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Analytical Standardization of Raw Guggulu and Navaka Guggulu

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ABSTRACT

'Analysis' means the detailed examination, which reveals the minor but important aspects regarding the drug. Analytical study of a product provides some standards to judge its quality. It is useful to decide future work plan and objective parameters to know the accurate status of a drug by conducting the comparative study of various samples during drug preparation. The important aims for analytical study of ayurvedic drugs are to know the particular chemical configuration and to point out the physico-chemical changes and effect of different processing (*samskara* e.g. *shodhana*, *marana* etc.) it also helps to know the probable role of a media during the pharmaceutical processing. Though analytical study one can interpret the probable pharmacokinetics and access the quality of selected material and final product. However, ayurvedic analytical techniques are not sufficient to answer the queries of modern science. Hence For better utilization of ayurvedic pharmaceutics, it is need of the hour to analyze the drug through both classical and modern qualitative and quantitative parameters. The quality of a dosage form should not only be tested at the end but must built into the product right from the moment of receipt of raw materials through processing until the final packaging. The Physico-chemical analysis provides the objective parameters to improvise the standards for quality of raw drugs as well as finished products.

INTRODUCTION

As it was observed that no previous work had been carried out concerning the Analytical standardization of raw *Guggulu* and *Navaka Guggulu* till date, the formulation was opted for the current research work. Sample of *Navaka Guggulu* was prepared under standardized conditions and for assurance of excellence, efficacy and performance, obtained samples were further subjected for Physico-chemical analysis. A very sincere attempt was made to set standardized parameters for the formulation. In the present research work, analytical analysis of Raw *Guggulu* and *Navaka Guggulu* has been studied.

AIM & OBJECTIVES

1. To prepare the sample of *Navaka Guggulu* as per the text *Chakradutta*.
2. To evaluate differentiation between Raw *Guggulu* and *Navaka Guggulu.*
3. To analyze Physico-chemical properties of both Raw *Guggulu* and *Navaka Guggulu*.

MATERIAL AND METHODS

Pharmaceutical Processing

It is done into following two steps-

1. *Guggulu Shodhana*
2. Preparation of *Navaka Guggulu*

1.Guggulu Shodhana

In order to maintain the quality and efficacy of the formulation, best quality *Guggulu* was procured from Una(Himachal Pradesh). Before preparation of *Navaka Guggulu*, *Shodhana* of *Guggulu* was done according to the AFI[(1)]. Physical impurities like stone, bark, wood etc. from raw material were removed manually. *Asudhha Guggulu* was broken into small pieces and taken in the *Pottali* (bundled in cotton cloth). It was hung in *Dola Yantra* (an instrument used to impregnate medicine in liquids) in *Gomutra* taken in a Steel vessel. *Gomutra* was heated at a temperature between 75 °C- 85 °C until all the *Guggulu* was passed into the fluid through the cotton cloth. After complete filtration, the filtrate was subjected for further heating to evaporate the liquid up to the semi-solid state. During the whole process temperature was maintained between 75^0C -85^0C later it was shifted into a stainless steel tray. The mass was dried in sunlight and then pounded with a pestle in a stone mortar.

2.Preparation of *Navaka Guggulu*

To prepare *Navaka Guggulu*, *Shudha Guggulu* was treated *Triphala*, *trikatu* and *trimad*. It was prepared, as mentioned in the classical text of *Chakradatta*[(2)]. All the ingredients of *Navaka Guggulu* were taken in equal quantities except *Guggulu* which will be equal to all the above ingredients as per AFI[(3)]. *Shuddha Guggulu* was heated and the fine powder of *Triphala*, *Trikatu*, *Trimada* were mixed with *Guggulu*. Gum acacia in 9% concentration of the mixture was taken in a beaker and sufficient amount of water was poured into it. The content was heated on a hot plate till a homogenous solution was obtained. This solution was added to the mixture and mixed thoroughly in a stainless steel vessel and passed through sieve no. 22 to obtain granules. These granules were dried in a hot air oven for two days at 50^0C temp. After that, the granules were compressed into tablets with the help of 12 station tablet punching machine. It was stored in an airtight container.

PARAMETERS STUDIED

Parameters were taken according to "**Protocol of testing of Ayurvedic, Siddha, and Unani Medicines",** written by Dr D.R. Lohar, published by Government of India, Department of Ayush, Ministry of Health and Family Welfare, and Pharmacopoeial Laboratory For Indian Medicines, Ghaziabad.

Place of work:

- Vasu Research Centre, Vadodara, Gujarat.

Classical Analytical Parameter-

1. ***Sugandhiyukta***- *Guggulu* has intense, pleasant balsamic odour.
2. ***Vigatashalya***- It was free from physical impurities like bark, thorn, sand etc.
3. ***Vahnau jvalanti***- The sample was immediately burnt on fire.
4. ***Tapane vilayam***- Guggulu started melting on exposure to sunlight/ heat.
5. ***Koshna salile payah samana***- when diluted in warm water, it produced milky emulsion.

Detail of Parameters for Analysis

Parameters	Characteristics
Organoleptic	*Rupa* (Colour), *Rasa* (Taste), *Gandha* (Odour), *Sparsha* (Consistency)
Physical	Hardness, Friability, Disintegration time, Uniformity of weight
Physico-chemical	pH, loss on drying, Total Ash value, Acid insoluble ash, Water soluble extractive, Alcohol soluble extractive, Assay of Guggulsterone
Heavy Metals	Lead, Arsenic, Cadmium, Mercury
Microbial limit Test	Total bacterial and Total fungal count

The various analytical tests which were performed on the samples are as follows:-

Organoleptic parameters-(lit. impression on the organ)

The word "leptic" is derived from the Latin word *Leptikos*; meaning "dispose to accept". The properties which are experienced by the sense organs, including taste, sight, touch, aroma and sound were observed.

2) Physical analysis –

a) Shape-Shapes of tablets are according to the shape of die and punches. The tablets are of various shapes viz. oval, rectangular, round, triangle, square, capsule, heart, diamond etc.

b) Size-The diameter and thickness of tablets were measured by using Vernier Caliper. Randomly selects ten tablets were measured for diameter and thickness individually.

c) Hardness test[4] –The finished *Vati* has to be hard, as it may not disintegrate in the required period of time and if the *Vati* is too soft, it may not withstand during packing and transporting. Therefore, it is necessary to check the hardness of tablets.

Method – (Monsanto Hardness test)

It is a soft portable hardness tester, which was manufactured and introduced by Monsanto chemical company. It consists of a spring, which can be compressed by moving the screw knob forward. The tablet which to be tested was held between a fixed and a moving jaw and reading of the indicator was adjusted to zero. The force was applied to the edge of the *Vati* and gradually increased by moving the screw knob forward until the *Vati* breaks. Reading was noted from the scale, which indicates the pressure required in Kg or in pounds to break the tablet. The hardness of 4 Kg considered suitable for handling of *Vati*.

Hardness in Kg $\geq$ 3.5+0.5

d) Uniform weight of tablets[5] –The average weight was determined by weighing 20 tablets. The tablets were also weighed singly. The deviation from the average weight in each case was calculated and expressed as a percentage. Not more than two of the tablets deviate from the average weight by a greater percentage. If 20 tablets are not available, ten may be used for the determination, not more than one deviate from the average weight by a higher percentage.

Limits according to U.S.P

· Weight of tablet 130 mg or less then %error = ±10%

· Weight of tablet 130-324 mg then %error = ±7.5%

· Weight of tablet 324 mg or more then %error = ±5%

e) Friability[6] –Friability test is performed to evaluate the ability of the tablet to withstand abrasion in packing, handling and transporting. The instrument used for the same is known as "Friability test apparatus"

Method –

It consists of a plastic chamber, which is divided into two parts and revolves at a speed of 25 rpm. Several tablets were weighed and placed in the tumbling chamber, which was rotated for four minutes of 100 revolutions. During each revolution, the tablets fall from a distance of 6 inches to undergo shock. After 100 revolutions the tablets were again weighed and the loss in weight indicates the friability. The acceptable limit of weight loss should not be more than 1%.

f) Determination of disintegration time[7]

Procedure –One pill was introduced into each tube of the disintegration apparatus. The disc was added to each tube. The assembly was suspended in a beaker containing water at 370 C and the apparatus was operated. The time was noted down with the help of stopwatch. The time taken for all the tablets to disintegrate completely is disintegration time.

3) Physico-chemical Parameters-

a)pH[8]-The pH value of an aqueous medium may be defined as the common logarithm of the reciprocal of the hydrogen ion concentration expressed in gram per litre. It was done by digital pH meter. pH meter was stabilized for 15-30 min. Now the electrode has been immersed in a standard buffer solution of pH 4.0 and stabilized for 1 min. and reading was adjusted at pH 4.0. The electrode was rinsed and immersed in the sample. The reading displayed on the monitor was noted.

b) Loss on drying[9]-The moisture content was estimated by determining the amount of volatile matter (water drying off from the drug). 10 gm of a drug (without preliminary drying) after accurately weighing (accurately weighed to within 0.01 gm) was placed in a tarred evaporating dish and dried at 105^0 C for 5 hours and weighed. The drying was continued and weighed at one-hour interval until the difference between two successive weights corresponds to not more than 0.25 percent. Constant weight is reached when two consecutive weights after drying for 30 minutes and cooling in a desiccator, showed not more than 0.01 gm difference.

c) Determination of total ash[10]-2-3 gm of accurately weighed ground drug was incinerated in a tarred platinum or silica dish at a temperature not exceeding 450^0 C until free from carbon. It was then cooled and weighed. By adding the filtrate, it was evaporated to dryness and ignited at a temperature not exceeding 450^0 C. The value of total ash was determined by calculating the percentage of ash with reference to the air dried drug.

d) Acid insoluble ash11-To the crucible containing total ash, 25ml of dilute HCl was added. The insoluble matter on an ashless filter paper was collected and washed with hot water. Filter paper containing the insoluble matter was transferred to the original crucible dried on a hot plate and ignited to constant weight. The residue was allowed to cool in suitable desiccators for 30 minutes and weighed without delay. The content of acid-insoluble ash was calculated with reference to the air-dried drug.

e) Determination of Alcohol Soluble Extractive[12]**-** 5gm of the air-dried coarsely powdered drug was macerated with 100 ml of alcohol of the specified strength in a closed flask for twenty-four hours, frequently shaking for six hours and allowed to stand for eighteen hours. After that it was filtered carefully, taking precautions against loss of solvent. Now 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, and allow drying at 105^0C to constant weight and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.

f) Water soluble extractive[13]-5 gm of the air-dried coarsely powdered drug was macerated with 100 ml of distilled water in a closed flask for twenty-four hours, shaked frequently for six hours and allowed it to stand for eighteen hours. After that, it was filtered, taking precautions against loss of water. Then 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dry at 105^0 C, to constant weight and weighed. The percentage of water soluble extractive was calculated with reference to the air-dried drug.

5) Test for Heavy/ Toxic Metals by AAS (Atomic Absorption Spectrometry)[14]

This technique is based on the fact that when atoms, ions or ion complexes of an element in the ground state are atomized in a flame, they absorb light at the characteristic wavelength of that element. If the absorption process takes place in the flame under reproducible conditions, the absorption is proportional to the number

of absorbing atoms. The measurement of the absorption of radiation by the atomic vapour of the element generated from a solution of that element is the basis of atomic absorption spectrometry. The determination is carried out at the wavelength of one of the absorption lines of the element concerned. The assay is done by comparing the absorbance of the test solution with that of the reference preparation.

5) Microbial Count

1. Total bacterial count
2. Total fungal count
3. Test for specific pathogen

- E. coli
- Staphylococcus aureus
- Pseudomonas aeruginosa
- Salmonella Spp.

➢ **Pretreatment of the sample-**

1gm of the sample was dissolved directly in Soybean Casein Digest broth.

Total bacterial count (Pour plate method)

To a 9cm diameter petri dish, 1mL of the sample from Soyabean Casein Digest broth was added in 15ml. Of SCDA at not more than 45^0C. The plates were incubated at 30-35^0C for three days after which the numbers of colonies were counted.

Limit – 10^5cfu/gm

Total fungal count

To a 9cm diameter petri dish, 1mL of the sample from Soyabean Casein Digest broth was added in 15ml of SDA with antibiotics at not more than 45^0C. The plates were incubated at 20-25^0C for 5 days after which the numbers of colonies were counted.

Limit – 10^3cfu/gm

RESULTS

Yield of *Guggulu*

Table 1. Detail of % yield and % loss of Raw *Guggulu*

Wt. of *Ashudha Guggulu* (kg)	**Volume of *Gomutra*(lt.)**	**Wt. of *shudha Guggulu*(kg)**	**Yield (%)**	**Residue**
3.2	29	2.9	90.62	9.37

Table 2. Detail of % yield and % loss of *Navaka Guggulu*

Observations	**Results**
Initial quantity of mixture	5.8kg
Obtained quantity of mixture	5.6kg
Loss in weight	200gm
% loss	96.55%
% Yield	3.44%

The data obtained from the analysis of different samples were presented in this section and have been categorized into-

- Raw drug testing
- Final Product

Table No 3. Organoleptic parameters of Raw *Guggulu* and *Navaka Guggulu*

Sr. No.	Test Parameter	Raw *Guggulu*	*Navaka Guggulu*
1.	Colour	Brown	Brown
2.	Odour	Balsamic	Gomutra
3.	Taste	Bitter	Characteristic
4.	Touch	Smooth	Smooth

Table No. 4. Physico-chemical Parameters of *Ashudha Guggulu* and *Navaka Guggulu*

S.No.	Parameters	*Ashudha Guggulu*	*Navaka Guggulu*	Limits
1.	Ph	4.97	-	-
2.	LOD%	5.48	-	-
3.	Total Ash%	2.51	-	NMT 5%
4.	Acid Insoluble Ash %	0.97	-	NMT 1%
5.	Water Soluble Extractive %	60.54	-	NLT 53%
6.	Alcohol Soluble Extractive %	27.03	-	NLT 27%
7.	Assay of Guggulsterone	0.50	2.55	-
8.	Hardness(kg/cm^2)	-	1.1	-
9.	Uniformity of weight(mg)	-	512.05	-
10.	Disintegration time (min)	-	110	-
11.	Friability%	-	0.06	

Table No. 5 Microbial Limit Test

Microbial growth	*Ashudha Guggulu*	*Navaka Guggulu*	Permissible limit
Total Bacterial Count	55 cfu/gm	148 cfu/g	NMT 10^5 cfu/gm
Total Yeast & Molds	142 cfu/gm	45 cfu/g	NMT 10^3cfu/gm
Escherichia coli	Absent	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent	Absent
Staphylococcus aureus	Absent	Absent	Absent
Salmonella enterica spp.	Absent	Absent	Absent

Table No. 6 Heavy Metal Content Test

Heavy metals	*Ashudha Guggulu*	*Navaka Guggulu*	Permissible limit (API)
Lead (Pb)	ND	ND	NMT10ppm
Cadmium (Cd)	0.016 ppm	0.16 ppm	NMT 0.3ppm
Arsenic (As)	1.80 ppm	1.19 ppm	NMT 3ppm
Mercury (Hg)	ND	ND	NMT 1ppm

ND= Not Detected

DISCUSSION

API was taken as a reference standard for all Physico-chemical test of *Guggulu.* Data evident from table no.4 the values of Raw *Guggulu* and *Navaka Guggulu* lies in physiochemical parameters permissible limit as per API standard.

Organoleptic test- *Guggulu* sample was examined by classical parameters like *Sugandhiyukta* (intense, pleasant balsamic odour), *Vahnaujvalanti* (free from physical impurities like bark, thorn, sand etc.), *Vahnaujvalanti* burn on fire), *Tapanevilayam* (melting on exposure to sunlight/heat), *Koshnasalilepayasasamana*(gives milky emulsion in warm water).

pH Value: pH value helps to determine the nature of the sample that is whether it is acidic or alkaline in nature.

In the stomach, drugs as weak acids are present mainly in their non-ionic forms and weak bases are in their ionic form. Since non-ionic species diffuse more readily through cell membranes, weak acids have a higher absorption in the highly acidic media of stomach. However, the reverse in the basic environment of the intestines weak bases diffuse more readily since they are non-ionic. Hence Raw *Guggulu* is weakly acidic, so it absorband enters the system and produces quick results.

Loss on drying (105^0C):

The loss on drying of any sample is directly related to its moisture content. Generally, the absorption of the moisture affects the appearance of the preparation and it may result in the decomposition of the preparation, if the components of the formulation are prone to hydrolysis, the preventive measurements should be adopted. It should always be kept in an airtight container. Loss on drying value for Raw *Guggulu* 5.48% is slightly less. It may be due to the absence of moisture content in the drug.

Total Ash - Ash value indicates the presence of inorganic contents-When herbal drugs are incinerated, they leave inorganic ash, which in case of many drugs (e.g. rhubarb) varies within the fairly wide limit and therefore it is evaluated. The total ash figure is of importance and indicates to some extent the amount of care taken in the preparation of the drug. In the determination of total ash values the carbon must be removed at low temperature (450□) as possible because alkali chlorides, which may be volatile at high temperature, would otherwise be lost. The total ash usually consists mainly of carbonates, phosphates, silicates and silica. Ash value of Raw *Guggulu* sample was 2.51% is indicative of the presence of less inorganic materials in that sample.

Acid Insoluble Ash -Acid Insoluble Ash is important to determine the amount of inorganic (minerals) impurities in the form of extraneous (non- physiological) materials in a plant/plant material.

Test for acid insoluble ash indicates the percentage of insoluble inorganic content or therapeutic efficacy. It indicates the physiological availability of the drug after passing through the gastric solution. Less is the acid insoluble ash more is the physiological availability in the human body. It indicates that *Guggulu* (0.97%) has contained more therapeutic efficacy because of its less acid insoluble ash.

Water Soluble Extractive Value -Water Soluble Extractive Value of Raw *Guggulu* is 60.54%. It also helps in indicating the nature of chemical constituents present in the drug. Water soluble extractive value is applied for the drugs which contain water soluble constituents.

Alcohol Soluble Extractive Value – Alcohol soluble extractive value of Raw *Guggulu* was 27.03% less is applied for the drugs which contain alcohol soluble constituents.

Therefore, water soluble extract indicates the total water soluble contents of the drug. Raw *Guggulu* has more value of water soluble extractive than alcohol soluble extractive.

Uniformity of Weight: Average weight of *Navaka Guggulu* was 512.05mg.

Disintegration time: The disintegration is the prime process for the oral solid dosage forms in the form of tablets as for absorption from gastro-intestinal tract (GIT), all these preparations are to be disintegrated, de-aggregated and finally are to be dissolute in gastrointestinal fluid. Those preparations which require very less time for disintegration (less than 2 min) are considered as fast dissolving and disintegration time increases the bio-availability of medicament. But in this study, the value of disintegration time was very high for classical formulations; higher disintegration time decreases the bioavailability of medicament.

Tablets comparatively have lesser disintegration time making it conventional release dosage form, after disintegration in GIT should release therapeutic agents effectively and systematically. Disintegration time of the sample *Navaka Guggulu* (110min) was found more.

Hardness Test-

The hardness of *Navaka Guggulu* (1.1) is less. It may be due to the *Shodhana* procedure of *Guggulu* and equal quantity of the ingredients which was used in the preparation of *Navaka Guggulu*.

Hardness indicates good mechanical resistance for the tablets. Hardness parameters give a clue to the compatibility and intrinsic strength of powdered materials. These include bonding strength, internal strain and brittleness (yet soft enough to disintegrate properly after swallowing). It is important in the sense that the tablet should not break while transporting or handling or before reaching the consumer.

Friability Test- Another measure of tablet's strength, its friability was carried out. This shows that it can withstand abrasion in packing, handling and transporting.

The weight of the tablets weighed before and after revolution showed the weight loss of 0.06% in *Navaka Guggulu*. This shows the stability to withstand the mechanical aberration in the sample.

Heavy metals: In Heavy Metal content analysis, raw sample and *Navaka Guggulu* were having heavy metal content, like lead, cadmium, arsenic and mercury under limit mentioned i.e. 10ppm for lead, 1ppm for mercury, 3ppm for arsenic and 0.3ppm for cadmium. Presence of cadmium and arsenic in raw and prepared samples may be due to chemicals used while farming herbs or during their preservation.

Total microbial count and **Total fungal count:** Total microbial and fungal count**s** are within Permissible limits in both samples. It depicts that no harmful pathogen was present in the drug and the drug is safe for application.

CONCLUSION

Analytical results demarked that *Navaka Guggulu* is brownish-black colour, smooth, formulation with characteristic odour and taste.

pH value denotes its acidic nature.

There was the absence of heavy metals and pesticide residue while the microbial count was found within the limit.

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