

PHYTO-CHEMICAL ANALYSIS OF GUGGULU (*COMMIPHORA MUKUL LINN*) W.S.R. TO ITS VARIOUS SHODHANA PROCEDURES

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ABSTRACT

Guggulu is the oleoresin of *Commiphora Mukul Linn.*, a plant that is native to India. Its extracts include compounds known for their hypolipidemic properties—the Z- and E-isomers of guggulsterone and its related guggulsterols. *Kalpana* is the process through which a substance can be transformed in to the form of medicine according to the need. During preparations of various formulations there are various *Samskaras* which are to be done for potentiating the drug or the formulation. Among all these pharmaceutical processes *Shodhana* is one of them. In our text, for a single drug many process of *Shodhana* in different ways have been mentioned. The present study includes *Shodana* of *Guggulu* as per Classical reference of *Rasendrasarasangraha* where *Shodana* of *Guggulu* is done by *Guduchi Kwatha*, *Triphala Kwatha* and *Godugdha*. Standard Operative Procedure of the process is done in the pharmaceutical study. The analytical study reveals the standards which can be given for *Ashuddha Guggulu* and *Shuddha Guggulu* of various Samples.

Keywords: Standard Operating Procedure (S.O.P),*Shodhana*, *Guggulu*, *Guduchi Kwatha*, *Triphala Kwatha*, *Godugdha*

INTRODUCTION: Since the evolution of life, diseases are also evolved to destroy it. To protect life, *Ayurveda*, the science of life is being practiced by *Aryans* from *Vedic* period. In the *Vedic* period *Guggulu* was a well-known drug of Indigenous System of Medicine. In *Atharvaveda* it is mentioned to be used both externally and internally. By just consuming the odour of *Guggulu*, it reduces many diseases. Many properties of *Guggulu* are described in our classics. Our ancient *Acharyas* like *Sushruta* describes, the utility and usefulness of *Guggulu* in the treatment of various diseases¹. *Guggulu* is the oleoresin of *Commiphora Mukul Linn.*, a plant that is native to India. Its extracts include compounds known for their hypolipidemic

properties—the Z- and E- isomers of guggulsterone and its related guggulsterols². *Guggulu* is used as a binding agent and also as a main ingredient in various formulations. To make it fit for internal use also, it has to undergo the process of *Shodhana*. *Shodhana* is the process of removal of physical, chemical impurities and potentiating of the drugs^{3,4}. There are different medias explained in literature for *Shodhana* of *Guggulu*^{5,6}. According to the media of purification the quality and pharmacological properties of *Guggulu* will vary. Depending on the change in properties the therapeutic effect may also vary. Various clinical studies have been carried out, but there is a need to ascertain

the changes likely, with difference in the media of *Shodhana*. The present study includes *Shodana* of *Guggulu* as per Classical reference of *Rasendrasarasangraha*⁷ and Good

Manufacturing Practice (G.M.P.) will be followed for preparing the various medias and *Shodana* of *Guggulu* mentioned below.

Sample	Raw Drug	Media	Process/Apparatus
1	<i>Guggulu</i>	<i>Guduchi Kwatha</i>	<i>Swedana/Dola Yantra</i>
2	<i>Guggulu</i>	<i>Triphala Kwatha</i>	<i>Swedana/Dola Yantra</i>
3	<i>Guggulu</i>	<i>Godugdha</i>	<i>Swedana/Dola Yantra</i>

AIMS AND OBJECTIVES:

1. Identification of *Guggulu* by Classical and Modern methods.
2. Phyto-chemical analysis of *Guggulu*, before and after *Shodhana* procedures.
3. An attempt was made to establish Standard Operating Procedure (S.O.P) for *Shodhana* procedures of *Guggulu* by *Guduchi Kwatha*, *Triphala Kwatha* and *Godugdha*.

PHARMACEUTICAL STUDY:

- *Aushadha* is a primary tool of *Vaidya* for combating various ailments. These *Aushadha* are prepared by different processing techniques applying to the raw drugs to get the desired effect. This processing results in transformation of good pharmacological action to that of substance. These pharmaceutical processes are called “*Samskaras*”.
- Practical study was done in 5 steps as
- Preparation of *Guduchi Kwatha* Practical 1.
- *Guggulu Shodhana* by *Guduchi Kwatha* Practical 2.
- Preparation of *Triphala Kwatha* Practical 3.
- *Guggulu Shodhana* by *Triphala Kwatha* Practical 4.

- *Guggulu Shodhana* by *Godugdha* Practical 5

ANALYTICAL STUDY: Analytical study of *Ayurvedic* drugs has become the need of present hour. In ancient days, the drugs were prepared by the physicians himself, with the help of experienced, assistants in their own pharmacies attached to their clinics. Now a days the trends have been entirely changed. The demand of *Ayurvedic* drugs have been increased by many folds and availability of raw materials are limited. So, there are of chances of production of low quality drugs for the commercial benefits.

To evaluate the quality of finished products, it becomes necessary to subject the drugs for various analytical studies. The drugs should be understood and interpreted in the light of advanced chemistry to provide scientific background. For *Guggulu*, which is an important drug of *Ayurveda*, *Shodhana* has been prescribed in various media and different methods are also available. For the present study, *Shodhana* of *Guggulu* as per Classical reference of *Rasendrasarasangraha*⁷ was followed for preparing the various medias and *Shodana* of *Guggulu* mentioned below.

Sample	Raw Drug	Media	Process/Apparatus
1	<i>Guggulu</i>	<i>Guduchi Kwatha</i>	<i>Swedana/Dola Yantra</i>
2	<i>Guggulu</i>	<i>Triphala Kwatha</i>	<i>Swedana/Dola Yantra</i>

3	Guggulu	Godugdha	Swedana/Dola Yantra
	Analysis was carried out at Central Laboratory, Bhagavathi Ana Labs Pvt. Ltd., Industrial Estate, Sanathnagar, Hyderabad. The analytical study was undertaken with an aim to suggest suitable parameters and their expected values for routine quality control of the below samples		primary idea about the quality of different formulations without using any chemical tests.
	Sample 1. Raw Guggulu (Resin of Commiphora Mukul Linn.)		II. Phyto-chemical parameters:
	Sample 2. Shuddha Guggulu (By Guduchi Kwatha)		1.Determination of foreign matter⁵: Raw drugs should be free from moulds, insects, animal fecal matter and other contaminations such as earthen, stones and extraneous material. Any matter not covered by the description of the drug in the monograph shall be regarded as a non-extraneous foreign matter.
	Sample 3. Shuddha Guggulu (By Triphala Kwatha)		Foreign matter is material consisting of any or all of the following: (i) In particular, parts of the organ or organs from which the drug is derived other than the parts named in the definition or for which a limit is prescribed in the individual monograph.(ii) Any organ or part of organ, other than those named in the definition and description. It was determined by taking the 100 gm weighed quantity of Sample 1 i.e <i>Ashuddha Guggulu</i> and it was spread in a thin layer. Foreign mater or foreign organs was separated out and weighed and percentage was calculated out.
	Sample 4. Shuddha Guggula (By Godugda)		2.Loss on drying at 110⁰ C.⁶: This test was conducted to find out the moisture content in the samples. About 1g, accurately weighed samples 1,2,3,4 were taken in a previously dried and weighed dish and heated in a hot air oven at 110°C till constant weight. It was cooled and the weight was noted. Difference between the weights was calculated and taken as the loss on drying. The loss on drying of the sample was expressed as % w/w.
	The 4 samples were analyzed by using the following parameters:		3.Determination of Acid Insoluble Ash⁸: Boil the ash obtained in (2.2.3) for 5 minutes with 25 ml of <i>dilute hydrochloric acid</i> ; collect the insoluble matter in a
	I. Organoleptic characters: Colour – <i>Rupa</i> , Odour – <i>Gandha</i> , Consistency - <i>Sparsha</i> , Taste - <i>Rasa</i>		
	II. Phyto-chemical parameters:		
	<ul style="list-style-type: none"> Determination of Foreign Matter (<i>Ashuddha Guggulu</i>) Loss on drying at 110⁰ C Ash Value (Water insoluble) Ash Value (Acid insoluble) Water Soluble Extractive Alcohol Soluble Extractive 		
	III. Chromatographic Studies:		
	<ul style="list-style-type: none"> Thin Layer Chromatography 		
	IV. UV Spectrophotometric analysis:		
	I. Organoleptic parameters: The <i>Sparsha</i> (Consistency), <i>Rupa</i> (Colour), <i>Rasa</i> (Taste) and <i>Gandha</i> (Odour) of all the 4 samples were noted. These characters correspond to the <i>Panchagyanedriya Pariksha</i> of Ayurveda. These various organoleptic characters provides an idea regarding the genuinely of the sample both to the physician and patient. These give an		

Gooch crucible, or on an ashless filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air dried drug.

4. Determination of Water Soluble Ash⁹:

Boil the ash for 5 minutes with 25 ml of water; collect insoluble matter in a Gooch crucible, or on an ashless filter paper, wash with hot water, and ignite for 15 minutes at a temprature not exceeding 450°. Substract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.

5. Determination of Water soluble extractive¹⁰:

This test was carried out to evaluate the water-soluble principles of the samples. 5g of sample was weighed accurately, 100 ml of distilled water was added to it and it was kept overnight. Next day, it was filtered. 20 ml of the filtrate was transferred to a dried and weighed evaporating dish. The solvent was evaporated on a water bath, dried till constant weight, cooled and weighed immediately. From the weight of the residue, the percentage of water-soluble extractive was calculated and expressed as %w/w.

6. Determination of Alcohol Soluble Extractive¹¹:

Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of Alcohol of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent,

evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

III. Thin Layer Chromatography¹²:

Chromatography is a process for separating the component of a mixture by producing different rates of movements for each component in a counter current system. The TLC of methanol extract and volatile oil of *Asuddha Guggulu*, *Triphala Shodhita Guggulu* and *Godugdha Shodhita Guggulu* were carried out by using the following conditions –

Absorption layer	- Silica gel G
Solvent system	- Taulene –Ethyl Acetate (93:07)
Detection	- UV Radiation - Anisaldehyde Sulfuric acid spray reagent followed by heating at 110°C for 10 min

IV. UV Visible Spectro Photometric Analysis^{3,4}:

Spectrophotometric analysis involves the measurement of the ability of the dissolved substance to absorb electromagnetic radiation of definite and narrow wavelength ranges. These absorption are measured at wavelengths that are generally a characteristics of the chemical composition of the dissolved absorbing substance, radiant energy waves ranging from 200nm to about 400nm is the UV region and from 400nm to around 750nm is the visible region. The UV or visible spectrum of a molecule is the result of change in energy of a molecule as a whole rather than of a particular bond.

OBSERVATIONS AND RESULTS:

Table no. 5.1- Showing Organoleptic Parameters of all Samples

Guggulu	Colour	Odour	Consistency	Taste
Sample 1	Golden brownish	Aromatic	Rough Granular	Kashaya
Sample 2	Light brownish	Aromatic	Dry Flakes	Kashaya, Tikta

	black			
Sample 3	Dark brownish black	Aromatic	Dry Flakes	<i>Kashaya Pradhana</i>
Sample 4	Light chocolaty brown	Milky Aromatic	Dry Moist Flakes	<i>Kashaya Madhura</i>

Table no. 5.2- Showing Phyto-chemical Parameters of all Samples

Parameters	Sample 1	Sample 2	Sample 3	Sample 4
Determination of Foreign Matter % w/w	25%	----	----	----
L.O.D at 110 ⁰ C w/w	6.6	8.5	8.0	5.7
Water Soluble Ash % w/v	13.8	8.6	7.3	8.2
Acid Insoluble Ash % w/v	5.7	3.9	2.9	3.0
Water Soluble Extractive % w/v	36.8	43.5	38.4	46.4
Alcohol Soluble Extractive % w/v	26.8	22.9	32.8	33.7

(Appendices) THIN LAYER CHROMATOGRAPHY: T.L.C. of the methanol soluble extract and volatile of the samples were carried out by using different conditions with an aim to develop suitable chromatographic conditions and also to compare the chromatographic pattern of the samples. Figure- 8 : The comparative T.L.C. of methanol extract of the samples under long wave uv, shows six fluorescent spots at Rf 0.06, 0.15, 0.20, 0.26, 0.38 and 0.66 in all the samples. The chromatograph shows similar pattern in *Asuddha Guggulu*, *Triphala Shodhita Guggulu* and *Godugdha Shodhita Guggulu*. Figure-9 : The comparative T.L.C. pattern of the volatile oil of the samples under long wave uv, has been presented in fig. 9. Both the *Shodhita Guggulu* samples show considerable difference from *Ashuddha Guggulu*. Number of fluorescent spots present in *Ashuddha Guggulu*, are absent in *Shodhita Guggulu*. The same after spraying with Anisaldehyde Sulphuric acid spray reagent followed by heating at 110⁰C for 10 min. shows number of the spots in all the three samples, but the pattern is quite different. Number of spots present in *Ashuddha Guggulu* are not present in

Shodhita Guggulu, indicating removal of certain chemical substances during *Shodhana* process. The comparative T.L.C. pattern of volatile oil shown in the Figure- 10 *Ashuddha Guggulu* shows 11 spots at Rf 0.16, 0.23, 0.30, 0.38, 0.45, 0.57, 0.62, 0.71, 0.78, 0.85 and 0.93 whereas the both the *Shodhita Guggulu* show only 6 to 7 spots. There is difference in the pattern of two *Shodhita Guggulu* samples. The spots at Rf 0.45 and 0.85 are present in *Triphala Shodhita Guggulu* (and also in *Ashuddha Guggulu*), but absent in *Godugdha Shodhita Guggulu*. Figure- 11 : The comparative T.L.C. pattern of the methanol extract of the samples obtained after spraying with Vanillin Sulphuric acid spray reagent also shows difference between *Ashuddha Guggulu* and *Shodhita Guggulu* as well as among two *Shodhita Guggulu* samples. The *Asuddha Guggulu* shows eight spots at Rf 0.23, 0.35, 0.47, 0.52, 0.62, 0.70, 0.76 and 0.97. The spot at Rf 0.62 is present in both *Ashuddha Guggulu* and *Godugdha Shodhita Guggulu*, but absent in *Triphala Shodhita Guggulu*, whereas the spot at Rf 0.52 and 0.97 is present in both *Ashuddha Guggulu*

and *Triphala Shodhita Guggulu* but absent in *Godugdha Shodhita Guggulu*.

U.V. SPECTROPHOTOMETRIC STUDY: The spectra of *Ashuddha Guggulu* is shown in Figure- 12 *Guduchi Shodhita Guggulu*, *Triphala Shodhita Guggulu* and *Godugdha Shodhita Guggulu* is shown in Figure- 13 as well as their comparison had been presented in Figure- 12 & 13 respectively. As could be seen from the figures that *Asuddha Guggulu* and *Shodhita Guggulus* have similar absorption pattern and give absorption peaks at wave length 232 and 324nm. The intensity of absorption at 232nm is almost same but that of the peak at 324nm is comparatively more in *Asuddha Guggulu*. The UV absorption pattern of *Shodhita Guggulu* is quite different from each other. It shows an absorption peak at 223nm. The comparative uv spectra of the three samples (Figure- 13) clearly shows difference in absorption pattern in *Guduchi Shodhita Guggulu*, *Triphala Shodhita Guggulu* and *Godugdha Shodhita Guggulu* indicating difference in their chemical composition and suggesting that *Shodhana* media may change the chemical nature of *Shodhita Guggulu*.

DISCUSSION: The present study was planned with an aim to establish Standard Operating Procedure (S.O.P) for *Shodhana* procedures of *Guggulu* by *Guduchi Kwatha*, *Triphala Kwatha* and *Godugdha*. Find out the effect of different *Shodhana* medias on the phyto-chemical properties of *Guggulu*. Go through the whole literature on *Guggulu* available from *Vedic* period to the advancement of present time. To achieve the goal of study, it has been divided in three major parts – Conceptual study which includes Drug review and Concept of *Shodhana*, Analytical study.

Analysis and results of each study are discussed in this section.

I. Organoleptic characters:

- Colour – *Rupa*, Odour – *Gandha*, Consistency - *Sparsha*, Taste - *Rasa*

II. Phyto-chemical parameters:

- Determination of Foreign Matter (*Ashuddha Guggulu*) Loss on drying at 110°c
- Ash Value (Water insoluble) Ash Value (Acid insoluble)
- Water Soluble Extractive Alcohol Soluble Extractive

III. UV Spectrophotometric analysis:

Table no 5.1 reveals the organoleptic studies. Sample 1 i.e. *Ashuddha Guggulu* was golden brownish colour, aromatic, rough granular and *Kashaya Rasa Pradhana*. Sample 2 i.e. *Shuddha Guggulu* (*Guduchi Kwatha Shodhita*) was light brownish black colour, aromatic, dry flakes and *Kashaya Tikta Rasa*. Sample 3 i.e *Shuddha Guggulu* (*Triphala Kwatha Shodhita*) was dark brownish black colour, aromatic odour, dry flakes with *Kashaya Pradhana Rasa*. Sample 4 i.e *Shuddha Guggulu* (*Godugdha*) was light chocolaty brown, milky aromatic, dry moist flakes and *Kashaya Madhura Rasa*. Table no. 5.2 reveals the phyto-chemical findings that *Asuddha Guggulu* was having 25% of foreign matter, which reveals the adulteration which is more for *Guggulu* due to its clinical importance. Loss on drying was found less in *Godugdha Shodhita Guggulu* and more *Guduchi Shodhita Guggulu*. Water soluble ash was found less in *Triphala Shodhita Guggulu* and more in *Ashuddha Guggulu*. Acid insoluble ash was found least in *Triphala Shodhita Guggulu* and more in *Ashuddha Guggulu*. Water soluble extractive was

found less in *Ashuddha Guggulu* and most in *Godugdha Shodhita Guggulu*. Alcohol soluble extractive was found less in *Guduchi Shodhita Guggulu* and more in *Godugdha Shodhita Guggulu*. By performing *Shodhana* procedure, moisture content was reduced, Ash value was reduced, water soluble extractive and alcohol soluble extractive were increased compared to *Ashuddha Guggulu*. This shows the role of different media in deciding the absorption, assimilation, effect and excretion of the drug. So due to these there may be changes in mode of action and also disease and disease condition.

THIN LAYER CHROMATOGRAPHY:

The comparative T.L.C. of methanol extract of the samples under long wave uv, shows six fluorescent spots at Rf 0.06, 0.15, 0.20, 0.26, 0.38 and 0.66 in all the samples. The chromatograph shows similar pattern in *Asuddha Guggulu*, *Triphala Shodhita Guggulu* and *Godugdha Shodhita Guggulu*. T.L.C. patterns of samples support the findings of uv – spectra. The analytical data suggest that the chemical composition of *Shodhita Guggulu* is affected by the medias used for *Shodhana* purposes.

U.V. SPECTROPHOTOMETRIC STUDY:

The comparative UV spectra of the three samples clearly shows difference in absorption pattern in *Guduchi Shodhita Guggulu*, *Triphala Shodhita Guggulu* and *Godugdha Shodhita Guggulu* indicating difference in their chemical composition and suggesting that *Shodhana* media may change the chemical nature of *Shodhita Guggulu*.

CONCLUSION:

- *Shodhana* of the *Guggulu* in the media i.e. *Guduchi Kwatha*, *Triphala Kwatha* and *Godugdha* were found pharmaceutically equal. But, *Guggulu Shodhana* in *Triphala Kwatha* and *Godugdha* was found better for the commercial benefits (more yield).
- All relevant analytical data of samples of *Ashuddha* and *Shuddha Guggulu* are showing difference in their physical and chemical values. It shows the importance of process of *Shodhana*, which is probably responsible for safe therapeutic uses of *Guggulu*.
- If *Guggulu* is intended to be used for *Rasayana*, *Vayasthapana* and treating *Prameha*, *Jwara*, *Kshaya* etc. *Guggulu Shodhana* should be done in *Guduchi Kwatha*.
- If intended in *Medoroga*, *Prameha*, *Kustha* then conducted in *Triphala Kwatha*.
- If *Guggulu* is used for *Rasayana*, *Balya* purpose *Shodhana* should be carried out in *Godugdha*.

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