ASSESSMENT OF FOUR MARKET SAMPLES OF ARQ MAUL LAHEM WITH REFERENCE OF PROTEIN

Mohammad Shahabuddin*, Tarannum, Mohammad Idris*****

*Research Associate, Central council for Research in Unani Medicine, New Delhi
** Medical Officer Unani, U.P.
***Principal & H.O.D., PG Deptt. Of Ilm-us-Saidla, Ayurvedic & Unani TIbbia College & Hospital, Karol Bagh, New Delhi, India

***Abstract*: Unani physicians paid a great attention towards *ilaj-bil-ghiza* (Dieto-therapy). They used to treat a number of ailments by alteration in quantity and/or quality of Ghiza. Their knowledge about its nutritive as well as its pharmacological activity was vast as evident from the Unani classical literature. For the management of disease as in *Ilaj bil ghiza* (Dieto-therapy), Unani physician designed a unique formulation as *arq maa-ul-leham* which contains meat as active ingredient (rich source of protein as much as 25%) and herbal drugs according to the need of patients. It provides nutrition along with medicinal activity as of mixing of both animal and herbal ingredients. It is prepared by the method of simple distillation. So, there is a quest behind that can protein obtained by the method of distillation? To answer this question and keeping in view of importance of *maa-ul-leham* present study was designed. This study aims to access protein content in four most common brands of market samples of *arq maa-ul-leham* (namely as sample A, B, C & D). Apart from this the quality of samples were also explored in the terms of some parameters of standardization. No significant variations were noticed in physical parameters of standardization namely viscosity, specific gravity and weight per ml. According to the Lowry's method amongst all the samples highest concentration of protein was found in sample A.**

Keywords*: Unani physicians, *ilaj-bil-ghiza* (Dieto-therapy), *arq maa-ul-leham

1) INTRODUCTION:

Historically, the healing art and practice was organised and relieved from the grip of magic, superstition and religion by Buqrat/Hippocrates. On the basis of Hippocrates's teachings, many other scholars flourished considerably and established the fundamentals of a scientific system of medicine like Jalinoos/Galen, Al Razi/Razes, and Ibn Sina/ Avicenna etc., which was rechristened as Unani system of medicine. Arabs developed and introduced the Unani system into India. It caught the attention and got the status of state medicine in Mughal period. But, during the British rule, it suffered from hindrance due to lack of government support. To overcome this hindrance, efforts were made by Sharifi family in Delhi, Azizi family in Lucknow and the Nizam of Hyderabad for the survival of Unani system of medicine in India during the British rule.

Ilaj-bil-ghiza (Dieto-therapy) has significant place in management of Unani system of medicine from ancient time. Unani physicians identified and devised different suitable dosage forms as dietothrepy in order to treat various disorders and maintain the health, like *fruits, vegetables, eggs, flesh, sikanjabeen, maa-ul-shaeer, maa-ul-jubn, maa-ul-leham, maa-ul-asl, maa-ul-fawakeh* etc (Masihi, 2007 & Ibn sina, 2010). Among of them *Arq-e- maa-ul-leham* is a unique Unani preparation owing to the presence of protein as much as 25%. It provides nutrition along with medicinal activity because of mixing of both herbal and animal ingredients. It has been also extensively described in the Unani classical literature. There is a mention of more than 100 different types of *arq maa-ul-leham*, in which more than 200 ingredients have been used. Apart from all these facts about *arq maa-ul-leham*, it is also important that it is prepared by the method of simple distillation. So, there is a quest behind that can protein obtained by the method of distillation?

To answer this question and keeping in view of importance of *maa-ul-leham* present study was designed. This study aims to access protein content in four most common brands of market samples of *maa-ul-leham*. Apart from this the quality of samples were also explored in the terms of some parameters of standardization.

2) OBJECTIVES:

The aim of the present study was to investigate the presence of protein in four different market samples of *Arq-e- maa-ul-leham*. Apart from this some parameters of standardization i.e. organoleptic and some physico-chemical parameters was also evaluated in market samples of *Arq-e- maa-ul-leham*.

3) MATERIALS & METHODS:

Four branded market samples of *arq maa-ul-leham* were collected at different prices in Delhi. They named as sample A, sample B, sample C, sample D. The following parameters are taken into consideration to achieve the objectives of study.

3.1) Organoleptic character analysis like appearance, colour, ordour and taste
3.2) Physic-chemical parameters analysis like viscosity, specific gravity and weight per ml
3.3) Qualitative as well as quantitative estimation of protein in all market samples.

3.1) Determination of organoleptic properties

Appearance

Appearance was noted as the state of consistency whether it was liquid, semi-liquid, semisolid, solid etc.

Determination of Colour

The four market sample was physically inspected with naked eye for its colour.

Determination of Odour

The determination of odour is totally based on the human perception. A small portion of all the samples was examined by slow and repeated inhalation of air over the sample by the several healthy volunteers having good sense of smell. The strength of the odour was noticed in low note, medium note, and high note.

Determination of Taste

It was performed by asking the healthy volunteers to taste all market samples one by one.

3.2) Physico-chemical parameter assessment

pH value of liquid preparations

The pH value of 1% liquid solution

The 1 ml of market samples was mixed in 100 ml of distilled water, and pH was measured with the help of pH meter.

The pH value of 10% liquid solution

The 10 ml of market samples was mixed in 100ml of distilled water, and pH was measured with pH meter (Anonymous, 2007; Khaleelullah & Rasheeda, 2009).

Determination of Refractive Index

The Refractive index was measured by using the Abbe's Refractometer.

Determination of viscosity

The viscosity of market samples was determined by the U-tube viscometer (Anonymous, 1986).

Determination of specific gravity by Pycnometer

A 25 ml pycnometer was taken for determination of specific gravity. It was weighed accurately, previously cleaned and dried, and the weight 'W' was noted. The stopper was removed, and pycnometer was filled with the market sample at 25 ºC temperature (Ueda *et al*, 2009).

Determination of weight per ml

It was determined by dividing the weight in air, expressed in the gram of the quantity of liquid which fills a pycnometer at specified temperature by the capacity expressed in ml of the pycnometer at the stated temperature.

3.3) Protein Estimation

3.3a) Qualitative analysis of protein

The various chemical tests were done for qualitative analysis of protein in market sample namely as sample A, sample B, sample C, sample C one by one. The details of performed test were given bellow:

Biuret Test

One ml of each market sample was taken in test tube and 1 ml of 40% sodium hydroxide solution and 2 drops of 1% copper sulfate solution were added and observed. The formation of violet colour indicated the presence of proteins (Salwaan *et al*, 2012).

Xanthoprotein Test

One ml of each market sample was taken and one ml of concentrated nitric acid was added. A white precipitate was formed which was boiled and cooled. Then, 20% of sodium hydroxide was added. The orange color indicated the presence of aromatic amino acids (Salwaan *et al*, 2012).

Lead Acetate Test

One ml of the each market sample was taken and one ml of lead acetate solution was added and observed white precipitate. The formation of white precipitate indicated the presence of proteins (Salwaan *et al*, 2012).

Ninhydrin Test

One ml of the each market sample was taken and two drops of freshly prepared 0.2% ninhydrin reagent was added, and it was heated and observed the blue colour. The blue color revealed the presence of proteins, peptides or amino acids (Salwaan *et al*, 2012).

Carbohydrate test

One ml of the test samples was taken and one ml of a mixture of equal parts of Fehling's solution 'A' and Fehling's solution 'B' was added. The contents of the test tube were boiled and observed. The appearance of brick red colour indicated the presence of carbohydrates (Anonymous, 1987).

3.3b) Quantitative estimation of protein

Total Protein estimation by the Lowry's method

Reagents Required

a). BSA stock solution (1mg/ml)
b). Analytical reagents : $CUSO_4$ (4%) solution was prepared by adding 2 g CuSO4 in 100 ml water with 4 g sodium carbonate in 100 ml water and 4 g Sodium potassium tartarate in 100 ml of water
c). Folin: Ciocalteau reagent solution (1N), dilute commercial reagent (2N) with an equal volume of water on the day of use (2 ml of commercial reagent + 2 ml distilled water).

Procedure

1. The different dilutions of BSA solutions were prepared by mixing stock BSA solution (1 mg/ ml) and water in the test tube. The final volume in each of the test tubes was 5 ml. The BSA range was 0.05 to 2 mg/ ml.
2. The 5 µL of these different dilutions mixed with 195 µL of water and 1ml of CuSO4 solution was added. It was incubated at room temperature for 10 minutes. The Follin reagent and water were mixed in the ratio of 1:1. Follin's solution 100 µL was added and incubated for 30 minutes. The OD (optical density) at 680 nm was taken. Results were noted.

3. Through this result, a standard calibration curve of absorbance against protein concentration was plotted.

4. The absorbance of the each market sample were taken and determined the concentration with the help of standard curve (Lowry *et al*, 1951).

4) RESULTS AND OBSERVATIONS:

4.1) Organoleptic characters of market sample of *Arq-e-ma'a-ul-lehem*:

Organoleptic character of market samples of *Arq-e-ma'a-ul-lehem* was summarized below in the table

names of different market sample of *Arq-e-ma'a-ul-lehem*	**organoleptic characters**			
	Appearance	**Colour**	**Odour**	**Taste**
sample A	Liquid	Dark orange	Medium note	Astringent aromatic
Sample B	Liquid	Dark red	Medium note	Astringent aromatic
Sample C	Liquid	Pink	Low note	Astringent aromatic
Sample D	Liquid	Red	Low note	Astringent aromatic

4.2) Physico-chemical parameters of market sample of *Arq-e-ma'a-ul-lehem*:

Physico-chemical parameters of market samples of *Arq-e-ma'a-ul-lehem* was mentioned below in the table

names of different market sample of *Arq-e-ma'a-ul-lehem*	**Physico-chemical parameters**		
	Viscosity	**Specific gravity**	**Weight per ml**
sample A	.892	1.003	1.023
Sample B	.895	1.005	1.029
Sample C	.891	1.003	1.024
Sample D	.888	1.003	1.024

4.3) Protein estimation in market samples of *arq-e-ma'a-ul-lehem*

4.3a) Qualitative analysis for protein

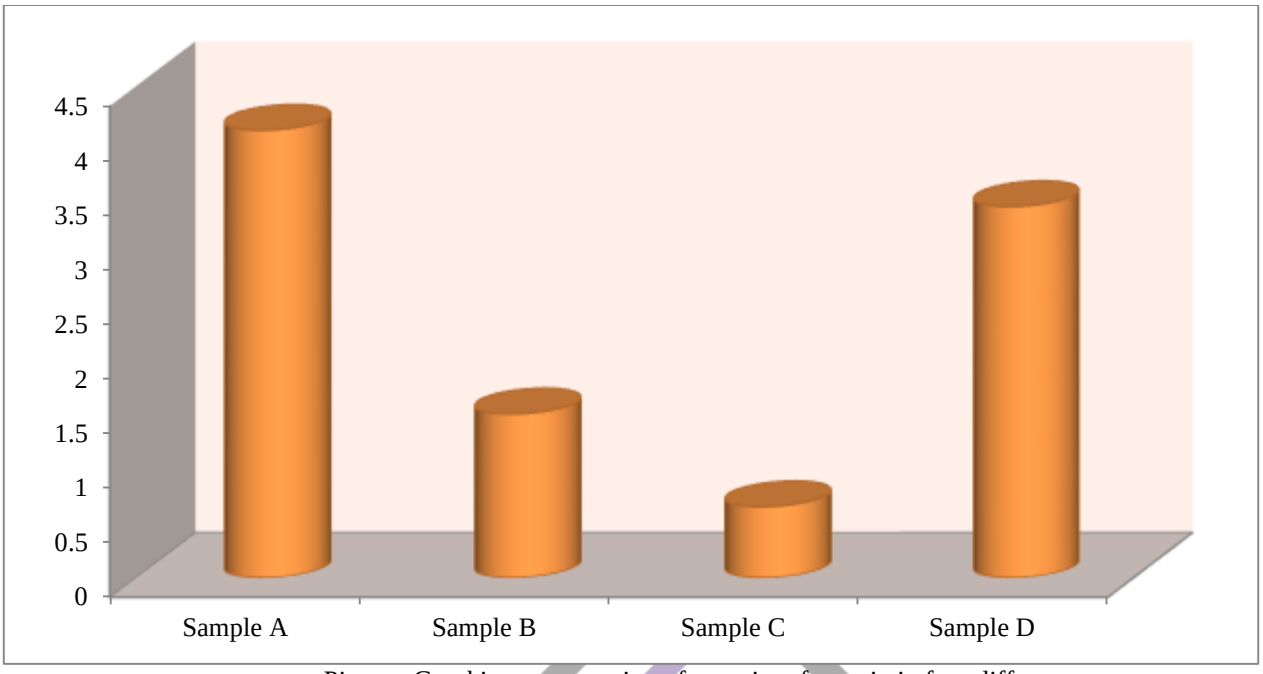
The result of various chemical tests performed on market samples of *Arq-e-ma'a-ul-lehem* was noted below in table.

names of different market sample of *Arq-e-ma'a-ul-lehem*	**Chemical tests**				
	Biuret test	**Xanthoprotein test**	**Lead acetate test**	**Carbohydrate test**	**Ninydrin test**
sample A	-ve	+ve	-ve	++ve	-ve
Sample B	+ve	+ve	++ve	-ve	-ve
Sample C	+ve	+ve	+ve	+ve	-ve
Sample D	+ve	+ve	+ve	+ve	-ve

4.3b) Quantitative analysis for protein

The amount of protein in four different market sample of *Arq-e-ma'a-ul-lehem* was given below in table

S.No	**Name of tests**	**Results of Lowry's method**
1	sample A	4.1 mg/ml
2	Sample B	1.5 mg/ml
3	Sample C	.65 mg/ml
4	Sample D	3.4 mg/ml



Picture: Graphic representation of quantity of protein in four different Samples of *Arq-e-ma'a-ul-lehem*

5) DISCUSSION:

According to the above mentioned physico-chemical analysis, many variations were noticed, such as:

5.1) In Organoleptic characters- all market samples were different in colour. No significant variations were noticed in physical parameters namely viscosity, specific gravity and weight per ml.

5.2) In protein estimation methods-Ninhydrin test is negative in all samples. In sample A only xanthoprotein and carbohydrate test was found positive. In sample B,C and D all test are positive.

According to the Lowry's method amongst all the samples highest concentration of protein was found in sample A.

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