Research

IDENTIFICATION OF RAW MATERIALS IN COMPOUND FORMULATIONS BY SIMPLE BASIC TECHNIQUES

Mohammad Zakir[1*], S.H. Afaq[2*], Tajuddin[3], and Shamshad Ahmad[2]

[*1]Regional Research Institute of Unani Medicine, Bhadrak, Odisha
[2]Department of Ilmul Advia, [3]Department of Saidla, A. K.Tibbiya College A.M.U. Aligarh.

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Abstract:

In Unani compound formulation the knowledge of quantity and quality of the raw material is a major task and the quality check of raw material is an important and necessary at the very beginning of manufacturing. The percentage of the costly, active and/or important constituent should be maintained in every batch for quality assurance. The assessment of any malpractice by adding the spurious, adulterated or low quality raw materials in compound formulation is an important but uphill task. In the present communication for identification of the genuine drugs, adulterants, limit of adulteration and quality of the drugs, various chemical methods (color reaction) along with thin layer chromatography (TLC) are employed. Now a days, although, many advance techniques are available which can be used for the quality control purpose but in present scenario in India it is not easy to employee these techniques because there are many small industries involved in manufacturing of these drugs and they have no facility for the quality check of finished products, hence, the basic need of the hour is to use the basic but specific technique for quality control. TLC fingerprinting and estimation of the active constituents in compound formulation is cost effective as well as easy task. In this paper two compound formulations i.e. Habb-e-mudir and Lauq-e-khyarshambar were chosen as a test drug for TLC fingerprinting and estimation of active ingredient in crude drug and finished products. The emphasis is given to the specific test for the active ingredient of the formulation e.g. *Rhein* in case of Lauq-e-khyarshambar (active constitute of the Maghz-e-floos-e-khayarshambar), and TLC finger printing of Saffron (*Crocus sativa* Linn.).

Keywords: TLC, Habb-e-mudir, Lauq-e-khyarshambar, Saffron, *Rhein* and Quality control.

Corresponding Author: Mohammad Zakir,
E-mail: urzakir@rediffmail.com

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INTRODUCTION:

Quality evaluation and assurance of herbal preparation is a fundamental requirement of industry and other organization dealing with Unani and other herbal products. Herbal products cannot be considered scientifically valid if the drug has not been authenticated and characterized in order to ensure reproducibility of the test results in the each and every batch of the product. The development of authentic analytical methods whose results can be a reliably profile of the product is a major challenge. The phyto-chemical composition, including quantitative analysis of marker/bioactive compounds and other major constituent can play a major role in this regard. Further to note that the conventional methods for standardization of herbal formulation that include botanical identification, microscopic examination and identification of chemical composition by various chromatographic techniques cannot be vomited. In order to have a good coordination between the quality of raw materials, in process materials and the final products, it has become essential to develop reliable, specific and sensitive quality control methods using a combination of classical and modern instrumental method of analysis. In the present communication two compound formulations i.e. "Habb-e-mudir" and "Lauq-e-khyarshambar" (used for the treatment of upper respiratory tract as well as lower respiratory tract problems by Unani Physicians) were selected as a test drug. The emphasis is given to the specific test for the active ingredient of the formulation e.g. *Rhein* in case of Lauq-e-khyarshambar (active constitute of the Maghz-e-floos-e-khayarshambar), and TLC finger printing of Saffron (*Crocus sativa* Linn.).

MATERIALS AND METHODS:

Raw materials were collected locally and those not available were purchased from the market and identified. The quality of the raw materials was also tested on the basis on Unani and/or Ayurvedic Pharmacopoeia. Three samples were prepared in lab and three market samples of different batches were tested simultaneously.

Physicochemical studies like total ash; acid insoluble ash; water soluble ash; alcohol and water soluble matter; water content; loss on drying were determined quantitatively according to methods mentioned in Indian Pharmacopoeia (Anonymous, 1978), WHO guidelines (Anonymous, 1998), For determination of carbohydrate, modified spectrophotometer method mentioned by Afaq *et al* (1994) were carried out. The preparative chromatography has also been employed for quantitative determination of *Rhein*. The determination of iron was carried out by the mentioned by Afaq *et al* (1994).

Assay of Rhein:

The laboratory made silica gel plates were used. The known quantity of ethanolic extract of "Lauq-e-khyarshambar" in µ liter was applied and the plate was eluted in the Toluene: Ethyl acetate: Formic Acid: Methanol (3:3:0.8:0.2) solvent and sprayed with 05% ethanolic potassium hydroxide solution. The pink spot that appears was scratched and the content was dissolved in ethyl alcohol. After filtration and evaporation of alcohol the reaming portion was weight and the percentage was calculated and mentioned as Rhein derivative. The assay of Rhein can be used to check the percentage of the Rhein in the samples that should not be less than 1.5.

Lauq-e-khyarshambar:

Lauq-e-khyarshambar is an important Unani compound formulation prepared by majority of the Unani pharmacies in India. It is a semisolid preparation having five ingredients.

1. Maghz-e-floos Khayarshambar, (fruit pulp of *Cassia fistula* Linn.) : 2 Kg.
2. Sapistan, (dried fruit of *Cardia latifolia* Roxb.) : 1.5 Kg.
3. Aslussoos, (dried root of *Glycyrrhiza glabra* Linn.) : 1.5 Kg.
4. Katira, (dried gum of *Cochlospermum religiosum* Linn.) : 1Kg.
5. Qand safaid, (White Sugar) : 18 Kg.

The main ingredient of this formulation is Maghz-e-floos Khayarshambar, (fruit pulp of *Cassia fistula* Linn.). The pharmaceutical active constitute of it is *Rhein* a chemical for the therapeutic activity of this ingredient.

Standards of Lauq-e-Khayarshambar:

The following are the standard fixed for the product. All the data is based on five observations. It is blackish coloured paste, sticky and sweet. The specific gravity is 1.11 and contains around 70% sugar (Sucrose). Various other physico-chemical standards are as follows.

Physico-Chemical Standards:

Total ash: Not more than 0.60
Acid insoluble ash: Not more than 0.15
Water soluble ash: Not more than 0.30
Percentage of alcoholic extract: Not Less than 16.00%
Percentage of water extract: Not Less Than 15.13%

Percentage of total carbohydrate:
Not less than 64.4 %
Moisture content: Is not higher than 57.87%
Percentage of Rhein: Not less than 1.5%

TLC Profile:

The chief ingredient of this preparation is Maghz-e-floos Khayarshambar and the market samples was found not satisfactory as it does not gives the test of rhein which is the physiological active compound of the Maghz-e-floos Khayarshambar. The decolorized ethanolic extract of the Maghz-e-floos Khayarshambar when eluted on Pre-coated silica gel (Silica Gel; 60 F_{254} Merk German) TLC plates in the solvent system Ethyl acetate: Methanol: Water (100:17:10) gives a pink colour spot when treated with 10% methanolic potassium hydroxide solution. The Rf was recorded as 0.75. When the market samples and the Laboratory samples of Lauq-e-Khayarshambar were put on this test and the TLC was made, pink colour spot was very clear in the Lab samples and the market samples were devoid of this spot. The colour reaction was also negative, suggesting that the Floos-e-khyarshambar that was used in the market samples was either replaced or exhausted or spurious. Pink colour spot is *Rhein* and is considered as a marker for the presence of Maghz-e-floos-e-khyarshambar in Lauq-e-Khayarshambar.

Habb-e-Mudir:

Habb-e Mudir is an important Habb of the Unani System of Medicine. It contains three ingredients they are:

- Sibr (Dried juice of leaves of *Aloe barbadensis* Mill.): 2 gm.
- Hira kasis (Ferrous sulfate): 1 gm.
- Saffron (style and stigma of *Crocus sativa* Linn.): 1 gm.

Sibr consist of dried juice of leaves of *Aloe barbadensis* Mill. Shrubs planted in many Indian gardens and found growing throughout India. Zafran consists of dried style and stigma from the flowers, a small bulbous perennial and 15 to 25 cm high and cultivated by corms in the Kashmir valley.

Standards of Habb-e-Mudir

The following are the standard fixed for the product. All the data is based on five observations. It is blackish, hard, pills and very light astringent. Diameter of the pill is not more than 1.1 cm. Disintegration time is not more than 23 minutes. Various other physico-chemical standards are as follows.

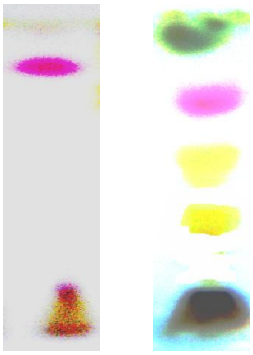
Physico-Chemical Standards:

Total ash: Not more than 16%
Acid insoluble ash: Not more than 12%
Water soluble ash: Not more than 14%
Percentage of alcoholic extract:
Not less than 33.0%
Percentage of water extract:
Not less than 66.0%
Moisture content: Not more than 18.0%
Total Iron: Not more than 31%

The chief constituent of the Habb-e-mudir is Saffron which is one of the costliest drugs of the Unani Medicine. The present cost varies from 70,000 to 1.4 lakh of Rs per Kg. The pharmacies are not using the standard saffron which is evident simply by the cost of the Habb. The Samples that are prepared in the lab after testing the authenticity of the Saffron and other material comes about 30 Rs. per Habb where as in the market Habb-e-mudir is about 2.5 Rs. per Habb.

TLC fingerprinting:

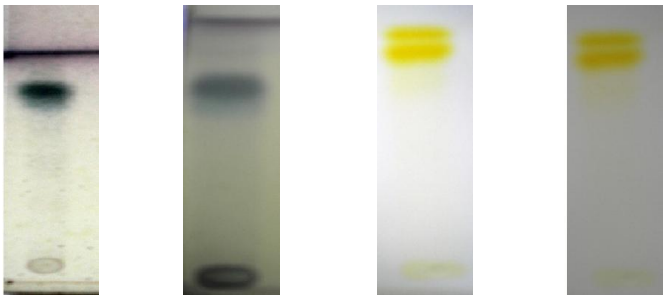
The market samples of saffron was tested for purity and added in the Habb. The Habb was powdered and refluxed with ethyl alcohol for five hours and filtered. After concentration the extract was used for TLC using Solvent System as (Methanol: ethyl acetate: water (5:2.5:0.5)). TLC of saffron was carried out on Per-coated Silica gel (Silica Gel; 60 F_{254} Merck German) TLC plates that gives two prominent yellow color spot in day light. The Rf was recorded as 0.86 and 0.95. When the market samples and the Laboratory samples were put on this test and the TLC was made these yellow colour spot were very clear only in the Lab samples and the market samples were devoid of this spot (Figure 2). The TLC shows two prominent yellow colour spot resembled with Saffron in day light, that disappear after spraying with vanillin sulfuric acid and heating at 110^0C for 5 min. After heating one violet colour spot (Rf. Value 0.76) appears which resembled with aloe.



A B

Figure 1. TLC profile of Lauq-e-Khyarshambar

A: Khyarshambar
B: Lauq-e-khyarshambar



A B C D

Figure 2: TLC Profile of Habb-e-Mudir

A: Aloe showing one spot after spraying vanillin sulfuric acid and heated it at 110^0C (Rf value 0.76)
B: Aloe in Hab-e-Mudir showing one spot after spraying vanillin sulfuric acid and heated it at 110^0C (Rf value 0.76)
C: Saffron showing two yellow color spots in day light (Rf value 0.95 and 0.86)
D: Saffron in Hab-e-Mudir showing two yellow color spots in day light (Rf value 0.95 and 0.86)

CONCLUSION:

Identification of the single drugs in powder form or in compound formulation is not easy. Testing the presence of authentic raw materials in the finished products is a tough job and Physico-chemical methods are not found suitable. The test for physiological active constituent of particular compound and the TLC finger printing can be the easiest way to check some, not all, the presence of the important and costlier ingredients in the formulations. These parameters are easy to carryout and can be employed in small places for the quality control purpose of the Unani compound formulations.

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