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Research Article

PHYSICO-CHEMICAL ANALYSIS AND IMPACT OF DIFFERENT *SHODHANA* METHODS ON *GUGGULU*

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KEYWORDS: *Guggulu, Commiphora wightii*, *Dolayantra Shodhana*, Guggulsterone (E and Z), HPLC, Physico-chemical analysis.

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ABSTRACT

Objective: *Guggulu* is one of the important *Dravya* (drug) used in Ayurvedic formulations since ancient time which means "Protection against diseases". *Ashuddhaguggulu* has physical and chemical impurities which need to be eliminated before using in formulation by *Shodhana*. The present study was conducted to evaluate and compare the effect of different *Shodhana* methods i.e*., Dolayantra Shodhana* and dissolving *Shodhana* on properties of *Guggulu* by employing various physic-chemical and chromatographic methods. **Method:** Physicochemical screening was done by evaluating ash, Acid Insoluble Ash, Loss on Drying, Water Soluble Extractive and Alcohol Soluble Extractive. Chromatographic analysis was performed to estimate guggulsterone (E and Z) content, using High Performance Liquid Chromatography (HPLC). **Result:** The Physico-chemical studies showed decrease in LOD, Ash and Acid Insoluble Ash content and increase in extractive values such as Alcohol Soluble Extractive of *Guggulu* after *Shodhana* process. The HPTLC analysis showed significant change in guggulsterone (E and Z) content in *Guggulu* after *Shodhana* process **Conclusion:** This study helps to understand the effect of different *Shodhana* methods on the efficacy of drug. In this study, we established qualitative profile of *Shodhit Guggulu* in terms of physicochemical parameters and phytochemical content by HPLC.

INTRODUCTION

Guggulu an oleo-gum resin is obtained from *Commiphora wightii* Bhandari of family Burseraceae. It is mentioned in Ayurvedic text to be used in the treatment of many disorders like obesity, lipid disorders, arthritis etc[1]. *Guggulu* has been reported to possess antioxidant[2-5], antitumor[6], thyroid stimulatory[7] and cardio protective activities[8]. It is reported that the E-guggulsterone showed good thyroid stimulating activity in rats which leads to reduction of cholesterol and serum lipids. Classics advocates to use purified *Guggulu* in therapeutics. Administration of raw *Guggulu* may sometimes lead to skin rashes, irregular menstruation, diarrhoea, headache, mild nausea and very high doses causes liver toxicity[9]. The literature indicates that unprocessed/unpurified *Guggulu* or partially purified *Guggulu* is use in treatment of several disease like hyperlipidemia, anti-cholesterol, melatonin induced hypothyroidism, obesity and immunomodulatory properties[10]. Studies also reported gastric irritancy reduced and increased pharmacological action found to be with purified *Guggulu*[11] In order to overcome the several unwanted effects and to make it suitable for internal administration along with enhancement of properties of *Guggulu*, a number of traditional purification process (*Śodhana*) have been described in Ayurveda with different methods like *Dolayantra* and Dissolving (*Vighatan*). *Shodhana* is a process of purification and detoxification by which physical and chemical blemishes and toxic materials are eliminated and substances are subjected for further processing[12].

The present study was carried out to evaluate the physico- chemical changes occurred in *Guggulu* using two different *Shodhana* process w.s.r. to same liquid media.

The purification process is not only removing the impurities but also increases the therapeutic effect. However, no study has been done to explore the changes during the different procedure and its probable effect on level of key constituents Guggulsterone. Therefore, the present study was carried out to raw *Guggulu* by using different methods as mentioned in Ayurvedic texts and to find the physicochemical changes and in the level of key constituents Guggulsterone.

Aims and objectives

- Physiochemical analysis of *Guggulu*, before and after *Śodhana*.
- Physiochemical analysis of both samples *Suddha Guggulu.*

Material and Methods

Crude drug *Guggulu*, exudates of *Commiphora wighitti* were purchased from Gujarat State forest Development Corp. Ltd. Vadodara (Mfg. Lic.No:GA/791) were subjected to authentication from Dravya Guna department of Rishikul Campus, Haridwar, UAU as per API protocol and Analytical lab of Multani pharmacy Roorkee. Cow's fresh urine was collected from Rishikul Vidyapeeth Haridwar.

Guggulu Śodhana* by *Dolayantra

Ashuddha (unpurified) *Guggulu* 100gm was taken and visible physical impurities were removed manually. *Pottali* (bundle) was prepared and then subjected to *Swedana* (steaming) in *Dolayantra* in the presence of *Gomutra* until all the *Guggulu* passes into the liquid through the cotton cloth. The *Pottali* was taken out after ensuring that all solubilized matter of the *Guggulu* has been digested in the fluid taken. After complete filtration, the filtrate was subjected for further heating to evaporate the liquid up to semi-solid state during the whole process temperature was maintained between 75^{0}C-85^{0}C then it was shifted into stainless steel tray. The mass was dried in sunlight and then pounded with a pestle in a stone mortar.

Table 1: *Guggulu Śodhana* by *Dolayantra*

Wt. of *Ashudha Guggulu* (gm)	Volume of *Gomutra* (ml)	Wt. of *Shudha Guggulu* (gm)	Yield (%)	Residue (%)
100	1300	90	90	10

***Guggulu Śodhana* by Dissolving Method**

Guggulu Śodhana was done by *Parīṣravaṇa* (dissolving and filtering) method[13]. External impurities like stone, bark, wood etc. from raw material were removed manually. Raw *Guggulu* was made into small pieces. 550ml of *Gomūtra* was added to 100gm of *Aśudhdha Guggulu* (AG) in a stainless steel vessel. The contents were subjected to mild heat maintaining temperature between 70-80^{0}C to facilitate dissolution of *Guggulu*. After complete dissolution, the contents were filtered through cotton cloth (madarpat fabric) in hot condition. Contents remained as residue in cloth were discarded. The filtrate was subjected to further heating at 70^{0}C till complete evaporation of liquid. Obtained semisolid mass was shifted to ghee smeared stainless steel trays, spread into thin uniform layers and dried in under sun. The dried mass was carefully collected as *Śudhdha Guggulu* (SG) and stored.

Physical impurities like stone, bark, wood etc. from *Ashudhdha Guggulu* (100gm) were removed manually. Then, it was shifted into a stainless steel vessel and *Gomutra* (550 ml) was added. The contents were subjected to mild heat maintaining temperature between 70-80^{0}C to facilitate gradual dissolution of *Guggulu*. After complete dissolution, the contents in hot condition were filtered through cotton fabric. Contents remained as residue in cloth were discarded. After complete filtration, the filtrate was subjected for further heating to evaporate the liquid up to semi-solid state. During the whole process temperature was maintained between 70^{0}C -80^{0}C then it was shifted into stainless steel tray for drying.

Table 2: *Guggulu Śodhana* by Dissolving Method

Wt. of *Ashudha Guggulu* (gm)	Volume of *Gomutra* (ml)	Wt. of *Shudha Guggulu* (gm)	Yield (%)	Residue
100	550	82	82	17

Results and Observations

Table 3: Organoleptic parameters of Raw *Guggulu* and *Shuddha Guggulu*

Sr. No.	Test Parameter	Raw drug	SGDo	SGDi
1.	Colour	Yellow brown	Dark Black	Dark Black
2.	Odour	Balsamic	*Gomutra*	*Gomutra*
3.	Taste	Bitter	Bitter	Bitter
4.	Touch	Smooth	Smooth	Smooth

Table 4: Physico-chemical test of raw and *Shuddha Guggulu*

S.No	Parameters	RG	SGDo	SGDi	Limits	Reference
1.	pH	5.11	6.61	6.25		API
2.	LOD%	5.84	6.2	11.0	Not >14	API
3.	Total Ash%	18.74	37.92	24.98		API
4.	Acid Insoluble Ash %	15.59	15.04	1.44	Not >1	API
5.	Water Soluble Extractive %	39.79	57.84	60.54	Not <53	API
6.	Alcohol Soluble Extractive %	37.32	16.56	20.63	Not<27	API
7.	Volatile oil content	.87%	-	-	Not <1	API
8.	Hardness(kg/cm^2)	-	7	14	-	API
9.	Average Wt (gm)	-	.650	.683	-	IP
10.	Disintegration time (min)	-	30	1.55	-	IP
11.	Dissolution time (min)	-	50	2.30	-	IP
12.	Friability test	-	.0090	8.375	-	IP

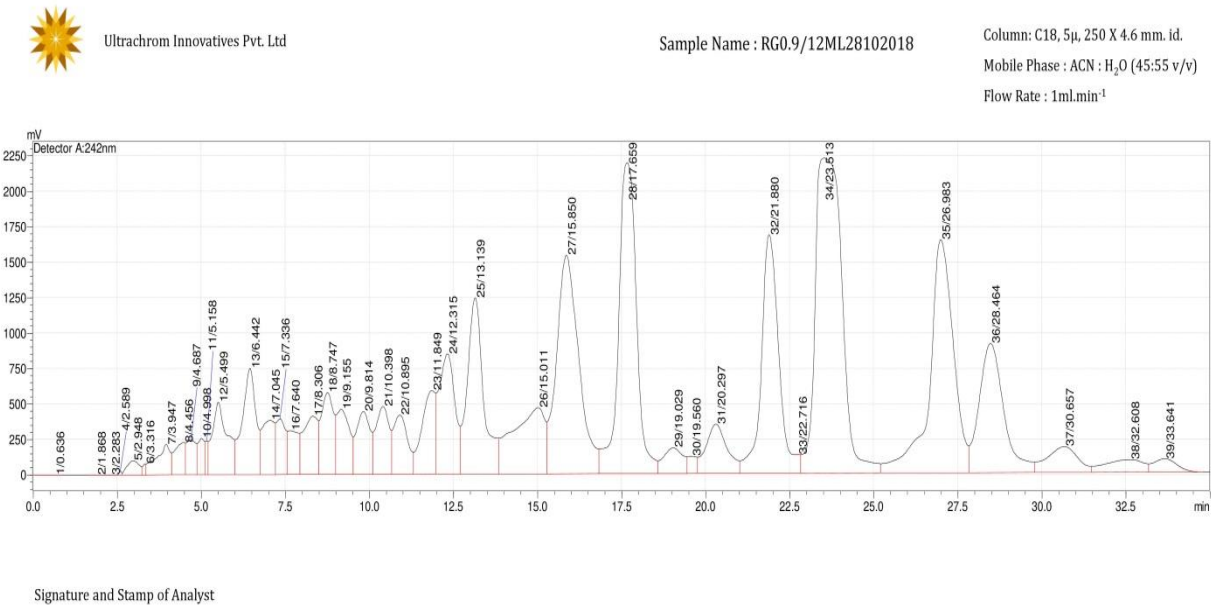
Discussion

API was taken as reference standard for all physico-chemical test of *Guggulu*. Data evident from table no. 4 the values of raw *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)[14]. In Organoleptic evaluation, Yellowish brown colour of AG was turned to dark brown after *Śodhana*in *Gomūtra*. Significant balsamic odour was perceived in Raw *Guggulu* sample while after *Śodhana*, mixed smell of *Gomūtra* and *Guggulu* were perceived in both samples. Taste of AG is bitter- astringent while SG has bitter taste. *Guggulu* became stickier after *Śodhana* due to *Gomūtra*. Final weight of *Shodhita Guggulu* depends upon the impurities which were removed from *Shodhana* process as well as extractive solid value of the used *Gomutra* for same process. 90% yield was obtained in SGDo and 82% yield was obtained in SSDi. pH value helps to determine the nature of the sample that is whether it is acidic or alkaline in nature. Physiochemical analysis of *Asudha Guggulu* sample was found within the limits given by API.[15] The pH of *Shodhita* samples was increased, as *Gomutra* was used in *Śodhana* media, which is slightly alkaline (ph=7.40) in nature may contribute to increase in pH (5 to 6 after *Shodhan*). Loss on drying was found more

(SGDo 6.2% and SGDi 6.25%) in *Shodhita* samples in comparison to *Ashodhita Guggulu* (5.11) indicating presence of more moisture content in *Shodhita Guggulu*. This loss in weight in the *Shudha Guggulu* would be due to *Gomutra* used in *Śodhana. Gomutra* consist a lot of *Kshariya* substances which are hygroscopic in nature. **Ash value**- SGDo 37.92% had more ash value than SGDi24.98%. This may be because of more quantity of *Gomutra* used in *Dolayantra* process as high amount of mineral contents present in *Gomutra*. **Acid insoluble ash** had less value was found in 1.44 in SGDi) than SGDo (15.05%). **Water soluble extractive** was more in SGDi (60.54%) than SGDo (57.84%). It may be because of *Śodhana* in *Gomūtra* that extracted some aqueous principles. **Alcohol soluble extractive** was found less (16.56% in SGDo than in SGDi20.63). **Disintegration time** of SGDo (30min) is less than the SGDi (1.55min). It may be because of adding ghee and hammering like pharmaceutical process which may reduce the particle size and the disintegration time.

HPLC:



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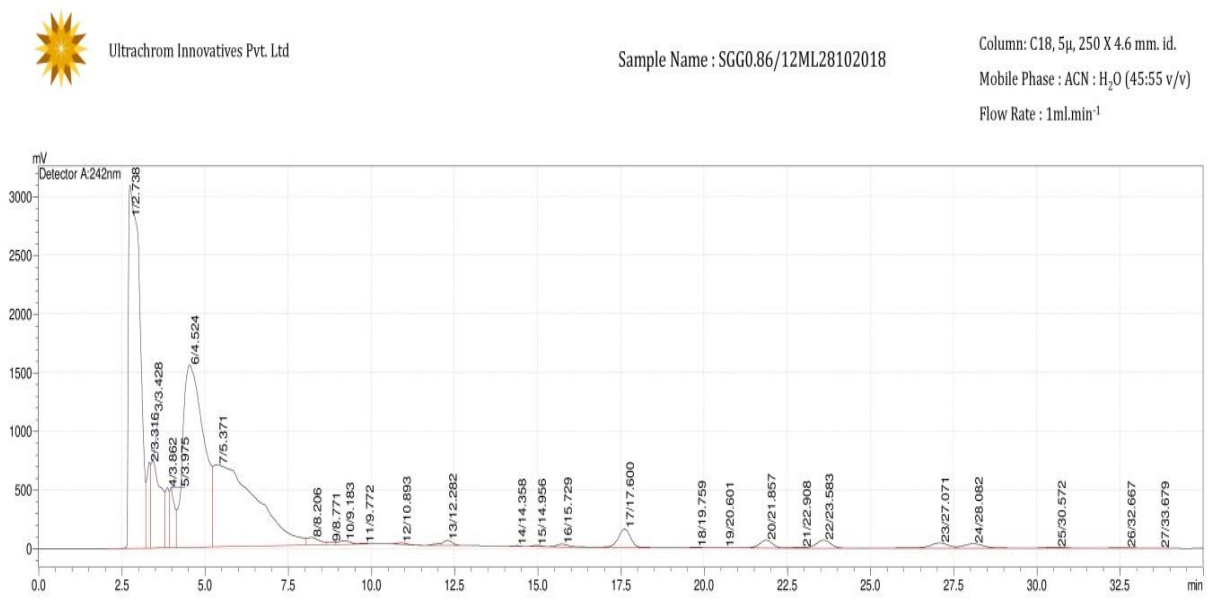

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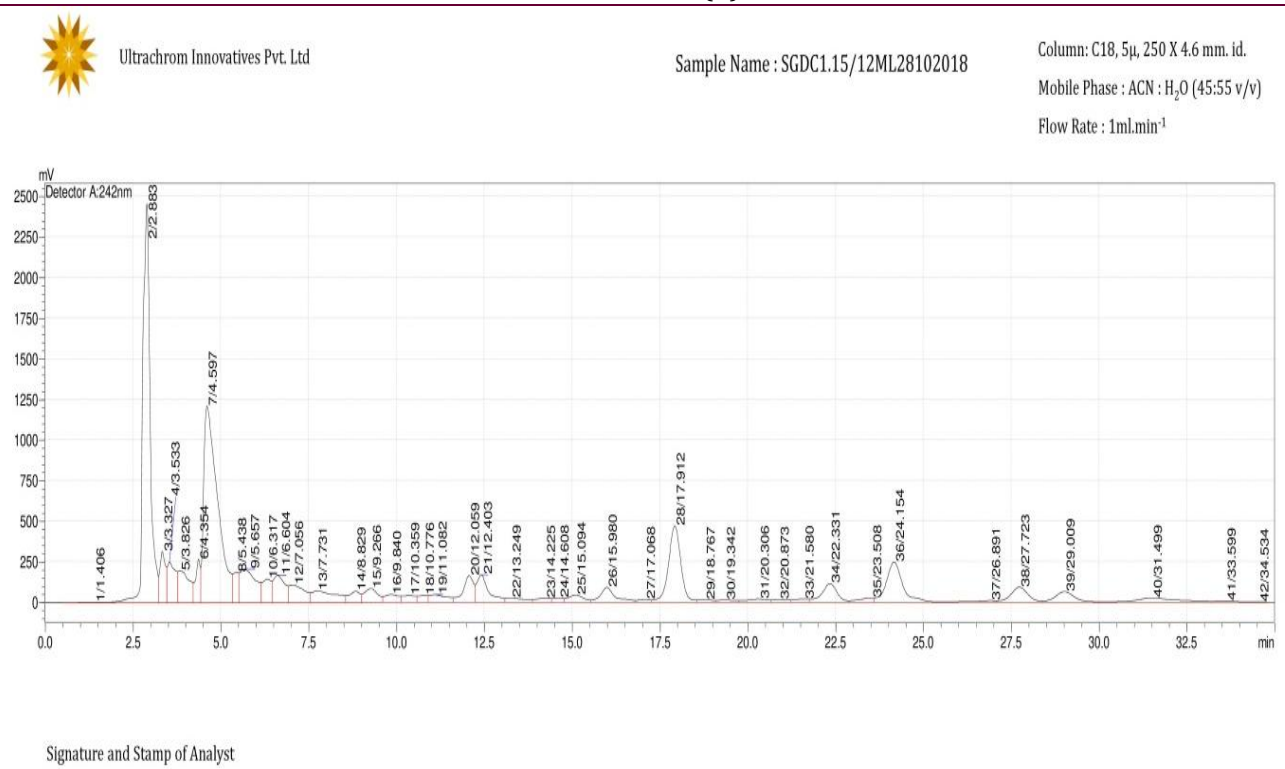

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HPLC method produced highly equal peaks with good resolution standards compound Guggulsteron-E at wavelength 242nm. Guggulsterone was detected in a range of 17.60 min to 17.95 min. retention time. The chromatograms of all samples showed peaks at retention time 17.60 min to 17.95 min, indicating the presence of Guggulusterone in both samples. The amount of guggulusterone was calculated from the calibration curve. HPLC profile of both *Guggulu* samples showed decreased concentration of Gugulsterone-E after *Śodhana* of *Guggulu.* The active components of the plant are the Guggulsterones, specifically the stereoisomers, Guggulsterone E and Guggulsterone Z.[16] *Guggulu* have a capacity to enhance the production of thyroxine (T4), triiodothyronine (T3) (thermogenic activity).[17] The total guggulsterone content was 10.3067% in raw *Guggulu*. The percentage contain varies with its processing since maximum concentration was observed in RG (10.3067%) and the lowest concentration was accounted in SGDi. The total Guggulsterone content (with respect to raw *Guggulu* were found highest in and lowest in SGDi (1.5563) when compared with raw *Guggulu*. The guggulusterone content was in order SGDo (7.04)>SGDi (1.55). The quantitative estimation by HPLC method has shown that all the samples of *Shodhit Guggulu* contain a higher amount of Guggulusterone E than the Guggulusterone Z. It can be concluded that the variation of Guggulsterone in the samples likely due to different procedure used in sample preparation.

Table 5: Comparative Data of column Chromatographic separation of five *Guggulu* samples

No.	Sample Name	Guggulsteron-E			
		Rt[min]	Area [mV.s]	Height [mV]	Area%
1	RG	17.659	81520072	2192026	10.3067
2	SGDo	17.912	11620560	472788	7.0463
3	SGDi	17.6	3917606	157093	1.5563

Microbial Limit Test

Table 6: Microbial limit test of RG, SDo, SDi, TG, BTG, Honey

Microbial growth	SGDo	SGDi	Permissible limit
Total plate Count	Absent	Absent	Max 10^5gm
Total fungal count	Absent	Absent	Max 10^3gm
Escherichia coli	Absent	Absent	Absent
Pseudomonas aeruginuosa	Absent	Absent	Absent
Staphylococcus aureus	Absent	Absent	Absent
Salmonella spp.	Absent	Absent	None

Total plate counts were within the normal range in all drug samples and total fungal count, *Escherichia coli*, *Psudomonos aeruginosa*, *Staphylococcus aureus* and *Salmonella spp* were absent.

Heavy Metal Content Test –ICP-MS

Table 7: Heavy metal analysis in *Raw Guggulu*

Heavy metals	RG	Permissible limit (API)
Lead (Pb)	6.77	10ppm
Cadmium (Cd)	0.09	.30ppm
Arsenic (As)	.76	3ppm
Mercury (Hg)	.57	1ppm

CONCLUSION

The study demonstrates that purification process significantly affect the physico-chemical parameters of *Guggulu* and quantity of its active compound Guggulsterone-E. The purification method employing *Dolayantra* method shows the best method for *Guggulu Śodhana* as more yield %, lesser disintegration time and maximum concentration of Guggulsterone-E were found by *Dolayantra* method. So, it may conclude *Guggulu Śodhana* by *Dolayantra* can be considered as easy, convenient. Data obtained from the present study is reproducible. The values of physicochemical parameters can be taken for quality assurance method was found to be best for *Guggulu Śodhana*.

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Guggulu shodhana* by *Dolayanyta

Asudha Guggulu

Gomuta

Pottali immersed in *Gomutra*

Pottali nirman

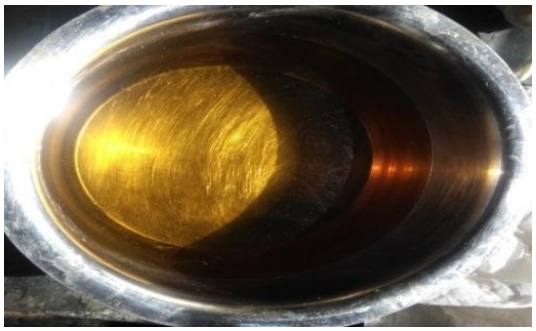
Sudha Guggulu

Evaporation of gum

Drying of *Guggulu*

Guggulu Shodhana by dissolving method

Asudha Guggulu



Gomutra

Dissolving of *Guggulu*

Mixing of *Guggulu* in *Gomutra*

Filtration of *Guggulu*

Evaporation of *Gomutra*

Drying of *Guggulu*