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Original Research Paper

PHYSICO-CHEMICAL STANDARDIZATION OF LAOOQ SAPISTAN KHYAAR SHAMBARI: A PHARMACOPOEIAL UNANI COMPOUND FORMULATION

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ABSTRACT

Physico-chemical standardization is a pre-requisite in quality control of unani drugs both single as well as compound formulation as the efficacy of the drugs mainly depends upon their chemical and physical properties. Therefore, the determination of physico-chemical characters for the authenticity of a drug is necessary before studying it for pharmacological activity. In the present study a pharmacopeial compound formulation Laooq Sapistan Khyaar Shambari (LSKS) has been selected to confirm its parameter according to the guidelines given in Unani Pharmacopeia of India (UPI) 2007. The parameters studied for quality assurance of LSKS includes Successive Extractive Value viz. petroleum ether 0.80 ± 0.05, diethyl ether 0.24 ± 0.01, chloroform 0.80 ± 0.05, ethanol 19.78 ± 0.85, aqueous 56.10 ± 0.95, moisture content 3.28 ± 0.02,total ash 0.97 ± 0.00, acid insoluble ash 0.63 ± 0.01,water soluble ash 0.31 ± 0.00 and water soluble matter 42.33 ± 1.20, and alcohol soluble matter 54.33 ± 0.88, specific gravity 1.360±0.011,viscosity at 70% 2310.07, pH Value at 1 % aqueous solution 6.43 ± 0.00 and at 10 % aqueous solution 5.80 ± 0.00, and loss on drying at 105 ^{0}C 6.27 ± 0.02. The qualitative analysis of various phytochemicals was estimated that revealed the presence of carbohydrates, glycosides, phenols, proteins, steroids and resins. The TLC profile of this pharmacopoeial formulation was also performed. This study will help in setting down pharmacopoeial standards in determining the quality and purity of compound drug formulation which is in use since time immemorial in URTI and it is also reported to have a potent in-vitro antibacterial activity.

Keywords: Laooq Sapistan Khyaar Shambari, Unani Formulation, URTI.

INTRODUCTION

The standardization of Unani drugs is an important part of the study for the quality assurance of the drug to be used for the welfare of mankind. It comprises of total information regarding its standardization and controls to necessarily guarantee consistent composition of all herbals in indigenous system of medicine. With the tremendous increase in the global use of medicinal plants, several concerns regarding the efficacy and safety of the herbal medicines have also been raised. Thus medicinal plant parts should be authentic and free from harmful materials like pesticides, heavy metals, microbial or radioactive contamination, etc. Hence it has become necessary to standardize the efficacy and safety measures of the indigenous/traditional drugs so as to ensure supply of medicinal plant materials with good quality. Usually the natural drugs are used as fresh plant juices, extracts as solid/ liquid/ semi-solid or semi-liquid preparation. Laooq Sapistan Khyaar shambari is one such Semi-solid preparation which is in use since ancient times for upper respiratory tract infections and diseases and mentioned in the National Formulary of Unani Medicine of India, Part-I, Vol. V 2008. This formulation is widely

used in Unani system of medicine for upper respiratory tract infection since a long time. An in-vitro study recently done by Latif *et al*. (2013) has validated its antibacterial efficacy on CLSI Guidelines. Therefore the physico-chemical and phyto-chemical analysis was also done to ensure the quality of the Laooq Sapistaan Khyaar Shambari.

In order to standardize and to lay down the standard operating procedures (SOPs) and pharmacopoeial standards, the formulation was prepared in the Saidla Lab of Department of Ilmul Advia, Faculty of Unani Medicine, AMU, Aligarh. It was subjected to analyze for physicochemical and phyto-chemical parameters. The present study describes the salient features of preparation and safety evaluation for the drug.

MATERIALS AND METHODS

The crude drug materials of Compound Unani Formulation 'Laooq Sapistan Khyaar Shambari was procured from Dawakhana Tibbiya College, Aligarh Muslim University, Aligarh. The drugs were properly identified by the Botanical Literatures available and then confirmed by Pharmacognosy Section of the Department of Ilmul Advia. A herbarium sample was prepared and submitted in the museum of the Department of Ilmul Advia, Aligarh Muslim University, Aligarh for the future reference. The Physicochemical parameters includes the organoleptic characters of compound drug, alcohol and water soluble matter, Specific gravity, moisture content, ash values, loss of weight on drying and pH values (Afaq *et al*., 1994; Jenkins *et al*., 1967; The Indian Pharmacopoeia, 1970). The phytochemical analysis includes determination of successive extractive values of the test drug in different organic solvents using soxhlet apparatus qualitative, estimation of the chemical constituents present in the drug sample and thin layer chromatography (British Pharmacopoeia, 1968; The Indian Pharmacopoeia, 1970, Anonymous, 1987; Anonymous, 1992).

Physico-Chemical Analysis

Extractive Values

The extractive values of the test drug in different organic solvents viz. petroleum ether, diethyl ether, chloroform, ethyl acetate, acetone, alcohol and distilled water were carried out by a soxhlet's apparatus. The heat was applied for six hours on a water bath for each solvent except water, which was heated directly on a heating mantle. The extracts were filtered and after evaporation of the solvents; the extractive values were determined with reference to the weight of drug. The procedures were repeated five times and the mean value was calculated.

Water and Alcohol Soluble Contents

5 gm of LSKS was taken into 100 ml of distilled water and alcohol, in a glass stoppard conical flask. The mixture was carefully shaken frequently for 6 hours and then allowed standing for 18 hour. It was filtered and 25 ml was evaporated to dryness on a water bath. The residue was dried at 105^0 C for 6 hours, cooled in desiccators for 30 minutes and weighed without delay. The percentage of water soluble matter was calculated with reference to the amount of air dried drug. The percentage of alcohol soluble matter was determined as above by using alcohol in place of water.

Moisture Content

The toluene distillation method was used for the determination of moisture content. 10 gm of drug was taken in the flask of the apparatus and 75 ml of distilled toluene was added to it. Distillation was carried out for 6 hours and the process was repeated for five times. The volume of water collected in receiver tube (graduated in ml) was noted and the percentage of moisture calculated with reference to the weight of the air dried drug taken for the process.

Ash Values

Total Ash

2 gm of drug was incinerated in a silica crucible of a constant weight at a temperature not exceeding 450^0 C in a muffle furnace until free from carbon, cooled and weighed and the percentage of ash was calculated by subtracting the weight of crucible from the weight of crucible

+ ash. The percentage of total ash was calculated with reference to the weight of drug taken.

Water Soluble Ash

The obtained ash was boiled with 25 ml of distilled water for 5 min. The insoluble matter was collected in an ashless filter paper, (Whatman No. 42) washed with hot water and ignited in crucible, at a temp no more than 450^0 C, the weight of insoluble ash was subtracted from the weight of total ash, giving the weight of water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug taken.

Acid Insoluble Ash

The total ash was boiled with 25 ml of 10% hydrochloric acid for 5 min. The insoluble matter was collected on ash less filter paper (Whatman No. 42), washed with hot water and ignited in crucible at a temperature not exceeding 450^0C and weighed after cooling in desiccator. The percentage of acid-insoluble ash was calculated with reference to the weight of drug taken.

Loss of Weight on Drying

10 gm of drug was taken, spread uniformly and thin layered in a shallow petridish. It was heated at a regulated temperature of 105^0C, cooled in a dessicator and weighed. The process was repeated many times till two consecutive weights were found constant. The percentage of loss in weight was calculated with respect to initial weight.

pH Value

Determination of pH was carried out by a digital pH meter (model no. 335) equipped with a combined electrode. The instrument was standardized by using buffer solution of 4.0, 7.0, and 9.20 to ascertain the accuracy of the instrument prior to the experiment. The pH value of 1% solution and 10% of powder drug solution was measured.

Chromatographic Studies

Thin Layer Chromatography (TLC)

It was carried out on TLC pre-coated aluminium plates with silica gel 60 of F_{254} (layer thickness 0.25 mm) of diethyl ether extract. Taking petroleum ether : diethyl ether in 1:1 ratio as the mobile phases. The R_f values of the spots were calculated by the following formula.

$$R_f \text{ value} = \frac{\text{Distance travelled by the spot}}{\text{Distance travelled by the solvent}}$$

Phytochemical Evaluation

Test for Alkaloids:

A drop of Dragendorff's reagent in the extract was added. The brown precipitate shows the presence of alkaloids.

Test for Carbohydrate / Sugars

Fehling's solution test

In the aqueous extract, a mixture of equal parts of Fehling's solution A and B previously mixed was added and heated. A brick red precipitate of cuprous oxide indicates the presence of reducing sugars.

Molisch's test

In an aqueous solution, α-napthol was added. Afterwards, concentrated sulphuric acid was gently poured. A purple colour ring at the junction of the two solutions indicates the presence of the reducing sugar.

Test for Flavonoids

Magnesium ribbon was added to the ethanolic extract of the material followed by drop wise addition of concentrated Hcl. Colour change from orange to red is a confirmatory test for flavonoids (Fransworth, 1966).

Test for Glycosides

The test solution is to be filtered and sugar is removed by fermentation with baker's yeast. The acid is removed by precipitation with magnesium oxide or barium hydroxide. The remaining ethanolic extract contains the glycosides which are subsequently detected by the following methods.

- The hydrolysis of the solution is to be done with concentrated sulphuric acid and after the hydrolysis sugar is determined with the help of Fehling's solutions.
- The Molisch's test is done for sugar using α-napthol and concentrated sulphuric acid.

Test for Tannins

Ferric chloride solution was added in the aqueous extract of the drug. A bluish-black colour, which disappeared on addition of dilute sulphuric acid followed by a yellowish brown precipitate, shows the presence of tannin.

Test for Proteins

Millon's reaction

For the test solution, Millon's reagent was mixed and white colour precipitate showed the presence of proteins.

Test for Starch

0.015 gm of Iodine and 0.015 gm of Potassium Iodide was added in 5 ml of distilled water; 2 ml of this solution formed was added to 2 ml of aqueous test solution, the presence of blue colour indicates the presence of starch.

Test for Phenol

5–8 drops of 1% aqueous solution of Lead acetate was added to aqueous or ethanolic test solution. The presence of yellow colour precipitate indicates the presence of phenols (Brewster and Mc Even, 1971).

Test for Sterol/Terpenes

Salkowski reaction

In the test solution of chloroform 2 ml sulphuric acid (concentrated) was mixed from the side of the test tube. The colour of the ring at the junction of the two layers was observed. A red colour ring indicates the presence of the sterols/terpenes.

Test for Amino Acids

The ethanolic extract was mixed with ninhydrin solution (0.1% in acetone). After heating gently on water bath for few minutes it gives a blue to red-violet colour that indicates the presence of amino acids (Brewster and Mc Even, 1971).

Test for Resins

The test solution was gently heated and acetic anhydride was added in it. After cooling, one drop of sulphuric acid was mixed. A purplish red colour that rapidly changed to violet indicates the presence of the resins.

RESULTS AND DISCUSSION

Physico-Chemical Studies

Physico-chemical study is also important, because it helps in characterization of constituent or group constituents that frequently lead to establish the structure-activity relationship and likely mechanism of action of the drug. Phytochemical constituent present in the drug vary, not only from plant to plant but also among different samples of same species, depending upon various atmospheric factors, storage and drying condition. Thus, keeping in view the above considerations, both the physico-chemical & Phytochemical studies were carried out and the results found are given in table 1 & 2.

Specific Gravity

The specific gravity of (LSKS) Unani compound formulation was determined at 25^0C by using a specific gravity bottle and was found 1.360±0.011.

Viscosity

The viscosity of (LSKS) compound formulation was determined by using 'G' size (U Shaped) viscometer and was found 1081.91±520.07.

Ash Values

Ash value is the residue that remains after complete incineration of the drug. Ash value plays an important role in ascertaining the standard of the drug, because the dust, earthy matters are generally added for increasing the weight of a drug resulting in the higher ash percentage. Therefore, the ash value determined for the basis of judging the identity and cleanliness of a drug and give information related to its adulteration in inorganic matter (Jenkins *et al*., 1967). The mean of percentage of total ash, acid insoluble ash and water soluble ash was found as 0.97 ± 0.00, 0.63 ± 0.01 & 0.31±0.00 respectively.

Extractive Value

The Extractive value is a parameter for detecting the adulteration in any drug. The amount of the extract that the drug yields in a solvent is often an approximate measure of the amount of a certain constituents that the drug contains. Therefore, for establishing the standards of any drug these extractive values play an important role, as the adulterated or exhausted drug material will give different values rather than the extractive percentage of the genuine one (Jenkins *et al.*,

1967) . The percentage of extractive values of LSKS in different organic solvents was found as: 0.80 ± 0.05, 0.24 ± 0.01, 0.80 ± 0.05, 19.78 ± 0.85, 56.10 ± 0.95 with petroleum ether, diethyl ether, chloroform, ethanol and water respectively.

Water and Alcohol Soluble Matter

Percentage of Solubility is also considered as an index of purity, as alcohol can dissolve almost all substances including glycosides, resins, alkaloids etc. The mean percentage of alcohol and water soluble matters was found to be 54.33±0.88 & 42.33±1.20 respectively.

Moisture Content

The moisture content of the drug s is variable because mostly herbal drugs are hygroscopic and excessive moisture content becomes an ideal medium for the growth of different type of micro-organisms like bacteria and fungi. They subsequently spoil the purity of drug. The percentage of moisture content by Toluene distillation method was found to be 3.28±0.02.

pH of 1% and 10% Solution

pH value of the drug is also an important parameter. The drugs in the opposite pH are unionized and absorbed rapidly from stomach. On account of having high acidic pH, the drugs get ionize in stomach because pH of stomach is reported to be about 3.5 (Gilman *et al*., 2001). The mean of pH value of 1% and 10% solution, was found to be 6.43 + 0.00 & 5.80 +0.00 respectively.

Loss of Weight on Drying at 105^0C

Percentage of loss of weight on drying at 105^0 C indicates towards the loss of volatile substance along with the water, which is determined by subtracting the moisture contents of the drug from the loss of weight in drying. So the percentage of loss of weight determined for LSKS was found to be 6.27 ± 0.02.

Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) is one of the important parameters used for detecting the adulteration for judging the quality of the drug, the resolution of different kinds of chemical components are separated by using TLC and calculating the R_f values after detecting the spots in order to standardize the drug for its identity, purity and strength. If the drug is adulterated, there might be appearance of the other components present in the adulterants; in turn the number of spots may increase. On the other hand, the extracted or deteriorated drugs may lose the components and the number of spots appeared might be less. Keeping this in mind, TLC studies of different extracts obtained in different organic solvents of the test drug (LSKS) have been conducted and R_f values of various spots appeared in different solvents system have been noted (table-3).

Qualitative Analysis for Various Chemical Constituents

Qualitative phyto-chemical analysis of the LSKS was also carried out for the determination of the presence of alkaloids, carbohydrates, flavonoids, glycosides, Tannins/phenols, proteins, starch, saponins, steroids/terpenes, amino acids and resins. As the therapeutic properties of the crude drugs are mainly due to physiologically active chemical constituents may cause lesser therapeutic values of the drugs and therefore, they are considered as low standard drugs. Our findings will be helpful in predicting the biological activity of the drug.

CONCLUSION

The compound Unani Formulation LSKS used in the present study is of the standard parameter as given in Unani Pharmacopoeia.

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Table 1: Physico-chemical analysis of LSKS

S. No.	Physicochemical Parameter	Mean±S. E. M.
1.	Loss of weight on drying at105 ˚C	6.27±0.02
2.	Specific Gravity	1.360±0.011
3.	Moisture content	3.28±0.02
4.	Ash Value (in %)	
	Total Ash Acid Insoluble Ash Water Soluble Ash	0.97±0.00 0.63±0.01 0.31±0.00
5.	pH Values (in %)	
	pH at 1% pH at 10%	6.43±0.00 5.80±0.00
7.	Solubility (in %)	
	Alcohol Soluble extractive Water Soluble extractive	54.33±0.88 42.33±1.20
8.	Extractive values in different organic solvent (%)	
	Pet Ether Diethyl Ether Chloroform Ethanol Aqueous	0.80±0.05 0.24±0.01 0.80±0.05 19.78±0.85 56.10±0.95

Table 2: Qualitative analysis of the phyto-constituents

S. No	Chemical Constituent	Tests/Reagent	LSKS
1.	Alkaloids	Dragendorff's reagent Wagner's reagent Mayer's reagent	_ _ _
2.	Carbohydrate	Molisch's Test Fehling's Test Benedict Test	+ + +
3.	Flavonoids	Mg ribbon and Dil.Hcl	_
4.	Glycosides	NaOH Test	+
5.	Tannins/Phenols	Ferric Chloride Test Liebermann's Test Lead Acetate Test	+ + +
6.	Proteins	Xanthoproteic Test Biuret Test	+ +
7.	Starch	Iodine Test	+
8.	Saponins	Frothing with $NaHCO_3$	_
9.	Steroid/Terpenes	Salkowski Reaction	+
10.	Amino Acids	Ninhydrin Solution	_
11.	Resin	Acetic Anhydride test	+

Table 3: Thin layer chromatography

Extract	Solvent System	Visible in	No. of Spots	R_f value
Diethyl ether	Benzene :Chloroform (10:1)	UV Long UV Short	1	0.33
Petroleum ether	Benzene Chloroform (1:1)	Day Light UV Long UV Short	1	0.17
Alcohol	Petroleum ether Ethyl Alcohol (95:5)	Day Light	2	0.14 0.29

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