



Research Article

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Comparative physico-chemical profiles of Fresh (*Naveena*) and Old (*Purana*) *Guggulu* (*Commiphora wightii* (Arn.) *Bhandari*)- A pilot study

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ABSTRACT

Introduction: *Kaala prakarsha* (Time factor) is one among the *Samskara* that will bring changes in a drug. It has been attributed to certain drugs like *Ghritha*, *Vidanga*, *Guggulu* etc. In classics Fresh (*Naveena*) *Guggulu* is attributed with *Brimhana* (Body mass increasing) effect while Old (*Purana*) with *Atilekhana* (scarificant). Possible physico-chemical changes that occur during *Kaala prakarsha* of *Guggulu* are not attempted till date. **Objective:** Current study attempted to evaluate physico-chemical changes between samples of Fresh and Old *Guggulu* before and after *Shodhana*. **Materials:** *Guggulu* was collected from Gujarat state Forest department corp. ltd. in two phases. Preservation of *Guggulu* was done for one year to make it old. Purification of both the samples (Fresh and Old) was done in presence of Cow's urine. Physico-chemical and chromatographic evaluation of both samples was carried out. Partial Least Square method-2 (PLS-2) was adopted for comparison of physicochemical parameters. **Results:** Principle Component Analysis (PCA) score showed difference between both samples. PLS-2 suggested a weak co-relation in between two samples. HPTLC profile showed more components in Fresh *Guggulu*, while HPLC reveals decreased Guggulsterones in old sample.

Keywords: *Commiphora wightii*, *Guggulu*, Guggulsterone, HPLC, HPTLC, *Kaala prakarsha*.

INTRODUCTION

Samskara^[1], an essential principles of ayurvedic pharmaceuticals is known to bring certain changes in a drug. *Kaala prakarsha* (Time factor), one of such *sanskara*, is unique and unavoidable. Few examples for drugs to be used under Time factor are *Bhallataka* (*Semecarpus anacardium* Linn.)^[2], *Ghee*, Honey, *Vidanga* (*Embelia ribes* Burm.), *Pippali* (*Piper longum* Linn.)^[3], *Amalaki Rasayana*^[4].

Guggulu (*Commiphora wightii* (Arn.) *Bhandari*) is one of the most popular drugs contributed by Ayurveda to the world. It is in Ayurvedic practice since centuries for the treatment of *Kustha* (skin diseases), *Medoroga* (lipid disorders), *Aamavata* (rheumatoid arthritis), *Sandhigata Vata* (osteoarthritis), *Gulma* (Abdominal lump), *Shotha* (edema) etc^[5]. Researches to extract active molecules from *Guggulu* were initiated way back in 1966^[6] and Guggulsterone E & Z still attracts scientists for their hypolipidaemic activity.

Ayurveda attributed different properties for fresh (*Naveena*) and old (*Purana*) *Guggulu*. Old *Guggulu* is said to be '*Atilekhana*' in comparison to Fresh *Guggulu*^[7]. Characteristics of both quality of *Guggulu* are also given by seer as *Guggulu* which is *Snigdha* (unctuous), *Kanchanashankasha* (golden yellow colour), *Sugandhiyukta* (with balsamic odor), and *Pichchila* (sticky) properties considered as Fresh while *Guggulu* having *Shushka* (dry texture), *Durgandhayukta* (with bad odor) and *Anyavarnaka* (detoriated coloured) is considered as old^[8].

This implies, storage for specific time period imparts certain changes in the drug. Classics indicated that some drugs should be used in fresh condition like *Guduchi* (*Tinospora cordifoliai* Willd.), *Kutaja* (*Holarrhena antidiysenterica* (Roth)), *Vasa* (*Adhatoda vasica* Nees.) etc. *Guggulu* is placed under this category^[9]. *Bhavamishra* also contraindicates the use of Old *Guggulu* in diseased condition^[10].

In addition, the sample needs to be subjected to *Shodhana* before its internal administration. Actual

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impact of this procedure needs to be evaluated, but may remove possible insoluble impurities from the sample. Till date, no work has been attempted on developing comparative physico-chemical profiles of both the samples, before and after *Shodhana*. Considering this, it is attempted to develop preliminary physico-chemical profile of fresh and old samples of *Guggulu*.

MATERIALS AND METHODS

Collection of drug

Guggulu widely cultivated at Dwaraka Forest Range, Gujarat was collected from Gujarat State Forest Development Corp. Ltd., Vadodara during February 2011 (Batch no. B 05, code-148600). This sample was preserved in air tight polyethylene bags and stored at room temperature (28-32°C) for one year to make it as Old. Though, specific time period is not mentioned for *Guggulu* to become old; the sample was stored for one year considering general terminology^[11]. Another sample of fresh *Guggulu* was collected in the same manner during February 2012 (Batch no. B 01, code-128500). Both the samples were purified by following classical method^[12].

Shodhana (treatment procedure): Physical impurities like stone, bark, leaves etc were removed manually. Four parts of *Gomutra* (cow's urine) was added to one part of *Guggulu*^[13] and subjected to mild heat maintaining temperature between 70-85°C to facilitate complete dissolution of *Guggulu*. Followed by filtration through cotton cloth, the filtrate was subjected to further heating at 70 °C for complete evaporation of liquid. Semisolid mass thus obtained was shifted to ghee smeared stainless steel trays, spread into thin uniform layers and dried in sun rays. The dried mass was collected as *Shuddha Guggulu* and stored in air tight containers.

Samples: Four samples under analysis are: *Ashuddha Naveena Guggulu* (ANG), *Shuddha Naveena Guggulu* (SNG), *Ashuddha Purana Guggulu* (APG), and *Shuddha Purana Guggulu* (SPG).

Table 1: Observations on organoleptic parameters

Sample	Colour	Odour	Taste	Touch
ANG	Golden yellow	Significant balsamic	<i>Tikta</i> (Bitter), <i>Kashaya</i> (astringent)	Smooth
SNG	Chocolate brown	<i>Gomutra</i> with slight balsamic	<i>Tikta</i>	Sticky
APG	Greenish yellow	Balsamic	<i>Tikta</i> , <i>Kashaya</i>	Smooth
SPG	Chocolate brown	<i>Gomutra</i> with slight balsamic	<i>Tikta</i>	Sticky

Table 2: Physico chemical parameters of all ANG, SNG, APG and SPG samples

Parameter	Results			
	ANG	SNG	APG	SPG
Loss on drying (110°C)%	7.38	8.58	4.53	12.39
Ash Value (w/w)%	4.82	9.21	1.4	4.7
Acid Insoluble Ash (w/w)%	2.19	2.01	0.82	0.62
Water Soluble Extract (w/w)%	31.54	39.39	21.54	39.00
Methanol Soluble Extract (w/w)%	37.3	45.16	57.80	49.7
Volatile Oil Content (w/w)%	0.99	0.97	0.93	0.91
pH (w/v)	4.98	6.30	4.23	7.18

Physico chemical analysis – Principle Component Analysis (PCA) is done by scoring all parameters in XY plotting. [Fig 1 a] PCA differentiated solubility parameters (Water soluble and Methanol soluble extractives) and other parameters (Loss on drying, Ash value, Acid insoluble ash, pH and Volatile oil). On observing the correlation loading plot for discrimination value between all the analyzed

Analysis of the samples: Organoleptic characters like odour, touch etc; physico-chemical parameters like loss on drying, ash value, acid insoluble ash, water soluble extractives, methanol soluble extractives, pH and volatile oil content were carried out by following standard procedures mentioned in Ayurvedic Pharmacopoeia of India^[14].

For High Performance Thin Layer Chromatography (HPTLC), 5 g of drug was extracted with methanol by soxhlet extract method. It was then combined with methanol to adjust the volume to 25 ml. A CAMAG (Switzerland) HPTLC system equipped with a sample applicator Linomat V was used for application of samples. CAMAG TLC Scanner 3, Reprostar and Wincats 4.02 were used for scanning the plates. CAMAG twin through glass chamber was used for developing the plates. Pre-coated silica gel GF 254 plate was used as stationary phase. Petroleum ether (60-80°C): Ethyl acetate: Methanol (6:2:0.5) v/v was used as mobile phase as per reference of ICMR database^[15]. After 30 minutes of chamber saturation, plate was developed, and then scanned under short UV (254 nm) and long UV (366 nm) and thereafter sprayed with Vaniline-sulphuric acid reagent for color reaction.

Chromatographic conditions for estimation of Guggulsterone-E and Guggulsterone-Z through High Performance Liquid Chromatographic (HPLC) are as follows: Column: ODS(Octadecylsilane) C 18(2), 5 microne size, 250x40 mm (Merck) RP-18 Lichrocart 250-4; Detection: SPD-20A prominence UV- Visible detector, Wavelength detection: 242 nm; Mobile Phase: Water:Acetonitrile (55:45); Temperature: Room temperature; Flow Rate : 1 ml/min; Pressure: 153 kgf; Note : 47.6 mg sample in 50 ml Acetonitrile.

RESULTS AND DISCUSSION

Changes in organoleptic characters are placed at Table1. Golden yellow coloured ANG was turned to greenish yellow after one year in APG. Observations in basic Physico chemical parameters are shown in Table 2. Here, significant balsamic was perceived in Fresh *Guggulu* sample than Old sample which may be due to gradually loss of volatile content from the one year Old sample.

parameters; difference was observed in between samples, which mainly due to solubility values [Fig 1 b]. Both figures reveal no variation in other thermal parameters between Fresh and Old samples. On other hand, marked variations were found in solubility parameters. Water solubility of Fresh sample was more than Old samples whereas alcohol solubility was more in Old samples.

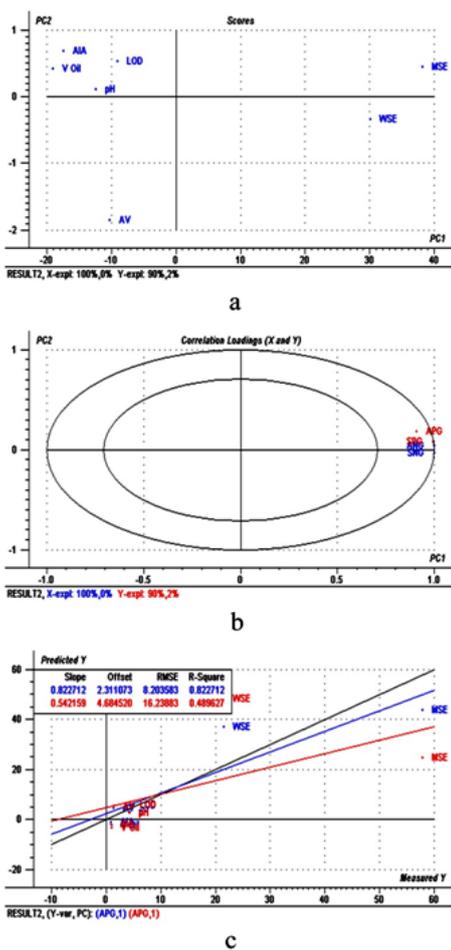


Figure 1: Fig. 1a: PC scoring; Fig. 1b: Principal Component Analysis of physico chemical parameters (LOD – Loss on drying, AV – Ash value, AIA – Acid insoluble ash, WSE – Water soluble extractives, MSE – Methanol soluble extractives); Fig 1c: Trade Line Discrimination Graph

Fig. 1 c shows R^2 (Trade Line Relation) around 0.82 and 0.48 suggests weak co-relationship between Fresh and Old samples on the basis of studied parameters.

Water soluble extractive was found more in *Shodhita* samples (SNG, SPG) than in *Ashudhdha* samples (ANG, APG). It may be because of *Shodhana* in *Gomutra* that extracted some aqueous principles. Water soluble extractive was more [31.54%] in ANG sample whereas it was 21.54% in APG. In vice versa, methanol soluble extractive was found more [57.8%] in APG than in ANG [37.3%]. In study, it was found that *Guggulu* fraction which is insoluble in acetylene acetate consists of the carbohydrate gum, which is about 55% of the gum resin [16]. From this, it can be interpreted that alcohol insoluble fraction may be carbohydrate which was found more in Fresh sample. Loss on drying was found more (8.58% for SNG and 12.39% for SPG) in *Shodhita* samples in comparison to *Ashudhdha* samples indicating presence of more moisture content in *Shodhita* samples. Ash value was found more (9.21%) in SNG followed by 4.82% in ANG. *Guggulu* is mixture of steroids, diterpenoids, aliphatic ester, carbohydrates, amino acids and variety of inorganic compounds [17]. Ash value was found more in Fresh samples (ANG and SNG) probably due to the presence of some inorganic contents in it. More Ash value was found in *Shodhita* samples than *Ashodhita* samples. It may be due to some inorganic substances incorporated during *Shodhana* procedure. Volatile oil content was found to be insignificantly decreasing with time factor.

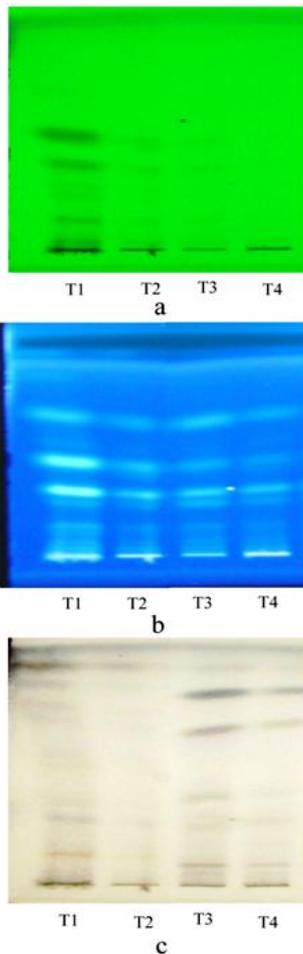


Figure 2: HPTLC profile of all Guggulu Samles; T1 – Ashudhdha Naveena Guggulu; T2 – Shudhdha Naveena Guggulu; T3– Ashudhdha Purana Guggulu; T4 – Shudhdha Purana Guggulu

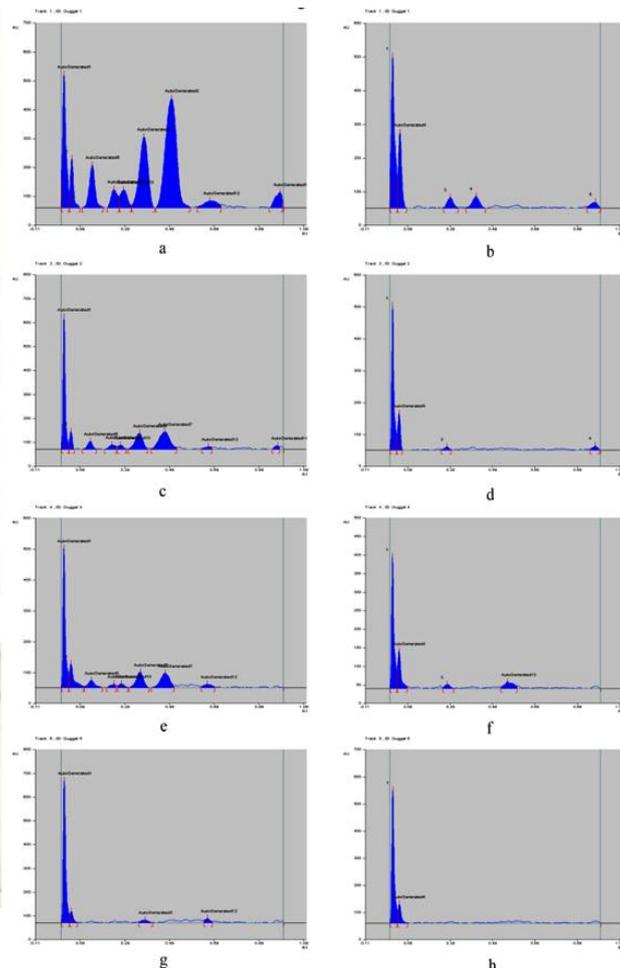


Figure 3: Peak Display of all samples of Guggulu

pH of the samples ANG, APG, SNG and SPG was found 4.98, 4.23, 6.30 and 7.18 respectively. Since *Gomutra* was used as *Shodhana* media, which is alkaline in nature the acidic pH of both the *Ashodhita* samples was reduced in *Shodhita* samples.

In HPTLC [fig.-2], methanolic extract of ANG, SNG, APG and SPG showed 9, 9, 8, and 4 numbers of spots at 254 nm wavelength; 5, 4, 4, and 2 spots at 366 nm wavelength and 11, 10, 9, and 6 spots at 400 nm after spraying with Vaniline sulphuric acid respectively. [Table 3 & fig 3]. In spectral comparison, common Rf values found in all *Guggulu* samples were 0.04, 0.37, and 0.66. Common Rf value found in ANG and APG samples were 0.14, 0.24, 0.28, 0.37, 0.47 and 0.67 whereas common Rf value found in SNG and SPG samples were 0.14, 0.36 and 0.66. Common Rf value found in ANG and SNG were 0.14, 0.23, 0.27, 0.37, 0.67 and 0.98 whereas 0.35 and 0.66 were found as common Rf values in SNG and SPG samples. Preliminary observations reveal that, Rf value one lesser in old samples in comparison to fresh samples.

In HPLC study, Guggulsterone-E was found more (0.37%) in Fresh *Guggulu* than in Old sample (0.19%). Simultaneously Guggulsterone –Z was found more (0.92%) in fresh samples than old sample (0.49%). In *Shodhita* Old samples also this reduction was observed [Table 4 & 5]. It may be due to changes in chemical composition of oleo-gum-resin by time factor.

Table 3: HPTLC of methanolic extract of different ANG,SNG, APG and SPG samples

Sample	UV spectrum					
	254 nm		366 nm		After spray with Vaniline sulphuric acid (400nm)	
	No. of spots	Rf	No. of spots	Rf	No. of spots	Rf
ANG	9	0.01, 0.05, 0.14, 0.24, 0.28, 0.37, 0.49,0.67,0.98	5	0.01, 0.05, 0.29, 0.41, 0.97	11	0.01, 0.06, 0.13, 0.23,0.28, 0.36, 0.49, 0.68, 0.74, 0.83, 0.91
SNG	9	0.01, 0.04, 0.13, 0.23, 0.27, 0.35, 0.46, 0.66, 0.97	4	0.01, 0.04, 0.27, 0.29	10	0.01, 0.12, 0.26, 0.34, 0.46, 0.65, 0.71, 0.83, 0.91,0.95
APG	8	0.01,0.04,0.14,0.24,0.27, 0.36, 0.47, 0.66	4	0.01, 0.04, 0.27, 0.56	9	0.09, 0.12, 0.24, 0.29, 0.37, 0.57, 0.64, 0.79, 0.90
SPG	4	0.01, 0.05, 0.38, 0.66	2	0.01, 0.05	6	0.09, 0.27, 0.40, 0.67, 0.81, 0.91

Table 4: Comparative data of column chromatographic separation of ANG, SNG, APG and SPG samples

Sample	Guggulsterone-E			Guggulsterone-Z		
	Rt	Area	Height	Rt	Area	Height
ANG	33.363	314.313	4.062	48.337	632.491	6.432
SNG	33.387	216.364	3.225	48.430	288.664	2.571
APG	33.453	193.040	2.902	48.490	400.236	4.058
SPG	33.497	183.280	2.689	48.590	208.021	2.086
Standard	31.820	19142.151	516.436	46.270	14994.370	287.442

Table 5: Percentage of Guggulsterone E and Z in all ANG, SNG, APG and SPG samples

Sample	Guggulsterone-E (%w/w)	Guggulsterone-Z (%w/w)
ANG	0.37	0.92
SNG	0.32	0.53
APG	0.19	0.49
SPG	0.26	0.36

CONCLUSION

As per results of Principle Component Analysis, Fresh and Old *Guggulu* can be differentiated by solubility parameters (Water and Methanol). Reduced components and decreased percentage of Guggulsteron observed through HPTLC and HPLC suggests that the drug undergo certain changes during Time factor. Possibly these changes may be responsible for the *Lekhana* property of old *Guggulu*. However, well defined clinical studies are needed to evaluate exact nature of efficacy in both the samples.

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CONFLICTS OF INTEREST

No conflicts of interest.

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