are given in (table 2). Fluorescence is an important factor of physiochemical estimation and exhibited by several chemical constituents exit in plant material. If the substances themselves are not fluorescent, they may often be transformed into fluorescent derivatives by reagents hence some crude drugs are often evaluated qualitatively in this way while ash values were used to detect the amount of siliceous contamination.

These values are important quantitative standards as it is useful in determining purity and authenticity of drugs. High total ash value indicates the presence of phosphates, carbonates, silicates and silica in the formulation (table 3). The high value of moisture contents in formulation is indicative of the chances of high microbial growth, which was prevented by addition of preservative (methyl paraben and propyl paraben) and 10% alcohol. Water-soluble and alcohol soluble extractive value plays a significant role in the evaluation of drugs. Solubility of extract is more found in alcohol as compare to water appeared as high extractive value as shown in the (table 3).

In the pharmaceutical parameter flow ability of the powder was evaluated by performing the angle of repose, porosity, compressibility and tapped density were found within the limits according to the standard as shown in the (table4).A stability study is one of the vital parameter for its evaluation. It was found that capsules ALG-06 were be stable at room temperature (RT) or ambient condition and no degradation occur regarding the odor, no variation in weights, no disintegration and least bio burden observed and all ranges were found within limits but only a slight change occur in the color and appearance in the accelerated condition at 40◦C 75% RH (table 6). In the light of results mentioned parameters it was revealed that the formulation (ALG-06) showed an excellent compatibility with all the excipients along with safety profile for clinical studies.

CONCLUSION

According to the WHO guidelines all parameters, which were performed are necessary in order to standardized any herbal formulation prior to clinical trials for achieving maximum effectiveness and safety of the formulation.

RESULTS

Table 3: Physiochemical parameter of ALG-06 powder

Parameter	ALG-06 powder
Total ash	13.97%
Acid insoluble ash	58.69%
Water soluble extract	7%
Alcohol soluble extract	12%
Moisture content	8.01%

Table 4: Flow characteristics of ALG-06 powder

Angle of repose	22.5
Porosity	13.3%
Tapped density	0.133
Bulk tapped	0.153
Compressibility index	0.13
Hausner ratio	1.153

Table 5: Organoleptic properties of Herbomineral powdered and capsule ALG-06

Parameters	Powdered and capsule
Color of powdered	Dark reddish brown
Color of capsule	Light and dark blue
Odor	Herbal smell
Appearance	Smooth shiny
Taste of powdered	Bitter taste

Fig. 1: ALG-06 capsules

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Table 6: Accelerated stability studies of ALG-06 capsules

Physical	Ambient RT				Accelerated Stability Condition (40ºC / 75%RH)			
	0D	10D	20D	30D	0D	10D	20D	30D
Color	--	--	--	--	--	-+	-+	-+
Odor	--	--	--	--	--	--	--	-+
Appearance	- -	- -	- -	- -	- -	-+	-+	-+
Weight Variations	605.5±14.4	601.8±16.7	603.7±19.4	616.3±14.0	610.3±22.2	609.4±20.7	607.2±21.48	611.6±22.3
Disintegration	5.1±0.87	4.54±0.51	5±0.63	5.1±0.75	5.3±0.938	4.96±0.68	4±0.63	3.86±0.76
Bio burden Test								
SDA	22	30	23	40	22	54	57	78
TSA	100	130	110	116	100	147	150	158
MaCkonkey agar	0	0	0	0	0	0	0	0

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