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PHYSICO-CHEMICAL STANDARDISATION OF UNANI HERBS USED IN POLYHERBAL FORMULATION FOR DANDRUFF.

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ABSTRACT

Objective: the objective of study was standardizing single Unani herbs used in Polyherbal formulation. In this article, we have provided standardized value of specimen of Nakhod (Cicer arietinum), Baqila (Vicia faba), Turmus (Lupinus albus), Khatmi (Althea officinalis) and Methi (Trigonella foenum). Method: These drugs were assessed on physico-chemical and analytical parameters viz: 1) macroscopic and microscopic features, 2) extractive values 3) moisture contents 4) ash values 5) loss of weight on drying 6) pH of 1% and 10% solution. Results and discussion: Results showed all the drugs involved in study were of standard quality as there moisture content less than 10%, total ash value less than 10%, water-soluble ash values of the individual drugs were in the range of 0.8 to 3% w/w which shows all drugs were of good quality. Acid insoluble ash up to 1% and pH of 1% and 5% of each drug was found to be around 7 with mild basic in nature which is good to neutralize acidic medium produced on scalp because of dandruff. Extraction values done in aqueous medium shows water soluble constituents such as tannins, sugars, plant acids and mucilaginous compounds. Alcohol soluble extraction values shows the presence of tannins, alkaloid and resins similarly ether/ chloroform extraction value show presence of volatile oils, fixed oils and resins. Conclusion: polyherbal formulation is standard quality and its application for desired action can give promising results.

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INTRODUCTION

Dandruff (pityriasis capitis, seborrheic dermatitis confined to scalp) is extremely common affliction of scalp; which is characterized by flaking of scalp regardless of origin. [2]Dandruff is a disease that has been around for centuries despite several treatment options. [3]The resemblance of clinical presentation of dandruff revolves around the Unani entities *Huzaz* and *Bafa*. [4], [5]

Huzaz is relapsed every now and then; its severity may also fluctuate with season as it often worsens in winter. Thus cost effective management is need of an hour. Thus, proper management of dandruff is also challenging for herbal world.

In the present study a Unani formulation having drugs such as [6]; *Nakhood* (*Cicer arietinum*), *Baqila* (*Vicia faba*), *Turmus* (*Lupinus albus*), *Khatmi* (*Althea officinalis*) and *Methi or hulbah* (*Trigonella foenum*) was selected for management in dandruff. The physico chemical analysis of test formulation was carried out for standardization (as per WHO guidelines) of above mentioned polyherbal formulation. Standardization is a system which ensures predefined amount therapeutic effect, quantity and quality control of herbal drugs. The authentication of herbal drugs and identification of adulterants from genuine medicinal herb is very essential for pharmaceutical companies and public health. It is important to ensure reproducible quality of herbal medicine. [1]Since the efficacy of drug mainly depends on physical and chemical properties therefore the determination of physico-chemical characters is important for authenticity of drug before subjecting it to pharmacological screening. In view of these, physical and chemical standardization of each drug used in herbal formulation was carried out to characterize the drug sample and set a standard of its quality.

Each Single drugs of the formulation were evaluated for morphological/organoleptic properties, microscopic structure (colour, odour, taste, consistency, weight of 100 seeds), microscopic structure Loss of drying (LOD): at 105°C, ash value (Total ash value, Acid insoluble ash, Water soluble ash), pH (1% solution, 5% solution), extractive values in different solvents such as Petroleum ether extract, Chloroform extract, Methanol extract, Aqueous extract

MATERIAL AND METHODS

Authentication

Collection of plant: Plants used in the formulation were bought from Khari Baoli market, wholesale market of herbs in Delhi.

Botanical identification: sample of drugs were given to Department of Botany, Jamia Hamdard for its identification. Identification was done with respect to its morphological characters and drugs were found to be authentic and identified as the same to be used.

Standardization of single drugs used in test formulation.

Morphology/organoleptic properties

Morphological characters such as colour, odour, taste, consistency and weight per 100 seeds was checked. Possible microscopic characters were noted in some drugs.

Loss on drying/moisture content

The crude drugs samples were placed on their respective petridishes. The petridishes along with drugs were dried at 105°C for 2 hrs in an oven and weighed. The drying was continued until two successive readings matched each other.

Ash value

The ash value was determined as total ash, the acid insoluble ash and the water soluble ash.

Total ash

Crucible was weighed (W_1) and then 5g of dry drug was kept in it and weighed again (W_2). Crucible with drug was kept in muffle furnace for two hours at 450°C for 4 hours. Crucible with resulting ash was cooled and weighed. Now, again crucible with ash was kept in muffle furnace for two hours at same temperature and weighed again. The weight was found constant (W_3). For all five drugs same procedure was repeated and results were noted.

Water soluble ash

Total ash was calculated by the above mentioned method and 20ml of water was put in the crucible and heated over water bath. The resultant was filtered through ashless filter paper and again the crucible was kept in Muffle furnace for 2 hrs at 450°C. The Crucible was cooled along with the ash and then weighed. With each drug same procedure was done.

Acid insoluble ash

Total ash of the drug was obtained as in previous experiments and about 25ml of 10% HCL was added to the crucible. Then it was heated on water bath for about 10-15 minutes and filtered through ashless filter paper no.42. Then insoluble ash and filter paper was placed in the crucible and incinerated in the furnace at 450°C for about 2 hours. The Crucible was cooled along with the ash and then weighed.

pH of crude drug

pH of 1% solution

One gram powder of the drug was weighed and dissolved in 100ml of distilled water. The resulting solutions/mixture was filtered and pH was measured with a standard glass electrode. For each drug same process was repeated.

pH of 5% solution

Five grams powder of drug was weighed and dissolved in 100ml of distilled water. The resulting solution/mixture was filtered and pH was measured with a standard glass electrode. For each drug same process was repeated.

Successive extraction

Successive cold and hot extraction was done.

Cold extraction:

Each drug was taken in 5g quantity and was coarsely crushed. Different solvents such as chloroform, ether, methanol and water was added in 100ml quantities to each drug respectively and kept for 4 hours and filtered. Then was evaporated to dryness and weighed.

Hot extraction:

Each drug was taken in 10g quantity separately and was coarsely crushed. Extraction was done by reflux method in distilled water for 5-6hrs below 60° temperature. The extraction value for each drug was determined.

RESULTS

Morphology/Organoleptic Properties: After proper identification and authentication, morphology/organoleptic properties noted as follows:

Khatmi (*Althea officinalis*) seeds

Organoleptic properties of khatmi as shown in table 1:

Table 1.

Colour	Brownish black
Odour	Aromatic
Taste	Mild sweetish and slight mucilaginous
Consistency	seeds kidney shaped smooth embryo curved
Weight per 100 seeds	1.38 g

Microscopic structure:

Embryo curved with thin cotyledons, endosperm almost absent.

Hulbah (*Trigonella foenum*) seeds

Organoleptic properties of *Methi* (hulbah) as shown in table 2:

Table 2.

Colour	Yellowish green
Odour	Aromatic
Taste	Very bitter, mucilaginous
Consistency	Small hard, angular somewhat compressed, have deep groove across One corner.
Weight per 100 seeds	1.32g

Microscopic structure:

Seeds are dicotyledons in nature having two whitish translucent endospermic halves enclosed in wrinkled brown-yellow seed coat or the husk. Between two halves of endosperm is placed yellowish germ portion which is mainly composed of good quality of edible proteins. Husk is fibrous in nature containing cellulose. Endosperm has galactomannans, which account for 50 % of dry seed. Galactomannans have gummy property and tend to bind with water.

Baqla (*Vicia faba*) seeds

Organoleptic properties of Baqla (*Vicia faba*) are as shown in table 3:

Table 3.

Colour	Dark brown externally / Dark yellow internally
Odour	Odourless
Taste	Slight sweet
Consistency	Round to oval in shape. 5-10 mm in diameter.
Weight per 100 seeds	22.5 g

Microscopic structure:

Seeds are Dicotyledons in nature it consists of seeds coat, cotyledons and embryo. Seed coats cross section showed characteristic palisade, parenchyma, tracheid and hour glass cells. It also has attachment point for funiculus. Inside it is cotyledons and embryo.

Cotyledons; cells are angular in shape and rich in starch. Embryo; heart shaped, embryo sac at two boundaries. Embryo is in whole sac. The ingrowths of epidermal cells with dense cytoplasm with few vacuoles were present.

Turmus (*Lupinus albus*) seeds

Organoleptic properties Turmus (*Lupinus albus*) seeds as shown in table 4:

Table 4.

Colour	Externally White in colour, internally orangish yellow
Odour	Slight
Taste	Bitter
Consistency	Oval shaped, somewhat compressed flat
Weight per 100 seeds	37.66 g

Microscopic structure:

It has seed coat which has tracheoid pits and other structures similar to other legumes. There are pores in this region for water transport during imbibitions.

Nakhood (*Cicer arietinum*) seeds:

Organoleptic properties Nakhood (*Cicer arietinum*) seeds are as shown in table 5:

Table 5.

Colour	Brown to black externally internally yellow in colour.
Odour	Slight
Taste	Slight sweetish.
Consistency	Sub-globose or obviate with beak.
Weight per 100 seeds	15.67 g.

Microscopic structure:

Largest embryo consists of two cotyledons joined at their adaxial surfaces, a small hypocotyls and a radical. The embryo enclosed by a seed coat (testa). The ventral side has hilum and micropyle a minute pore. Both surrounded by a corona (hilum rim). Seed coat: Cross section of seed coat show cotyledons hourglass cells of the hypodermis inner cuticle, inner palisade, cuticle, outer palisade and inner parenchymatous region. Cotyledons: It has a complete single layer of epidermal cells. Two types one close to seed coat (abaxial) and other close to cotyledons (adaxial). Sub-epidermal cells on the abaxial are similar to epidermal cells but arranged in one to three layers irregularly. On adaxial side larger sub epidermal cells are seen. No endosperm seen. Inner cotyledon structure is composed of parenchymal cells containing numerous starch granules surrounded by protein. Parenchymal cells are round to ellipsoid. Hilum region contain hilum and micropyle here seed is attached to pod. Sclerenchyme cells form palisade layer and with hourglass cells are on outer edges of seed beak. Parenchymal cells are inside the hypodermal layer while the proximal ends of sclerenchyme cells are found in central region of hilum.

Loss on drying/moisture content

Loss on drying helps to measure amount of moisture and volatile constituent in a test drug. It helps to maintain quality of test drug. Low moisture content is always desirable for higher stability of drugs. Highest quantity of moisture was found in Khatmi seeds and lowest in Baqla. All the drugs had moisture content less than 10% as shown in table 6:

Table 6: Loss on drying/moisture content.

S.no.	Name of drug	Initial weight of sample. (W ₁) g	Final weight after drying. (W ₂) g	Percentage (%) W ₁ -W ₂ /W ₁
1.	Khatmi	5	4.67	6.6
2.	Methi	5	4.80	4
3.	Nakhood	5	4.85	3
4.	Baqla	5	4.92	1.6
5.	Turmus	5	4.88	2.4

Ash value

The ash value of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in ash residue consisting inorganic material (metallic salts and silica). A high ash value is indicative of contamination, substitution, adulteration, or carelessness. The ash value was determined as total ash, the acid insoluble ash and the water soluble ash.

Total ash

Highest value of total ash was found in Nakhood. Lowest value of ash was found for Hulbah. All the drugs have total ash value less than 10% thus good quality is maintained of drugs is as shown in table 7:

Table 7 Total ash.

S.No.	Drugs	Weight of crucible + drugs (W ₂) g	Weight of Crucible + Ash (W ₃) g	Weight of Ash (W ₃ -W ₁) g	Percent % W ₃ -W ₁ /W× 100
1.	Khatmi	36.76	32.25	0.49	9.8
2.	Hulbah	38.27	33.29	0.02	0.4
3.	Nakhood	36.43	31.30	0.13	2.16
4.	Baqila	36.20	31.52	0.32	6.4
5.	Turmus	35.96	31.00	0.04	0.8

Water soluble ash

Water-soluble ash is the part of the total ash content, which is soluble in water. It is a good indicator of either previous extraction of water-soluble salts in the drug or incorrect preparation. Thus, it is the difference in weight between the total ash and the residue obtained after treatment of total ash with water. The water-soluble ash values of the individual drugs were in the range of 0.8 to 3% w/w. This shows a normal quality of the drugs as shown in table 8.

Table: 8 Water soluble ash.

S.no.	Drugs	Weight of crucible + drugs (W ₂) g	Weight of crucible + ash G	Weight of insoluble Ash + crucible (W ₃) g	Weight of water Soluble ash (total ash- WIA) g	Percentage Of water soluble ash. %
1.	Khatmi	36.76	32.25	32.15	0.10	2
2.	Hulbah	38.27	33.29	33.14	0.15	3
3.	Nakhood	36.43	31.30	31.24	0.06	1.2
4.	Baqila	35.96	31.02	30.98	0.04	0.8
5.	Turmus	36.34	31.53	31.47	0.06	1.2

Acid insoluble ash

It helps to determine earthy matter and calcium oxalate contents in crude drugs. It helps to maintain quality of drugs. Maximum of 1% was noted in test drugs thus all drugs were good in quality as shown in table 9.

Table: 9 Acid insoluble ash.

S.no.	Drugs	Weight of crucible + Drug (W ₂) g	Weight of Crucible + acid Insoluble ash. (W ₃) g	Weight of acid Insoluble ash. (W ₂ -W ₃) g	Percentage %
1.	Khatmi	36.00	31.02	0.02	0.4
2.	Hulbah	36.21	31.10	0.03	0.6
3.	Nakhood	36.36	31.35	0.01	0.2
4.	Baqila	36.20	31.24	0.04	0.8
5.	Turmus	35.96	31.01	0.05	1

pH of crude drugs

pH of 1% and 5% of each drug was found to be around 7 with mild basic in nature. As a result it has helped in desired action in Dandruff as free fatty acids cause itching and irritation to scalp. Results are tabulated as follows as shown in table 10:

Table 10 pH of crude drugs.

S.No.	Drugs	pH of 1%	pH of 5%
1.	<i>Khatmi</i>	8.05	7.49
2.	<i>Hulbah</i>	8.18	7.46
3.	<i>Nakhood</i>	8.14	7.58
4.	<i>Baqila</i>	8.30	7.46
5.	<i>Turmus</i>	7.93	7.45

Successive extraction

Cold extraction

It helped in giving rough idea of chemical constituents of different drugs. It also helped to determine adulteration if any. Maximum extraction of all drugs was more in aqueous extract and minimum in chloroform. As aqueous extraction shows water soluble constituents such as tannins, sugars, plant acids and mucilaginous compounds. Alcohol soluble extraction shows the presence of tannins, alkaloid and resins. The ether and chloroform extract shows presence of volatile oils, fixed oils and resins. The cold extraction results for different solvents are as shown in table 11:

Table: 11 Cold extractions of test drugs

S.No.	Drugs	Methanol	Ether	Water	Chloroform
1.	<i>Khatmi</i>	1.32%	0.58%	4.6%	0.98%
2.	<i>Hulbah</i>	1.12%	0.94%	4.2%	2.06%
3.	<i>Nakhood</i>	1.98%	0.8%	1.98%	1.26%
4.	<i>Baqila</i>	0.88%	0.08%	0.24%	0.32%
5.	<i>Turmus</i>	7.8%	0.7%	8.26%	1.34%

Hot extraction

Hot extraction of all drugs was done and maximum extraction value was found for Baqila and minimum for Khatmi as shown in table 12.

Hot extraction of test drugs Table 12.

S.no.	Drugs	Results (%)
1.	<i>Khatmi</i>	19.3
2.	<i>Hulbah</i>	26.7
3.	<i>Nakhood</i>	29.3
4.	<i>Baqila</i>	41.6
5.	<i>Turmus</i>	22.3

DISCUSSION AND CONCLUSION

Morphological characterization of a drug helps in identification of the native plant as well as detection of adulteration. In some cases, quality of crude drug can be checked on the basis of morphology only. In our study, we have observed that all test drugs had moisture content less than 10%. Low moisture content is always essential for stability of drug. [7] High ash value of 9% suggests presence of high inorganic matter. Lower value of the acid insoluble ash suggests the greater physiological availability of drug. Extractive values gave information about availability of soluble phytoconstituents in particular solvent. [8] Water soluble extractive measures are more as compared to ethanol, petroleum ether and chloroform extractive values suggesting that aqueous extract would be more beneficial for desired action.

Thus a physical and chemical standardization of each drug in polyherbal unani formulation was carried out to characterize the drug sample and set a standard for its quality.

Conflict Of Interest

There is no conflict of interest between the authors.

Future scope

Pre-clinical trials with simple shampoo base and Clinical trials on larger scale can be carried out to evaluate the safety and efficacy of polyherbal unani formulation used in dandruff.

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List Of Abbreviation

L.O.D	loss on drying
W.H.O	world health organization
g	grams
$^{\circ}\text{C}$	centrigrade
hrs	hours
ml	mili liter
mm	millimeter

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