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Standardization of Unani polyherbal formulation (Sundrus, Luk-e-maghsool and Marzanjosh)

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Abstract

To obtain effective drug with proven efficacy, it is necessary to establish standards through modern scientific and technical procedures which would certainly enhance the reputation and acceptance of traditional medicines. It means conformation of its identity and determination of its quality and purity and determination of nature of adulterants by various parameters i.e., morphological, microscopical, physical, chemical and biological observations.

Unani polyherbal Formulation comprising of Sundrus, (*Trachylobiumhornemannianum)* Luk-e-maghsool*(Lacciferlacca / Coccus lacca)* and Marzanjosh*(Origanum vulgare* L.) mentioned by Hakim Azam Khan in his treatise Akseer-e-Azam for treatment of obesity was selected for standardisation. This formulation was used in a powdered form. In the present paper an effort has been made to discuss the various parameters of standardization like morphological characters, (colour, odour, size, shape, and taste) microscopic evaluation, fluorescence analysis, physicochemical studies (extractive values, moisture content, ash values, pH values), solubility test (alcohol soluble, water soluble and ether soluble matter), heavy metal analysis and thin layer chromatographic studies. The details will be discussed in full-length paper.

Keywords: Standardization, Sundrus, Luk-e-maghsool, Marzanjosh, thin layer Chromatography

Introduction:

In the Recent times, peoples are getting aware of adverse effects of sybthetic modern medicine and there has been a change in the global trend of medicine selection from synthetic to herbal medicine. Humans are using Medicinal plants since centuries with great success. Natural medicinal plants are highly valued all over the world as a and treatment of different of diseases and ailments.[1] The revival of interest in herbal medicines in almost all parts of globe, has led to an increase in their demand leading to a decline in their quality, mainly due to lack of enough regulations pertaining to drugs.[2] WHO has stressed the need to ensure quality control of medicinal plant products by applying modern techniques and by applying suitable parameters and standards. In order to overcome certain dooming shortcomings of the pharmacopoeial texts other quality control measures must be researched. In other words, to obtain effective drug with proven efficacy, it is necessary to establish standards through modern scientific and technical procedures which would certainly enhance the reputation and acceptance of traditional medicines. [3,4]

Unani polyherbal Formulation comprising of Sundrus, (Trachylobiumhornemannianum) Luk-e-maghsool(Lacciferlacca / Coccus lacca) and Marzanjosh(Origanum vulgare L.) mentioned by Hakim Azam Khan in his treatise Akseer-e-Azam for treatment of obesity was selected for standardisation. [5]

This formulation was used in a powdered form. In the present paper an effort has been made to discuss the various parameters of standardization like morphological characters, (colour, odour, size, shape, and taste) microscopic evaluation, fluorescence analysis, physicochemical studies (extractive values, moisture content, ash values, pH values), solubility test (alcohol soluble, water soluble and ether soluble matter), heavy metal analysis and thin layer chromatographic studies.

Material and methods:

Collection and Authentication of Ingredients:

This sufoof is consist of 3 ingredients, viz. Sundrus (TrachylobiumHornemannianum) Luk-e-Maghsool, (Lacciferlacca), Marzanjosh, (Origanum vulgare).

All the crude ingredients of the test drug were procured from DawakhanaTibbiya College, Aligarh Muslim University, Aligarh. The samples were properly identified by the Botany Department, Aligarh Muslim University, Aligarh. All these materials were observed carefully and foreign metterswer removed. The materials was kept in oven 40+2 for drying. The completely dried drugs were finely powdered and preserved in air tight container. A herbarium sample of each drug was submitted to the *Mawalid-i-Salasa* Museum of the Department of Ilmul Advia.

Preparation of Powder/Extract

All the ingredients of the test drug were dried in shade and stored in an air tight container. However, they were dried again in hot air oven at a temperature not exceeding 40°C before subjecting them for pulverization.

All the ingredients were powdered together with the help of an electric grinder at pharmacy lab of Department of IlmulAdvia.

Ingredients of *Sufoof* [12,13]

S. No.	Ingredients	Botanical name	Dose
1.	Sundrus	TrachylobiumHornemannianum	5gm
2.	Luk-e-Maghsool	Laccifer lacca	5gm
3.	Marzanjosh	Origanum vulgare	5gm

STANDARDISATION OF TEST DRUG:

Physicochemical Studies:

Evaluation of Organoleptic characters of crude drugs

Organoleptic characters mean evaluating the powdered drug by its colour, odour, taste and texture.

Determination of Extractive Values

The successive extractive values of the test drug in different solvents viz. Petroleum ether, Chloroform, Acetone, Ethanol, and distilled water (Aquas) were determined with the help of Reflux method (Successive method). The heat was applied for 6 hours for each solvent on a heating mantle.[6]

Physicochemical analysis such as the total ash, acid insoluble ash, water soluble ash, were calculated according to the methods described by the Afaq*et al*, 1994; Jenkins *et al*., 2008; Anonymous, 1968). water soluble extract, pH of 1% and 10% aqueous solution and loss of weight on drying at 105 °C, moisture content by Dean and Stark Method were also done.[7,8]

Phytochemical Studies:

Qualitative Analysis

The qualitative analysis of different chemical constituents present in test drug was carried out according to the scheme proposed by Bhattacharjee and Das (1969).

Different tests for Alkaloids, Glycosides, flavonoids, tannins, proteins, amino acids, resins etc were also carried out. [7,9]

Thin layer chromatography of different extract was carried out was carried for separation of compound. [6,7]

Results and Discussions:

Table 1-Successive Extractive values

S.No.	Petroleum Ether %	Di-ethyl Ether %	Chloroform %	Acetone %	Alcohol %	Water %
1.	1.46	0.72	0.38	2.3	5.08	24.88
2.	1.25	0.53	0.46	2.35	4.33	19.69
3.	1.39	0.68	0.42	2.29	4.89	22.86
Mean ± SEM	1.36± 0.061	1.36± 0.061	0.42± 0.023	2.31± 0.01856	4.76± 0.225	22.47± 1.510

Table 2- Ash values

S.NO	Total ash %	Acid insoluble ash	Water soluble ash %
1.	3.78	0.3	1.6
2.	3.73	0.6	1.3
3.	3.59	0.43	1.2
Mean ±SEM	**3.7±0.056**	**0.44±0.086**	**1.36±0.012**

Table 3-PH values of 1% and 10 % solution of powder

S. NO.	1% Solution	10 % Solution
1.	6.70	5.93
2.	6.83	5.76
3.	6.85	5.84
Mean±SEM	6.79±0.047	5.85±0.058

Table 4-Water soluble contents of Powder

S. No.	Alcohol Soluble Contents (%)	Water Soluble Contents (%)
1.	13.6	26.3
2.	17.06	25.88
3.	14.94	27.4
Mean±SEM	15.2±1.007	26.52±0.453

Table 5-Moisture content

S.NO.	Moisture %
1.	1.34
2.	1.20
3.	1.30
Mean±SEM	1.28±0.014

Table 6- Loss of weight on drying and Bulk Density

S.NO	Loss on drying %	Bulk Density (gm/ml) %
1.	17.4	2
2.	13.5	1.92
3.	19.39	1.97
Mean±SEM	16.76± 1.72	1.96±0.023

Table 7- Qualitative tests

S. No.	Chemical Constituents	Result
1.	Alkaloid	+ve
2.	Glycoside	+ve
3.	Tannin	+ve
4.	Phenol	- ve
5.	Flavonoid	+ve
6.	Carbohydrate	+ve
7.	Starch	+ve
8.	Sterol	+ve
9.	Resin	+ve
10.	Protein	- ve

Table 8-Fluorescence analysis of powder of Non Pharmacopeial compound formulation

S. No.	Powdered Drug	Day light	UV Long	UV Short
1.	Formulation as such	Light Brown	Brown	Clay colour
2.	Formulation +25%HCL	Brown	Green	Black
3.	Formulation+ conc. H2SO4	Dark Brown	Black	Dark Green
4.	Formulation + Glacial acetic acid	Reddish Brown	Green	Dark Brown
5.	Formulation + conc. HNO3	Reddish Brown	Green	Blackish Brown
6.	Formulation+ Drangendorf reagent	Light Orange	Green	Black
7.	Formulation+ NaOH	Bluish Black	Bluish Black	Bluish Black
8.	Benedict reagent	Bluish Black	Orange	Orange
9.	Formulation+ Fehling	Brownish Green	Green	Black

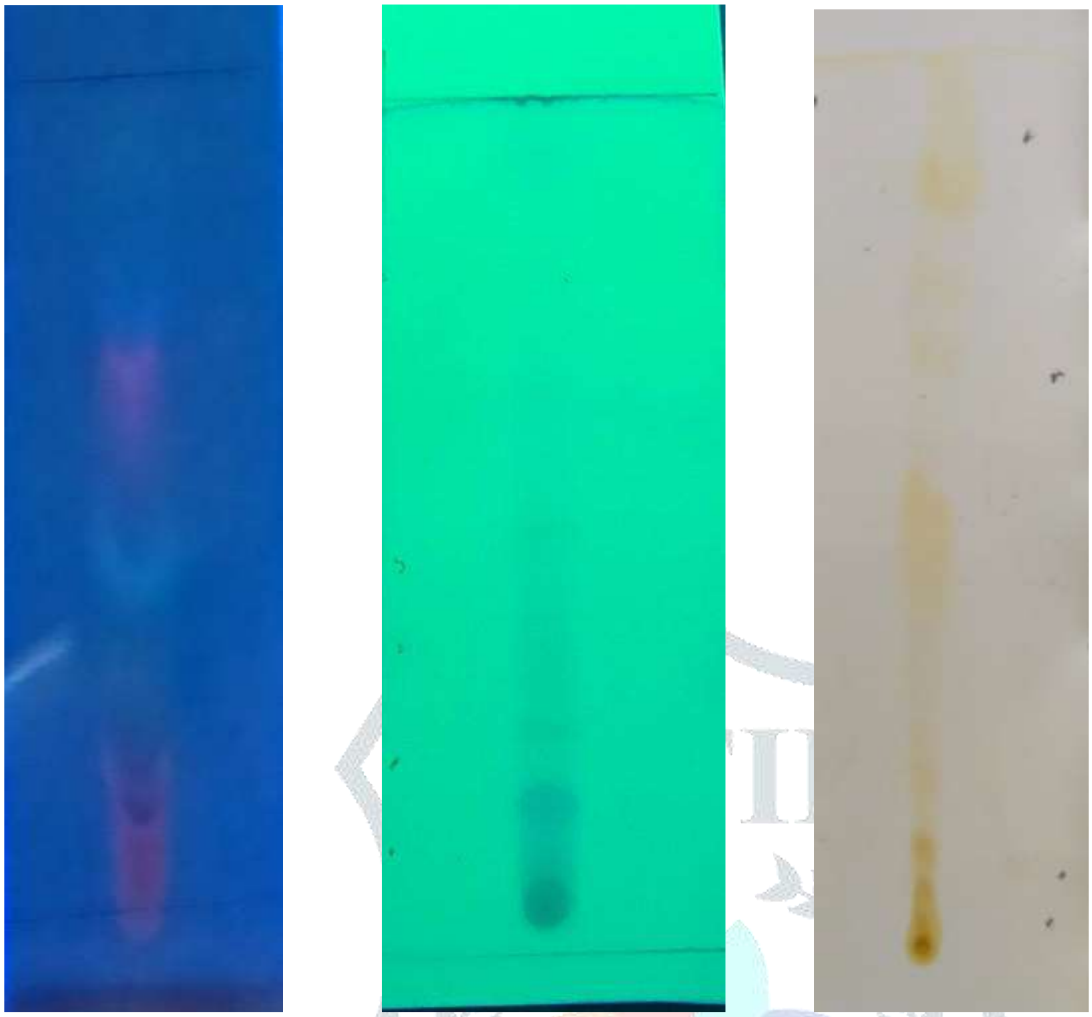
Table 9-Fluorescence analysis of Non pharmacopeial compound extract in different lights

S. No.	Extract	Day Light	UV Long	UV Short
1.	Pet. Ether	Brownish yellow	Brownish Green	Greenish Brown
2.	Di-ethyl ether	Yellow	Light Brown	Greenish Yellow
3.	Chloroform	Brownish yellow	Dark Brown	Brownish Green
4.	Acetone	Transparent	Dark Brown	Light Brown
5.	Alcohol	Reddish Brown	Yellow	Brownish yellow
6.	Aqueous	Greenish Brown	Light Brown	Dark Black

Table 10-Thin Layer Chromatography profile of Non pharmacopeial compound

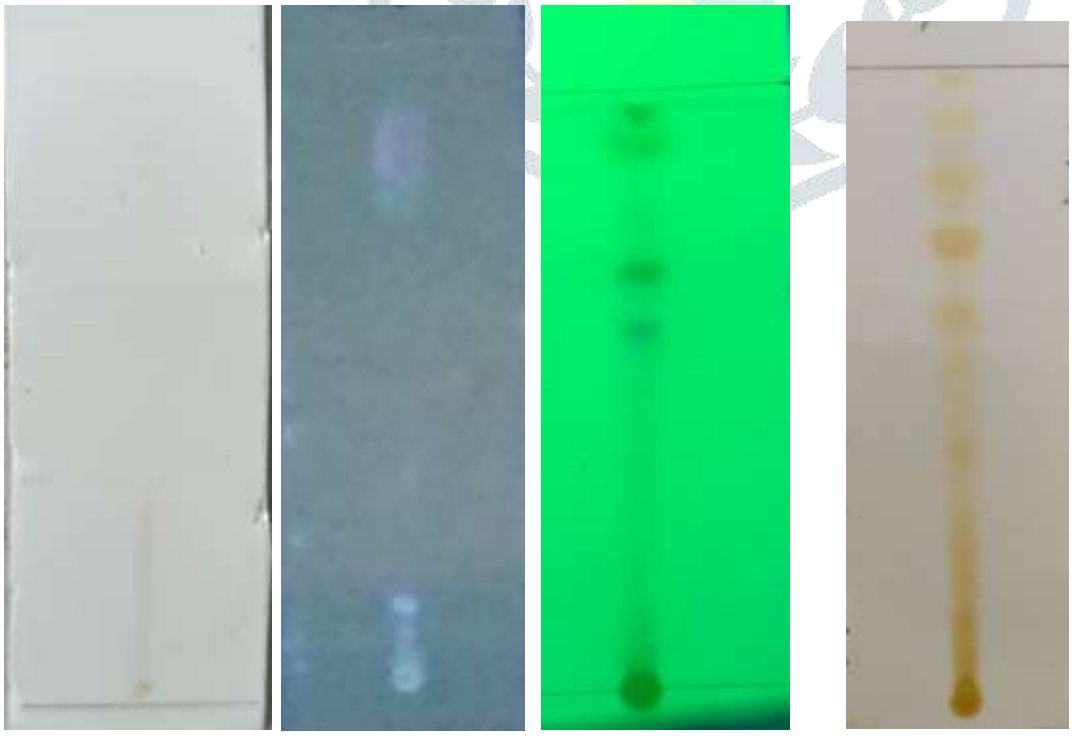
Treatment	Mobile Phase	No of spots	Rf values and colour of spots
Petroleum ether extract			
UV short	**a) Petrolium ether: Ether 4:1**	**4**	.36(LB), .42(LP), .56(LB), .76(BP)
UV Long		**6**	.22(BW), .29(LB), .34(FW), .56(BW), .78(BW), .76(LY),
Iodine vapours		**3**	.23(DB), .78(LY), .88(LY)
b) Chloroform extract			
Day light	**Toulene: Ethyl Acetate (8:2)**	**3**	0.23(DB),0.34(LB),0.39 (LY)
UV short		**2**	.36(LB), .56(LP)
UV Long		**5**	.22(BW), .30(LB), .34(FW), .56(BW), .78(BP)
Iodine vapours		**3**	.23(DB), .78(LY), .88(LY)

Note: Y= yellow, FW= Florescent white, LB = light blue, LY= Light yellow, LP = Light Pink, DB= Dark Brown LB= Light Brown, BW= Bluish White.



UV Long **UV Short** **Iodine Vapour**

Fig. 1: TLC profile of Petroleum Ether Extract of Non pharmacopeial compound



Day Light **UV Long** **UV Shor** **Iodine Vapour**

Fig.2: TLC Profile of Chloroform Extract of Non pharmacopeial compound

Discussion:

Physicochemical Study

- The efficacy of a drug mainly depends upon its physical and chemical properties therefore, the determination of physicochemical characters for the authenticity of a drug is necessary before studying it for pharmacological activity. Following parameters were used for the physicochemical study of NPC:
- Organoleptic characteristics of NPM (2) Alcohol and Water-soluble contents (3) Successive extractive values (4) Ash values (5) Moisture content (6) pH values of 1% and 10% solution (7) Loss of weight on drying, Bulk Density (8) Qualitative analysis of various constituents present in NPM (9) Thin Layer Chromatography/TLC studies of the extracts of test drug (NPM) were carried out using different organic solvent systems. [6,7,8]
- **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 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.[8] The mean percentage of the Extractive values of NPM were found as 1.36±0.061, 0.42±0.023, 2.31±0.0185, 4.76±0.225, 22.47±1.510. with petroleum ether, diethyl ether, chloroform, alcohol and water respectively. (Table No.1)
- **Ash value** is the residue that remains after complete incineration of the drug. Ash value plays an important role in ascertaining the standard of a drug, because the dust, earthy and un-required matters are generally added for increasing the weight of a drug resulting in the higher ash percentage. Therefore, the ash value determination furnishes the basis of judging the identity and cleanliness of a drug and give information related to its adulteration with inorganic matter. [8] The mean percentage of the total ash, acid insoluble ash and water-soluble ash were found to be 3.7±0.056, 0.44±0.086 and 1.36±0.012 respectively. (Table No.2)
- **Moisture content** of the drug is variable because mostly herbal drugs are hygroscopic and excessive moisture content becomes an ideal medium for the growth or different type of micro-organisms like bacteria and fungi. They subsequently spoil the purity of drug. [10] So, it is important to point out that accurate scientific works where the drug is to be sold with guaranteed assay, the percentage of active constituents must be calculated on the basis of moisture free drugs. [8] The percentage of moisture content of the drug was therefore, determined. The mean percentage of the moisture content was found to be 1.28±0.014. (Table No.3).
- **The pH** value of an aqueous liquid may be defined as the common logarithm of the reciprocal of the hydrogen ion concentration expressed in gram per litre. Although this definition provides a useful practical means for the quantitative indication of the acidity and alkalinity of a solution. [6] The mean of pH value of 1% and 10% solution were found to be 6.7±0.047 and 5.85±0.058 respectively. (Table No.4)

Phytochemical Studies[11,15]

- **The Thin Layer Chromatography** is one of the important parameters used for detecting the adulteration and determining the quality of the drugs. Rf values of various spots appeared in different solvent systems were noted in order to set the standard. The **R*f* value** may be used as an indicator of the number of constituents present in test drug. The TLC profile may also be used as the basis for quantitative analysis of the active constituents present in a drug. (Table No.10)

- **Qualitative phytochemical analysis** was also carried out for the determination of the presence of flavonoids, phenols, sterols, reducing sugars and tannins. The therapeutic properties of the crude drugs are mainly due to physiologically active chemical constituents present in the drugs, and the lower percentage of chemical constituents may cause lesser therapeutic value. Even a high percentage may induce increased biological effect which may be unpredictable and may be responsible for the side effects. Therefore, our findings will be helpful in predicting the biological activity and dose response relationship of the drug. [7,11] (Table No. 8)

Conclusion:

- Traditional Unani medicines are time tested and efficient medicines but most of these lacks standardization
- The present study has determined and set certain standards of the test drugs Sundrus, Luk-e-Maghsool and Marzanjosh through the process of standardization so that in future the studies may be carried out on these drugs by comparing with these determined characters, that will help in ensuring the purity and genuineness of the drugs by reproductivity of the scientific study on these drugs.

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