

Research Article

Quality assessment and standardization of the MAJOON NAJAH, a polyherbal Unani Formulation.

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Abstract:

The present work reports on the evaluation of quality control standards of Majoonnajah which is one of the Unani formulations which is known for its proved effectiveness in melancholic aliment. Melancholic aliment means, which increase or enhances the melanin in the hairs hereby, retains black colour of the hairs. It gives improvement of the hair falling and growing of the hair within the 3 weeks. Keeping in view the high medicinal importance of drug, it has been studied on the basis of physico-chemical and biological aspects. The parameters applied for the present study include qualitative and quantitative phytochemical tests, TLC fingerprinting, antimicrobial activity and stability studies.

In the light of results obtained it can be concluded that this study play very important role in the herbal drugs development and its quality control standardization. Present study is based standardizing physico-chemical and biological basis which can be useful to supplement information with regards to its identification and shall be helpful in establishing the medicinal use.

Keywords: Physico-chemical studies, Phytochemical studies, Standardization, Quality control.

(Received 05 Jan 2017, Revised: 10 Feb 2017, Published: 16 Mar 2017)

Introduction:

The traditional Indian system of medicine has a long history ^[1] of use of plants in various forms. They lack adequate scientific documentation, particularly in light of modern scientific knowledge. These natural compounds are the basis of modern drugs that we are using today. There are many families of phytochemicals those help the human body in a variety of ways. Phytochemicals may protect human from various diseases. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive ^[2] properties. Phytochemicals are basically divided into two groups ^[3], primary and secondary metabolites according to their functions in plant metabolism. Primary metabolites comprise common sugars, amino acids, proteins and chlorophyll while secondary metabolites consist of alkaloids, flavonoids, tannins and so on.

Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles. The Department of AYUSH (Ayurveda, Siddha, Unani & Homeopathy) renamed in November, 2003 is responsible for designing, formulating & implementing policy for promoting & propagating the Indian systems of Medicines. It emphasis on upgradataion of AYUSH educational standards, quality control & standardization of drugs, improving the availability of medicinal plant material, research & development &

awareness about the efficacy of the systems domestically & internationally.

The importance of safety, quality and efficacy in such products has universally been acknowledged. There is an increasing demand for ASU / botanical drugs / dietary supplements in the developing countries and the industrialized developed world is also looking for the standardized botanical products. The need of the time is therefore, to subject Ayurvedic, Sidha & Unani (ASU) drugs / products to rigorous modern scientific testing and develop standards so as to maintain quality for global competitiveness (Patel et al., 2006)

The present study was designed to screen the Standardization of Unani formulation, Majoonnajah to know quality standards in this formulation.

Materials and methods:

Preparation of Majoonnajah: The formulation was prepared on the basis of the specifications laid down by the standard guidelines.

Take a clean and dry vessel add 500 gm honey i.e. 3 times weight of formulation.

On a slow flame heat the honey collect the honey forth and remove it, let the honey cool.

Take separate clean dry vessel take Halelasiyah and Balela roast it in 5-7ml desi ghee till smell arises Mix it thoroughly with other ingredients properly i.e.

| Name | Weight |
|---------------------|--------|
| 1. Bisfaij fustugi | 15gm |
| 2. Aftimoon vilaiti | 15gm |
| 3. Ustakhudoos | 15gm |
| 4. Turbud | 15gm |
| 5. Halelasiyah | 35gm |
| 6. Balela | 35gm |
| 7. Amlakushk | 35gm |

Add roasted ingredients in cooled honey mix it thoroughly and uniformly.

Storage and preservation: It was preserved in dried, airtight, fungus free clean glass or china clay container.

Experimental procedure: *Physico-chemical analysis and Biological analysis*

Organoleptic analysis, Total Ash value, acid insoluble Ash, water soluble extractive value, ethanol soluble extractive value, fixed oil content, loss on drying were determined (Iyengar, 1995; Trease and Evans Wc., 1989).

Phyto-chemical Analysis: Preliminary tests were carried out on methanolic extract for the presence / absence of phyto-constituents like alkaloids, carbohydrates, flavanoids, glycosides, saponins, sterols, terpenes and tannins (Sazada *et al.*, 2009). **TLC was performed for tannins with mobile phase Chloroform: Ethyl acetate: Formic acid (2.5:2.0:0.8) and detecting reagent as 5% FeCl₃ solution.**

A) Test for phytochemical constituents

| Sr. No | Test | Observation |
|--------|---|---|
| 1. | Test for Tannins:- Methanolic extract + water (1:1). Boil for 5 minutes and filter. Filtrate + Ferric Chloride (FeCl ₃) | Dark colour/ppt |
| 2. | Test for Flavanoids :- Methanolic extract + Lead acetate solution (1:1) | Yellow colour |
| 3. | Test for Saponin glycoside (Foam Test) :- Shake the drug extract or drug powder vigorously with water. | Peristant Form |
| 4. | Test for Steroids :- Methanolic extract + 2ml Chloroform (CHCl ₃) + 2ml Conc. Hydrochloric acid (HCl). Shake well | CHCl ₃ layer appear red and acid layer shows greenish yellow fluorescence. |

B) Biochemical Analysis

Test for Carbohydrates

| Solution | Reagents added | Observation |
|-------------------|---|------------------------|
| Standard | 2ml of Std. + 5ml of Fehling's A + 5ml of Fehling's B | Blue coloured obtained |
| Majonnajah | 2ml of sample + 5ml of Fehling's A + 5ml of Fehling's B | Blue coloured obtained |

Test for Proteins

| Solution | Reagents added | Observation |
|-------------------|--|------------------------|
| Standard | 1ml of Std. + 5ml of Alk. CuSO ₄ + 0.5ml of F.C reagent | Blue coloured obtained |
| Majonnajah | 1ml of sample + 5ml of Alk. CuSO ₄ + 0.5ml of F.C reagent | Blue coloured obtained |

Microscopic Analysis: The microscopic Character of each ingredient and final product were carried out (Anonymous, 1992). Permanent slides were prepared and stained with Safronin (1%) + Glycerin (Selvakumar *et al.*, 2010).

Microbial Screening: For the finished product microbial analysis was done. (Gopala *et al.*, 2008).

Standard plate count: This method was followed in order to enumerate the total aerobic count in a sample (Gopala *et al.*, 2008).

Antimicrobial test: Formulation was checked for its antimicrobial activity against *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhae*, *Staphylococcus aureus*, *Escherichia coli*,

Candidaalbicans by Agar diffusion method (Gopala *et al.*, 2008).

Stability studies and HPTLC Profile: Comparison of the finished product (formulation) stored at room temperature for second, third & fourth month was carried out by conducting tests for the parameters tannin content, using HPTLC technique (Gopala *et al.*, 2008).

1 gm of sample was extracted with 10 ml of methanol in a reflux condenser for 1 hr, filtered and concentrated. Plates were developed using a mobile phase (consisting of Toluene: Ethyl acetate: Methanol (7:2:1 v/v), for comparing finished product with raw material.

For stability studies, Toluene: Ethyl Acetate: GAA (7.5:3:0.2) was used as the

mobile phase. After that Densitometric scanning was performed on Camag TLC

scanner III in the operated by win CATS planer chromatography version 1.4.3.

| Formulation | TLC plate dimension | Standard used | Sample used (Methanolic extracts of raw materials and formulation) | Volume of sample taken(μ l) |
|-------------|-------------------------|----------------------|---|----------------------------------|
| Majoonnajah | 10 x 10 cm ² | Gallic acid solution | <i>Bisfaijfastugi</i> <i>Aftimoonvilaiti</i> <i>Ustakhudoos</i> <i>Turbud</i> <i>Halelasiyah</i> <i>Balela</i> <i>Amlakhushk</i> Majoonnajah extract | 10 μ l each |

Result and discussion:

Botanical parameters revealed that brownish brown in color, with bitter odour; sweet taste (Table 1). Biochemical analysis showed the presence of carbohydrate, aminoacids and proteins (Table 2). Phytochemical analysis showed presence of tannins, flavonoides while glycosides and steroids were absent (Table 3 and table 4). Microscopic analysis of sample showed the presence of identifying diagnostic characters, which are not overlapping. It shows presence of Ca-oxalate, cells of mesocarp, sclerides (Fig. 1). TLC fingerprint profiles were established for Majoonnajah along with its ingredients using the marker component Tannins i.e. Gallic acid as standard (Fig. 2 and Fig. 3).

Majoonnajah: Bisfayej Fustuqi, Aftimoon Vilaitiand Turbud didn't show any spot

under UV and even after treatment of the TLC plate with the detecting reagent. Hence, it doesn't contain tannin. Rest all of the raw materials showed the presence of tannins and their R_f values approximately matched to Gallic acid Standard. Formulation extract also showed a faint spot of tannin and aR_f value nearly matching to that of the standard and other raw materials. Thus, it can be concluded that this formulation contains these raw materials as its major ingredients.

For the finished product, microbial analysis was done. Pathogens *Escherichia coli*, *Salmonella typhi*, were found to be inhibited by formulation (Table 5). Total aerobic count was done and bacteria, fungi and coliforms were found to be within limits.

A) Organoleptic characteristics (table 1)

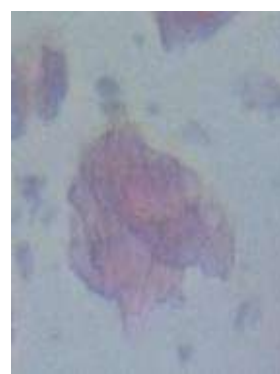
| Characteristics | Observation |
|-----------------|--------------|
| Color | Brown |
| Taste | Sweet-Bitter |
| Odour | Sweet |
| Texture | Semisolid |
| consistency | Viscous |

B) Microscopic analysis (fig. 1)

MAJOON NAJAH - Microscopic evaluation of Majoonnajah showed presence of following:



(a) Ca-oxalate



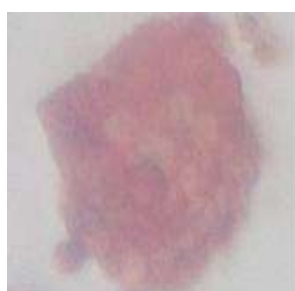
(b) Cells of Epicarp in macerate



(c) Tracheary element (vessel)



(d) Rectangular sclereids of mesocarp



(e) Thick walled cells of Epicarp

C) Phytochemical and Biochemical Analysis

a. Biomolecules tests (Table 2)

| Biomolecules | Test | Majoonnajah |
|-------------------|--------------|-------------|
| Carbohydrate test | Fehling test | Present |
| Amino acid test | Ninhydrin | Present |
| Proteins tests | Folin-Lowry | Present |

b. Phytochemical tests (Table 3)

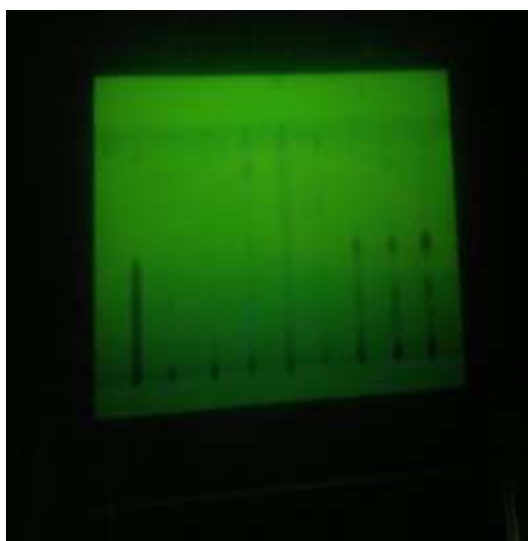
| Test | Tannins | Flavonoids | Glycosides | Steroids |
|-------------|---------|------------|------------|----------|
| Majoonnajah | Present | Present | Absent | Absent |

c. Physico-Chemical Parameters (Table 4)

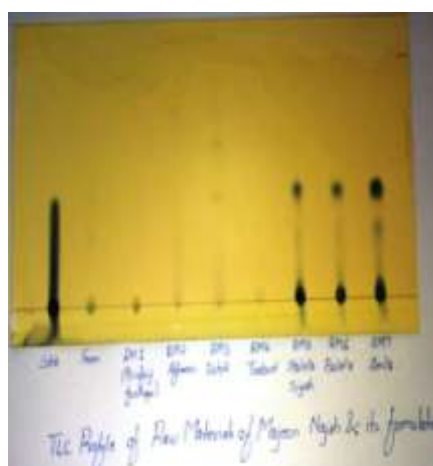
| Test | Majoonnajah |
|----------------|-------------|
| Acid Value | 5.600 |
| Saponification | 354.80% |

d. Thin layer chromatography and stability studies –

TLC plate was developed with suitable solution system and was made to run under 254 nm & 366 nm. The results are as follows.



TLC fingerprint of methanolic extracts
Under UV at 254 nm (Fig. 2).



TLC fingerprint of methanolic extracts
after treatment with detecting reagent (Fig.3)

D) Antimicrobial analysis (Table 5)

Observation table:

| Test Organisms | <i>Staphylococcus aureus</i> | <i>Escherichia coli</i> | <i>Salmonella typhae</i> | <i>Candida albicans</i> | <i>Klebsiella pneumonia</i> |
|----------------|------------------------------|-------------------------|--------------------------|-------------------------|-----------------------------|
| Majoonnajah | Negative | Positive | Positive | Negative | Negative |

Conclusion:

The analysis of sample Majoonnajah by different integrated approaches including physicochemical and biological parameters like total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive, microscopic analysis, biochemical phytochemical analysis, HPTLC chromatogram, and microbial screening and stability studies showed reproducible fingerprints between batches. So it can be concluded that these

parameters can be used for the evaluation of Majoonnajah. The same protocol may be applied for as a regular development of drug, its quality control and standardization for polyherbal formulations.

Further studies are required to determine its mechanism of action and *in vivo* studies.

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