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Granules Formation of Majoon Aarad Khurma Prepared with Stevia Rebaudiana as Sweetening Agent and its Standardization

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Abstract - There is a huge treasure of Compound drugs described in various pharmacopoeias that have developed as a result of painstaking and cumulative efforts of elite scholars of Unani medicine. However, there has always been scope for inclusion of new compound drugs whose safety and efficacy has been proved scientifically. Majoon Aarad Khurma which is widely used as an effective aphrodisiac is prepared with sugar as base. Therefore the present study is aimed to develop granules of Majoon Aarad Khurma with natural sweetening agent *Stevia rebaudiana*. Granules are more convenient and comfortable in usage and dispensing. Granules of Majoon Aarad Khurma were evaluated for physico-chemical parameters as recommended in Physicochemical Standards of Unani formulations by CCRUM. The granules of Majoon Aarad Khurma were found to be more stable, convenient and comfortable in usage and dispensing, and also safe, light, efficacious, cost effective and quality controlled.

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I. INTRODUCTION

A number of drugs have been used in different traditional system of medicine which are claimed to have sex improving ability. Since, these drugs act in different ways therefore have been categorized in different groups. Drugs supporting the sagging libido or sexual performance which are not up to the desire level or enhance the sexual performance are called "Aphrodisiacs"^{1, 2}. A number of Unani drugs of plant, animal and mineral origin have been claimed to possess aphrodisiac properties such drugs are named as Muqawwie Bah, which may be single drug or a formulation. In present study an important Unani compound formulation Majoon Aarad Khurma has been modified into granular form using natural sweetening agent *Stevia rebaudiana* which has sweetening property as well as hypoglycemic activity, the granules of Majoon Aarad Khurma become palatable and will not cause any harm to diabetic patients who are suffering from sexual dysfunction. This study also includes evaluation of physicochemical standards granules of Majoon Aarad Khurma there is a need for standardization of GMAK

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following scientific parameters including organoleptic characters, Physical analysis, chemical analysis, chromatographic pattern. The work was undertaken to standardize and validate Unani medicine, Granules of Majoon Aarad Khurma used in the treatment of Sexual weakness. Standardization of GMAK was carried out following Good Manufacturing Practices (GMP) for preparation of Unani medicines. Standardization guidelines for herbal products provided by World Health Organization (WHO) have been followed.

In present study an important Unani compound formulation Majoon Aarad Khurma has been modified into granular form using natural sweetening agent *Stevia rebaudiana* by the process of granulation.

Granulation is the process in which primary powder particles are made to adhere to form larger multi particle entities called granules. Pharmaceutical granules typically have a size range between 0.2 and 0.4 mm, depending on the subsequently use of the granules. The Majoon was converted into granules because Granules are used

1. To prevent segregation of the constituent of the powder mixture.
2. To improve the flow properties of the mixture.
3. To improve the compaction characteristics of the mixture.
4. Other reasons:
 - i. The granulation of toxic materials will reduce the hazard associated with the generation of toxic dust which may arise when handling powder. Thus granules should be non-friable and have a suitable mechanical strength.
 - ii. Material which are slightly hygroscopic may adhere and form a cake if stored as a powder. Granulation reduces these hazards as the granules will be able to absorb some moisture and yet retain their flow ability because of their size.
 - iii. Granules, being denser than the powder. They are therefore more convenient for storage or shipment³.

Stevia is a genus of about 240 species of herbs and shrubs in the sunflower family (Asteraceae), native to subtropical and tropical regions from North west

America to South America. The species *Stevia rebaudiana*, is widely grown for its sweet leaves. As a sweetener and sugar substitute, *Stevia's* taste has a slower onset and longer duration than that of sugar, With its steviol glycoside extracts having up to 300 times the sweetness of sugar, *Stevia* has garnered attention with the rise in demand for low-carbohydrate, low-sugar food alternatives. Because *Stevia* has a negligible effect on blood glucose, it is attractive as a natural sweetener to people on carbohydrate-controlled diets.⁴ It is perennial shrub which grows up to 2 meter height and leaves are 2-3 cm long. Small white flowers appear after 50-100 days of transplantation. It generally survives in warm weather but treated as annual in cold region. *Stevia* is grown commercially as well as in gardens and kitchens⁵. It is useful in obesity, diabetes, gingivitis, acne, digestive problems, cuts, wounds, mouth sores, heartburn, seborrhoea, eczema, dermatitis, inflammations, and high blood pressure. It is used in mouthwash, toothpaste and used to kill mouth bacteria. Sweetener in confectionery, beverages and food industry. It is also used in cosmetic industries in skin shinning and anti-wrinkle creams. It helps the body sustain a feeling of vitality and well-being and used externally for blemishes. Stimulate alertness and counter fatigue, facilitate digestion and gastrointestinal functions⁵.

The quality control of herbal medicines has a direct impact on their safety and efficacy.¹¹⁰ Physico-chemical standardization are a pre-requisite in quality control of Unani medicines, both single as well as compound. The efficacy of a drug mainly depends upon its physical and chemical properties therefore, the determination of physico-chemical characters for the authenticity of a drug is necessary. A little deviation from the normal in terms of quality and quantity of the constituents may alter the effect of the drug. Apart from the degradation in the quality of the drugs that occurs due to above conditions, adulteration also contributes to variability.

In this study Granules of Majoon Aarad Khurma were prepared and subjected to Physico-Chemical evaluation under the following parameters:

(1) Organoleptic properties such as the appearance, colour, smell, and taste (2) Alcohol soluble matter and Water soluble matter (3) Successive extractive values (4) pH value (5) Bulk density and Tapped density (6) Ash value (7) Volatile oil (8) Saponification value (9) Iodine value (10) Acid value (11) Estimation of total Alkaloids (12) Resin (13) Reducing and non-Reducing sugars (14) Crude fibers (15) Thin layer chromatography (TLC) was also conducted for identification of compounds.

II. MATERIAL AND METHOD

a) Formulation of Granules

All the required ingredients of Granules of Majoone Aarad Khurma were procured from the raw drug dealers under the supervision of the Guide, and all the raw drugs were identified and authenticated by the expert Dept. of Ilmul Advia, NIUM Bangalore, (Karnataka). Granules are prepared in the laboratory of Dept. of Ilmul Saidla, NIUM Bangalore, as per the formulation mentioned in the National Formulary of Unani Medicine, Part-1, Govt. of India. The composition is as given in table 1: All the dried ingredients were powdered and sieved in (sieve number 80). All the Maghaziyaat (kernels) were powdered separately and sieved in (sieve number 40), and dates were separately dried in a hot air oven at 100 °C for 4 hours and then powdered and passed through sieve number 60. *Stevia* plant extract was prepared with 120 ml water at low temperature for 15 minutes, and sieved through muslin cloth; the total quantity of this extract obtained was 80 ml. All the dried drugs were mixed one by one in *Stevia* extract, and subjected into the granulator (sieve number 20) for formation of granules and then stored in container at room temperature for further study⁶.

Table 1 : Ingredients of granules of Majoon Aarad Khurma

Sl. No.	UNANI NAME	BOTANICAL NAME	PART USED	QUANTITY
1	Khurma	<i>Phoenix dactylifera</i>	Fruit	200gm
2	Kamagh arbi	<i>Acacia arabica</i>	Gum	200gm
3	Singhara khushk	<i>Trapa bispinosa</i>	Fruit	200gm
4	Satawar	<i>Asparagus rasemosus</i>	Root	50gm
5	Jaiphal	<i>Myristica fragrans</i>	Nutmeg	1.25gm
6	Javitri	<i>Myristica fragrans</i>	Mace	1.25gm
7	Qaranfal	<i>Myrtus caryophyllus</i>	Fruit	2.5gm
8	Maghaze Badam	<i>Prunus amygdalus</i>	Fruit	25gm
9	Maghaze Chilghoza	<i>Pinus gerardiana</i>	Fruit	25gm
10	Maghaze Fundaq	<i>Corylus avellana</i>	Fruit	25gm
11	Maghaze Pambadana	<i>Gossypium herbaceum</i>	Fruit	5gm
12	Stevia plant powder	<i>Stevia rebaudeana</i>	leaves	3.50gm

Physico-Chemical Evaluation

The Physico-Chemical studies were carried out on Granules of Majoon Aarad Khurma in the laboratory of Dept of Ilmu Saida, NIUM, Bangalore.

Organoleptic Properties^{7,8}.

Organoleptic properties of GMAK like appearance, colour, smell and taste were noted.

Determination of alcohol and water soluble matter

b) *Cold maceration method:*⁹

Accurately weighed 5.0 g of the samples of GMAK were placed in glass Stoppard conical flask separately. Macerated with 100 ml of the Ethyl Alcohol for 6 hours, shaking frequently, and then allowed to stand for 18 hours and filtered rapidly through dry filter paper, and evaporated to dryness on a water-bath. Then dried at 105 °C for 6 hours in hot air oven and cooled in desiccators for 30 minutes and weighed instantly. The percentage of water soluble matter was determined as above by using distilled water instead of alcohol.

c) *Successive extractive values*¹⁰

The extractive values of GMAK in different solvents viz. petroleum ether, chloroform, ethyl alcohol and water were carried out by percolation in Soxhlet's apparatus separately and heated for six hours on a water bath for each solvent except water, which was heated directly on a heating mantel. Granules of test samples was taken and subjected to successive extraction with each solvent. The extracts were filtered on water bath; the percentage of extractive values was calculated.

d) *Determination of pH*¹⁰

pH of freshly prepared 1% w/w suspension and 10% w/w suspension in 100 ml distilled water of the test samples GMAK was determined using simple glass electrode digital pH meter.

e) *Bulk density & tapped density of granules*¹¹

30 gm of drug (GMAK) was filled and carefully added them into cylinder with the aid of a funnel without any loss. The cylinder was tapped by a digital tap density apparatus (Lab-India Tap Density Apparatus, Model-TD 1025). The initial volume was noted and the samples were then tapped until no further reduction in volume was noted.

$$\text{Bulk Density} = \frac{\text{Mass}}{\text{Bulk Volume}}$$

$$\text{Tapped Density} = \frac{\text{Mass}}{\text{Tapped Volume}}$$

Carr's Index

$$\text{Carr's Index} = 100 \times \frac{1 - D_b}{D_t}$$

Where D_b = Bulk density, D_t = Tapped density

Hausner Ratio

$$\text{Hausner Ratio} = \frac{D_t}{D_b}$$

Where D_b = Bulk density and D_t = Tapped density.

f) *Ash values determination*¹²

i. *Total Ash*

2 gm of drug GMAK was incinerated in a silica dish at a temperature not exceeding 450°C until free from carbon, cooled and weighed and the percentage of Total Ash was calculated.

ii. *Acid insoluble Ash*

The ash was boiled with 25ml of dilute hydrochloric acid for 5 minutes. The insoluble matter was collected on an ash less filter paper washed with hot water and ignited at a temperature not exceeding 450°C and weighed after cooling. The percentage of Acid insoluble Ash was calculated.

iii. *Water soluble Ash*

The ash was boiled with 25 ml of distilled water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited. The weight of insoluble ash was subtracted from the weight of the total ash, giving the weight of the water soluble ash. The percentage of Water soluble Ash was calculated.

g) *Estimation of volatile oils*¹⁰

50 gm of GMAK was taken with 30ml of glycerine and 300ml of water in 1litre distilling flask separately; and the condenser were fixed. The content of the flask was now heated and stirred by frequent agitation until ebullition commenced. At the end of specified time (3-4 hrs.) heating was discontinued, the apparatus was allowed to cool for 10 min and the tap was opened and the tube lowered slowly, as soon as the layer of the oil completely entered in to the graduated part of the receiver the tap was closed and the volume was recorded.

h) *Estimation of total alkaloids*¹³

2.5 g of the test drug sample GMAK was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added by drop to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitates was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried and weighed.

i) *Determination of resin*¹⁰

10 gm of drug GMAK was weighed accurately and rapidly refluxed with acetone to exhaust the drug for the resin content, the excess solvent was removed by distillation and placed on water-bath and the last traces of acetone was removed under vacuum, the residue was suspended and transferred to a separating funnel,

repeatedly extracted the suspension with ether to extract all the resin content was filtered, dried and distilled the excess of ether was dried over water-bath, then transferred to a weighed Petri-dish and dry out under vacuum to constant weight. The percentage of Resin was calculated.

j) *Determination of reducing sugar and non-reducing sugar*^{10, 14}

Dissolve 2gm of drug GMAK in about 250 ml of distilled water separately, 12 gm saturated solution of lead acetate (neutral) to produce flocculent precipitate, shake thoroughly and let to stand for 15 mints. Filtered through a dry filter paper and add 3.17gm anhydrous sodium carbonate or potassium oxalate to make sure that all the lead had been removed then filtered.

III. STANDARD FEHLING'S SOLUTION PREPARATION

10 ml of freshly mixed Fehling's solution A & B was taken in a conical flask, then 40 ml of distilled water was added. Then dextrose solution added which was prepared by dissolving 1.25gm dextrose in 250 ml of distilled water, Fehling's solution was constantly heated at a low flame until the solution was completely reduced and the blue colour nearly discharged, 3-5 drops of aqueous Methylene Blue was added while continuing the titration until the indicator was completely decolourised and the liquid was orange red. The weight of the glucose was calculated equivalent to 1 ml of Fehling's solution¹⁴.

a) *Reducing sugar*:¹⁴

The sample solution prepared earlier was taken into a burette and titrated as above. The titration was completed within a total boiling time of 3-4 mints. The amount of reducing sugar was calculated with the help of dextrose used in the above titration.

b) *Non-Reducing sugar*:¹⁴

50 ml of prepared sample solution of GMAK was taken separately into a flask, 15 ml of 1N HCl (86ml/1 liter) and boiled for 3-4 mints. The solution was cooled rapidly, neutralized with the help of NaOH solution adding phenolphthalein as indicator and the volume was made upto 250 ml. The percentage of Non-Reducing sugar was calculated.

c) *Determination of crude fibers*^{10, 12}

15 gm of drug MAK and GMAK was weighed; leach out all the sugars, the material was extracted first in volatile ether (100 ml) for the removal of fats and waxes which, being immiscible in aqueous solution, prevented penetration of acid and alkali into the drug particles. After that 200 ml of boiling sulphuric acid (1.25%) was added (the sulphuric acid was prepared by diluting 51 ml of 1N sulphuric acid to 200 ml of distilled water at 25°C), the mixture of acid and drug was cooled

and then heated to boiling and then the flame was adjusted for slow steady boiling for 30 minutes. The time was noted when the mixture started to boil and not from the time when it is kept on flame. A continuous stirring is necessary where as a current of air should be passed into the mixture through a capillary tube to prevent frothing. Acid insoluble residue was collected on a filter and washed with water to remove the acid, the residue was put into the flask with 200 ml of boiling 1.25% sodium hydroxide solution (70 ml of recently standardized carbonate 1N sodium hydroxide was dissolved in 200 ml distilled water). The mixture was boiled for 30 mints under the refluxed condenser, then filtered and washed with hot water to remove all the alkali, after drying the residue at 100°C, until constant weight. The percentage of Crude fibres was calculated.

IV. CHEMICAL EVALUATION

a) *Thin layer Chromatography*:^{12, 15}

Thin layer chromatography was carried out on T.L.C. pre coated aluminium plates, silica gel 60 F 254 (layer thickness 0.25 mm) for ethanolic extract of the test drug samples GMAK in various mobile phases (Toluene: Ethyl acetate (7: 3, with 2 drop Sulphuric acid), .. The R_F values of the spots were calculated of the test drug by the following formula.

$$R_f \text{ Value} = \frac{\text{Distance travelled by Spot}}{\text{Distance travelled by Solvent}}$$

V. RESULTS AND DISCUSSION

The organoleptic properties of Granules of Majoon Aarad Khurma was determined on the basis of appearance, color, smell, and taste, was found to be, granular, light brown, pleasant, and sweet.

The **Alcohol and Water soluble matter** of GMAK were determined. The percentage of alcohol and water soluble matter was found to be 24.6 ± 0.61 and 36.6 ± 0.50 respectively in Granules of Majoon Aarad Khurma.¹⁰. (shown in table no.2)

Extractive values of the drug were determined. The percentage of extractive values of Granules of Majoon Aarad Khurma was found to be 4.2 ± 0.11 in Petroleum Ether, 0.73 ± 0.06 in Chloroform, 19.13 ± 0.17 in Ethyl-alcohol and 37.2 ± 0.23 in water. The **Extractive value** is a parameter for detecting the adulteration in any drug 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 one¹⁶. When the values were compared with values of MAK mentioned in physico-chemical standard of Unani Medicine by CCRUM the values of GMAK were higher than the values of MAK. This indicates the efficacy of GMAK is higher than MAK¹⁰.

pH of the drug were determined and was found to be acidic for each drug the values being 5.82 ± 0.008 in 1 % aqueous solution and 5.27 ± 0.008 in 10 % aqueous solution of Granules of Majoon Aarad Khurma. The **pH value** of various dosage forms of plant drugs may also be considered a parameter for the purity of a drug. The pH and hydronium ion concentration also play an important role in the study of drug receptor-site interactions, an area of research which has gained considerable impetus during recent years¹².

The **bulk density, tapped Density, Carr's index and Hausner ratio** of Granules of Majoon Aarad Khurma found to be, 0.56 gm/ml, 0.65 gm/ml, 12%, and 1.12 respectively. **Bulk density** of a compound varies substantially with the method of crystallization, milling, or formulation.

Compressibility Index and the Hausner's ratio are the simple, fast and popular methods of predicting

powder flow characteristics, the compressibility index has been proposed as an indirect measure of bulk density, size and shape, surface area, moisture content and cohesiveness of materials.

The percentage of Ash values of Granules of Majoon Aarad Khurma were found to be 2.5 ± 0.28 total ash, 0.66 ± 0.16 acid insoluble ash and 1.16 ± 0.16 water soluble ash. **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 unrequired 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 gives information related to its adulteration with inorganic matter.¹⁷

Physico-Chemical data of GMAK

Sl. No.	Physico Chemical Properties	GMAK
1.	Organoleptic properties Apearance Odour Smell Taste	Granules Brownish Pleasant Sweet
2.	Alcohol Soluble Matter	24.6%
3.	Water Soluble Matter	36.6%
4.	Successive Extractives Petroleum Ether Chloroform Ethyl Alcohol Aqueous	 4.2% 0.6% 19.13% 37.2
5.	PH Value 1% 10%	 5.82 5.27
6.	Ash Value Total Ash Acid Insoluble Ash Water Soluble Ash	 2.5% 0.66% 1.16%
7.	Bulk Density Tapped Density Carr's Index Hausner Ratio	 0.6gm/ml 0.68gm/ml 12% 1.13
8.	Volatile oils Traces	0.1%
12.	Alkaloids Total	3.52%
13.	Resin Estimation	37.2%
14.	Determination of Resin	37.2%
15.	Reducing sugar	15.6%
16.	Non-Reducing sugar	24.2%
17.	Crude Fibers	2.62%
18	TLC Rf Values	 0.31 0.36 0.50 0.68 0.75

TLC plates high lighting the spots in GMAK



In present study an important Unani formulation i.e. Majoon Aarad Khurma has been modified into granules using *Stevia rebaudiana* as a sweetener. Thus, keeping in view the above consideration, physico-chemical standardization of the test drugs GMAK was carried out and subjected to Physico-Chemical evaluation under the following parameters:

(1) Organoleptic properties such as the appearance, colour, smell, and taste (2) Alcohol soluble matter and Water soluble matter (3) Successive extractive values (4) pH value (5) Bulk density and Tapped density (6) Ash value (7) Volatile oil (8) Estimation of total Alkaloids (9) Resin (10) Reducing and non-Reducing sugars (11) Crude fibers (12) Thin layer chromatography (TLC) was also conducted for identification of compounds.

The **percentage of Volatile oil** in the in the Granules of Majoon Aarad Khurma was found to be 0.1 ± 0.00 . The volatiles oils are mixed and mostly contain hydrocarbons, alcohols, aldehyde, ketones, phenols, acids and sulphur compounds.

The percentage of **Total alkaloids** estimation of Granules of Majoon Aarad Khurma was found to be 3.52 ± 0.09 . As a class of medicinal agents, **alkaloids** are characterized by their high potency. A slight deficiency of alkaloids in a preparation may cause a marked decrease in physiological effect; on the other hand, a slight excess may cause toxic effects when the preparation is administered. It therefore followed that the accurate estimation of the quantity of alkaloids present

in a medicinal substance as an important subdivision of pharmaceutical analysis.

The percentage of **Resin** of the drug Granules of Majoon Aarad Khurma was found to be 15.1 ± 0.05 and 37.2 ± 0.06 . **Resins** are the complex mixture of resin acid, resin alcohol, esters and resins. The resins are present as a homogenous mixture of volatile oil, and /or gum. If they contain volatile oil they are oleo-resin, if they contain gum then gum-resin and if both are present they are known as oleo-gum-resin.¹⁶

The percentage of reducing sugar estimation of Granules of Majoon Aarad Khurma was found to be 15.6 ± 0.06 . **A reducing sugar** is any sugar that in a solution has an aldehyde or a ketone group. The ability of a sugar to reduce alkaline test reagents depends on the availability of an aldehyde or keto group for reduction reactions. Sugars which are capable of reducing the oxidizing agents, Fehling reagents (CuO) in alkaline solution are called the Reducing Sugars¹⁸.

The non-reducing sugars that cannot reduce oxidizing agents. The percentage of Non-Reducing sugar estimation of the drug Granules of Majoon Aarad Khurma was found to be 24.2 ± 0.13 .¹⁹

The percentage of Crude Fiber content of the drug Granules of Majoon Aarad Khurma was found to be 2.48 ± 0.08 . **The crude fiber** content of a drug is of considerable importance in the examination of certain drugs and particularly of spices, since the commonly used adulterants consist of waste or refuse material derived from the drugs or spices themselves or from other food products. Frequently, this material is the outer cellular layer or protecting coating, which contains a larger proportion of lignified tissues and, consequently, more crude fiber.¹⁸

TLC studies of Alcoholic extract of the test drug Granules of Majoon Aarad Khurma was performed and R_f values of various spots appeared in Toluene: Ethyl acetate (7: 3, with 2 drop sulphuric acid) solvents system was found to be 0.31, 0.36, 0.50, 0.68, 0.75 respectively. In GMAK five spots were observed. **Thin layer chromatography** is one of the important parameter used for detecting the adulteration for judging the quality of the drugs. If the drug is adulterated there might be appearance of the other compounds present in adulterant, in turn may increase the no. of spots. On the other hand the exhausted or deteriorated drugs may lose the component and the number of spots appeared might be less.

VI. CONCLUSION

The Physicochemical standards for scientific evaluation of Granules of Majoon Aarad Khurma were estimated and the standards were evaluated as recommended by CCRUM.

Based on the finding it is concluded that:

- Granules possessed the same principles and maintained same characteristics as traditional dosage form Majoon Aarad Khurma.
- The granules of Majoon Aarad Khurma were found to be more stable, convenient and comfortable in usage and dispensing, and also safe, light, efficacious, cost effective and quality controlled.
- Stevia a natural sweetening agent can be used as safe and efficacious sweetening agent in preparation of granules as well as in other Unani formulations.

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