AYUSHDHARA
ISSN: 2393-9583 (P)/ 2393-9591 (O)
An International Journal of Research in AYUSH and Allied Systems

Research Article

A COMPARATIVE PHARMACEUTICAL AND ANALYTICAL STUDY OF *SHODHITA GUGGULU* IN DIFFERENT *SHODHANA* MEDIA W.S.R. TO ESTIMATION OF GUGGULSTERONE ISOMERS (E&Z)

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Article info

Article History:
Received: 26-03-2022
Revised: 04-04-2022
Accepted: 10-04-2022

KEYWORDS:
Shodhana, Guggulu, Safety, Comparative, Pharmaceutical, Analytical, Guggulsterone isomers E&Z.

ABSTRACT

To eliminate the poisonous character and to increase the therapeutic efficacy, the texts have advocated *Shodhana* for *Guggulu* before their therapeutic application. Methodology: A comparative pharmaceutical analytical study of *Shodhitha Guggulu* purified by four different *Shodhana* methods with special reference to the comparative analytical study of Guggulsterone isomers E & Z by HPLC method. Heating and dissolving in four different *Shodhana* media is followed in the current study. Four types of *Shodhita Guggulu* got after pharmaceutical study by using four different *Shodhana* media. *Guduchi kwatha Shodhita Guggulu* (GKSG), *Triphala kwatha Shodhita Guggulu* (TKSG), *Gomutra Shodhita Guggulu* (GMSG), *Nirgundi Swarasa* with *Haridra Choorna Shodhita Guggulu* (NHSG). The essential analytical studies as per API were conducted and a comparative study was done. The yield of *Shodhita guggulu* was more in TKSG (79.6%) followed by (NHSG) which (71.6%), (GKSG) 68.8%, and the least yield (GMSG) Total 156g of *Shodhita guggulu* yielded by 62.4%. The analytical profiles of *Shuddha guggulu* varied in 4 different samples.

In the comparative analytical parameters of four *Shodhitha guggulu* by HPLC with special reference to Guggulsterone isomer E was found highest to lowest in GKSG (0.084%), NHSG (0.0697%), GMSG (0.0074%) & TKSG (0.006%) respectively.

The analytical study of Guggulsterone isomer Z was found highest in both sample GKSG (0.39%) & NHSG (0.39%). And the second highest value got in GMSG (0.194%) and the least value of Guggulsterone Z was noted in TKSG (0.008%).

Conclusion: Considering the active ingredient Guggulsterone isomer E; the best sample among the four different *Shodhita Guggulu* in the present study is *Guduchi Kwatha Shodhita Guggulu.* And when considering the therapeutic efficacy by Guggulsterone isomer Z; both *Guduchi Kwatha Shodhita* and *Nirgundi Swarasa with Haridra Choorna Shodhita Guggulu* are the best among the present study samples.

INTRODUCTION

Guggulu (*Commiphora wightii, (Arn) Bhandari*) is a well known herbal drug, which is being used in vast range of diseases since *Vedic* period.[1]

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	https://doi.org/10.47070/ayushdhara.v9i2.920

It is one of the oldest and the most prominent herbs in Ayurvedic medicine. This plant contains a number of bioactive constituents including terpenoids, steroids, flavonoids, guggulstetrols, lignans, sugars, and amino acids. Guggulsterones E and Z are the chief bioactive constituents of this resin and are endowed with immense pharmacological value[2]. Understanding the poisonous character and to increase their therapeutic efficacy of *Guggulu,* Ayurvedic texts have advocated *Shodhana* for *Guggulu* before their therapeutic application. Different types of *Shodhana* methods are explained in various classical texts and API. But the actual impact of different *Shodhana* methods on

Guggulu has to be understood in the context of pharmaceutical study and analytical study with special reference to Guggulsterone isomers E & Z. Considering these, it has been planned to do a comparative pharmaceutical analytical study of *Shodhitha Guggulu* purified by four different *Shodhana* methods with special reference to the comparative analytical study of Guggulsterone isomers E & Z by HPLC method.

Methodology

Pharmaceutical Study

In the present study *Guggulu Shodhana* have been performed in 4 different medias *Guduchi Kwatha*[3], *Triphala Kwatha*[4], *Gomutra*[5], *Nirgundi Swarasa* with *Haridra choorna.* [6]

Place of Study: PG Department of Rasashastra and Bhaishajya Kalpana, KVG Ayurveda medical college and Hospital, Sullia.

It was done in following different steps

- 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 *Gomutra*- practical 5
- Preparation of *Nirgundi patra swarasa* with *Haridra choorna*- practical 6
- *Guggulu Shodhana* by *Nirgundi patra Swarasa* with *Haridra Choorna*- practical 7

Procedure

Preparation of different media

- ***Guduchi Kwatha***

Guduchi Yavakuta Churna (500gm) was kept soaked in water (8 litres) for overnight. Next day it was boiled on slow heat without covering its mouth. Water was evaporated slowly and reduced till the quantity became ¼th. It was filtered with clean cotton cloth and filtered liquid was collected as *Guduchi Kwatha.*

- ***Triphala Kwatha***

Prepared similar to *Guduchi Kwatha* replacing *Guduchi Yavakuta churna* with *Triphala Yavakuta churna.*

- ***Gomutra:*** Was freshly collected (2 litres), filtered through clean and dry cloth to remove the impurities.
- ***Nirgundi Patra Swarasa* with *Haridra Choorna***

Fresh leaves of *Vitex negundo (Nirgundi patra)* were collected. The leaves were washed with water and cut into small pieces. These small pieces were soaked in water for overnight. The slurry was prepared with the help of hand mixture. The small amount of *Haridra* powder (10gm) was added. The mixture was filtered. The filtrate was used as *Nirgundi swarasa* with *Haridra churna* for *Shodhana* process of *Guggulu.*

Guggulu Shodhana in Different Media

Guggulu Shodhana

Physical impurities like stone, sand, wood, bark pieces etc., were manually cleaned. *Shodhana* media was taken in a clean vessel and *Ashuddha Guggulu* was added in it and stirred well and heated for 1-2 hours, then it was macerated well and rubbed with hands. When the complete *Guggulu* dissolved in the media it was filtered by cotton cloth. After filtration the residue in the cloth was discarded. Now the filtered liquid was heated on gas stove on *Madhyamagni* with continuous stirring. (Gas knob was set on low fire) as water got evaporated its consistency increased gradually. When it started to become *Ghana (Avalehavat)*, the soft mass was transferred in to a wide stainless steel plate smeared with *Ghrita* and was kept for drying. After completely drying the *Shuddha Guggulu* was collected and preserved.

Analytical Study

The study has been carried to evaluate preliminary physico-chemical profiles of

- *Guduchi Kwatha Shodhitha Guggulu*
- *Thriphala Kwatha Shodhitha Guggulu*
- *Gomutra Shodhitha Guggulu*
- *Nirgundi Swarasa* with *Haridra Choorna Shodhitha Guggulu*.

Physico-chemical Analysis [7]

Place of work: QC lab of KVG Ayurveda Pharma and Research centre, Sullia.

HPLC Analysis was done in Care Keralam, Thrissur, Kerala.

- pH
- Loss on drying
- Ash value
- Acid insoluble ash
- Alcohol soluble extractive
- Water soluble extractive
- HPLC have been estimated for Guggulsterone isomers E&Z.[8]

For standard methods of analysis, relevant sections of Ayurvedic Pharmacopoeia of India (API) have been referred.

Loss on Drying[9]: It determines the amount of volatile matter (i.e. water drying off from the drug). Accurately weighed 10g of sample has been placed in an evaporating dish, dried at 105° for 5 hours and again weighed.

Ash Value [10]: 2gm of accurately weighed sample was incinerated in a cubicle at 450° allowed to become cool

and weighed. The percentage with reference to the air dried drug was calculated to get the total ash value.

Acid-insoluble Ash [11]

To the crucible containing total ash, 25ml of dilute hydrochloric acid was added. The insoluble matter on an ash less filter paper (Whatman 41) was collected and washed with hot water until the filtrate became neutral. The 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 become cooled in desiccators for 30 minutes and weighed without delay. The content of acid insoluble ash with reference to the air dried drug was calculated to get acid-insoluble ash.

Alcohol Soluble Extractive [12]

5gm of sample was macerated with 100ml of alcohol in a flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. Carefully filtered, evaporated 25ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 105°, to constant weight and weighed. The percentage of alcohol soluble extractive with reference to the air dried drug was calculated to get the alcohol soluble extractive.

Water Soluble Extractive [13]

5gm of sample was macerated with 100ml of chloroform water in a flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours carefully filtered, evaporated 25ml of the filtrate to dryness in tarred flat bottomed shallow dish, and dry at 105°, to constant weight and weighed. The percentage of alcohol soluble extractive with reference to the air dried drug was calculated to get the water soluble extractive.

Determination of pH Value [14]

Accurately weighed 1gm and 10gm of *Shodhitha Guggulu* were dissolved in 10ml and 100ml of distilled water respectively. When the sample was completely soluble, it was filtered. The pH of the filtrate was determined using pH meter.

Quantitative evaluation of Guggulsterone isomers E & Z in *Guggulu* samples (Four *Shodhitha guggulu*) using HPLC method[15]

Instrumentation of HPLC- High-performance liquid chromatography (Shimadzu) was performed using photodiode array detector (PDA). The PDA was set by optimizing wavelength to give the best response for all samples at 245nm to acquire the chromatogram. The work was done at Care Keralam, Thrissur, Kerala.

HPLC: CKL/ANL/E-027- Agilent Technologies 1200 Infinity Series

Balance: CKL/ANL/E-036

Method

Mobile phase: Acetonitrile: Water (45:55)

Standard preparation [16]- Weigh 5mg Guggulsterone E and Guggulsterone Z standard in a 5ml standard flask. Make up to volume with acetonitrile to get 1000ppm. From this 100ppm of mixed standard were prepared by diluting with acetonitrile.

Sample Preparation [17,18]**-** Weigh accurately about 3g of sample and transfer to a 250ml beaker. Extract with 50ml acetonitrile by boiling on a water bath for 20 minute and transfer the extract in a beaker. Repeat the process till the extract is colourless. Concentrate to below 100ml cool to room temperature and make up the volume to 100ml with acetonitrile. Mix well filter before injection.

Chromatographic Conditions

Column: C_{18} 4.6×100mm×3µm

Flow rate: 1.5ml/Minute

Inj. Volume: 20µl

Wave length: 242nm

Run time: 15 minute

Ref: Quality standard of Indian Medicinal Plants Volume 3

OBSERVATIONS AND RESULTS

Table 1: Showing Results of *Shodhita Guggulu* in different media

Batch	Weight of *Ashudha Guggulu*	Weight of *Shodhita Guggulu*	Yield (%)	Loss (g)	Loss (%)	Residue (gm)
Guduchi Kwatha Shodhita	250g	172g	68.8%	78g	31.2%	72g
Thriphala Kwatha Shodhita	250g	198g	79.6%	52g	20.4%	47g
Gomutra Shodhita	250g	156gm	62.4%	94g	37.6%	89g
Nirgundi Swarasa with *Haridra Choorna Shodhita*	250g	179gm	71.6%	71g	28.4%	64g

Organoleptic Evaluation

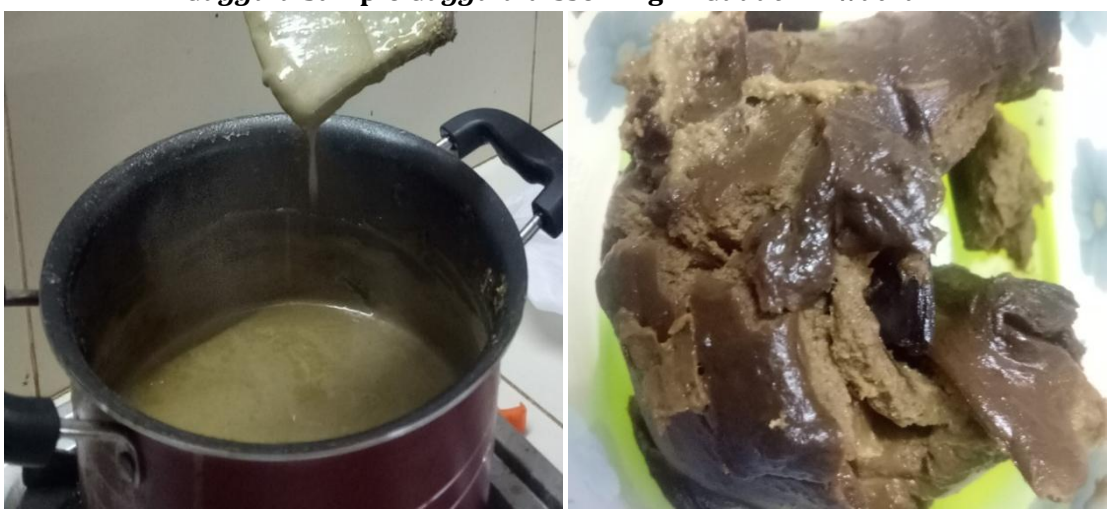
Table 2: Showing Various Parameters such as Color, Taste, Odor, Texture

Batch	*Guggulu*	Colour	Taste	Smell	Texture
1	*Ashodhita Guggulu*	Yellowish brown, black	Bitter, astringent	Balasmic, good odour	Gummy, harder, shining
2	*Guduchi Kwatha Shodhita Guggulu*	Greenish brown	Bitter	Pleasant odour	Sticky, flaky, softer
3	*Triphala Kwatha Shodhita Guggulu*	Dark brownish black	Bitter, astringent, sour	Mixed odour of *Thriphala* & *Guggulu*	Semisolid, sticky
4	*Gomutra Shodhita Guggulu*	Light chocolate brownish	Bitter, salty	Mixed smell of *Gomutra* and *Guggulu*	Softest of all samples
5	*Nirgundi Swarasa* with *Haridra Choorna Shodhita Guggulu*	Greenish brown	Bitter	Mixed smell of *Swarasa* and *Guggulu*	Flaky, soft

Pharmaceutical Study Pictures

Shodhana* in *Guduchi Kwatha

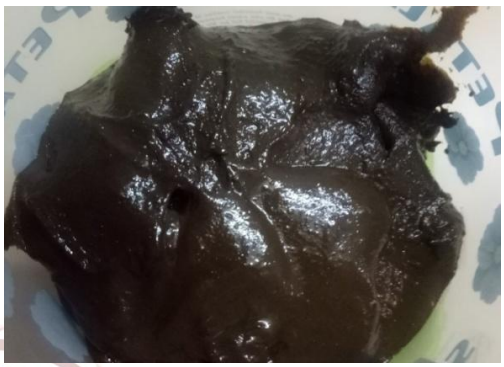
Guggulu* Sample *Guggulu* dissolving in *Guduchi Kwatha



Evaporating after filtration *Guduchi Kwatha Shodhitha Guggulu*

Shodhana* in *Triphala Kwatha

Guggulu* Sample *Guggulu* dissolving in *Triphala Kwatha



Evaporating after filtration *Triphala kwatha Shodhita Guggulu*

Shodhana* in *Gomutra

Guggulu* for *Shodhana-Gomutra

Guggulu* dissolving in *Gomutra **Evaporation after Filtration**

***Gomutra Shodhitha Guggulu* (GMSG)**

Guggulu Shodhana* in *Nigrundi patra swarasa* with *Hardidra Choorna

***Guggulu* sample**

***Guggulu* dissolving in media**

Evaporation after filtration *Nirgundi patra Swarasa* with *Haridra Choorna Shodhitha Guggulu* (NHSG)

Physico-chemical Analysis

Table 3: Showing Comparative Analytical Parameters of four *Shodhitha guggulu*

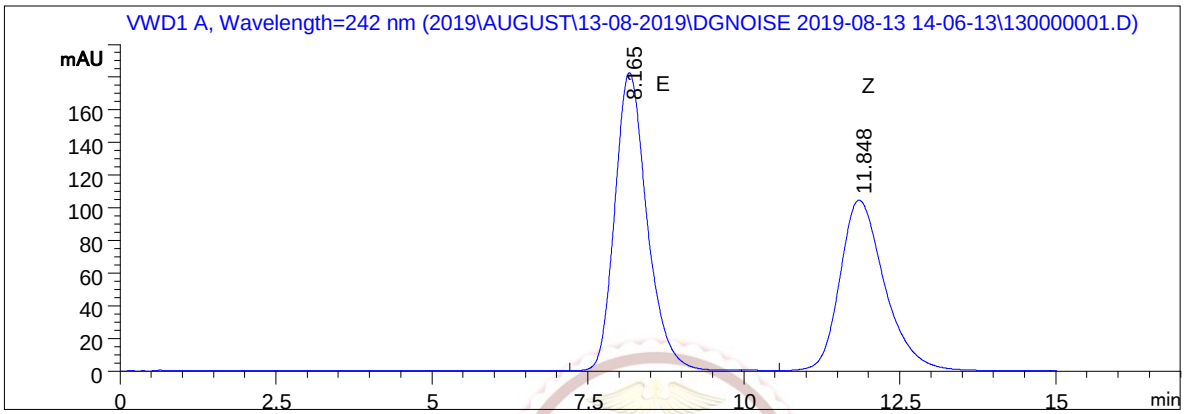
Batch No	Sample	pH	Loss on drying	Total ash value	Acid insoluble ash	Alcohol soluble extractive	Water soluble extractive
1	GKSG	6.1	7.38%	3.05%	1.06%w/w	23.79%	36.15%
2	TKSG	3.6	12.3%	2.5%	1.54%w/w	30%	23.6%
3	GMSG	5.5	18.6%	7%	2.42%w/w	28.73%	32.18%
4	NHSG	5.4	4.86%	2.34%	1.32%w/w	11%	30.38%
API Limits			Not>14	Not>5	Not>1	Not<27	Not<53

Table 4: Showing Comparative analytical parameters of four *Shodhita Guggulu* by HPLC with special reference to Guggulsterone isomers (E&Z)

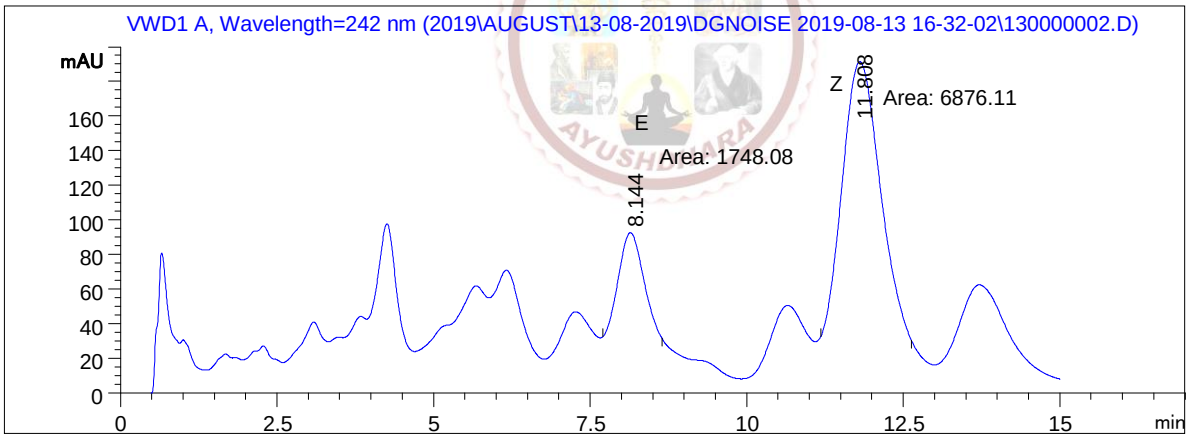
Batch	Sample	ID	Date of test	Test method	Result of Guggulusterone E	Result of Guggulusterone Z
1	GKSG	W1364	13/08/2019	CKN/ANL/HPLC/037	0.084%	0.39%
2	TKSG	W1363	13/08/2019	CKN/ANL/HPLC/037	0.006%	0.008%
3	GMSG	W1361	13/08/2019	CKN/ANL/HPLC/037	0.0074%	0.194%
4	NHSG	W1362	13/08/2019	CKN/ANL/HPLC/037	0.0697%	0.39%

Graph No: 1 Showing Chromatogram of Guggulsterone isomers E & Z.

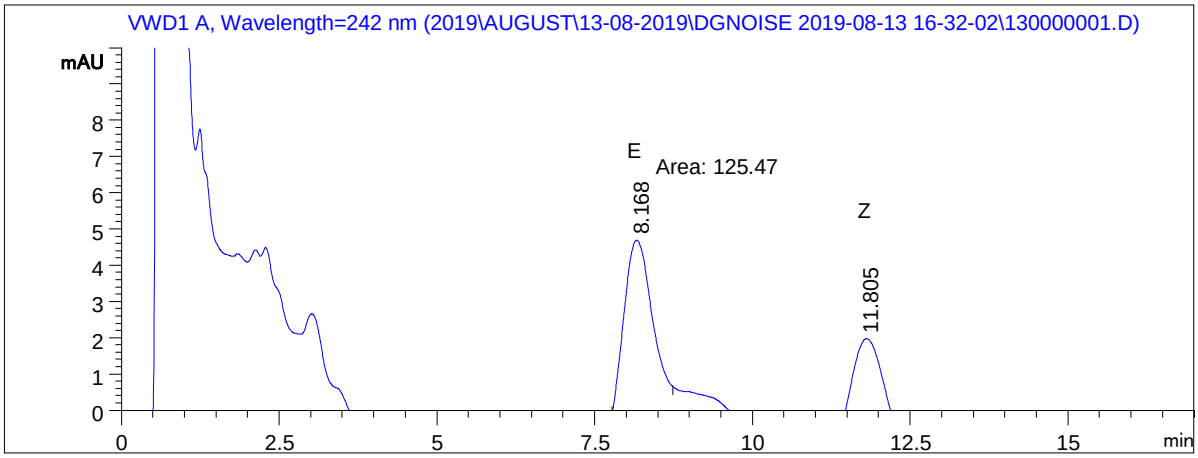
Sample Name: Guggulsterone E & Z



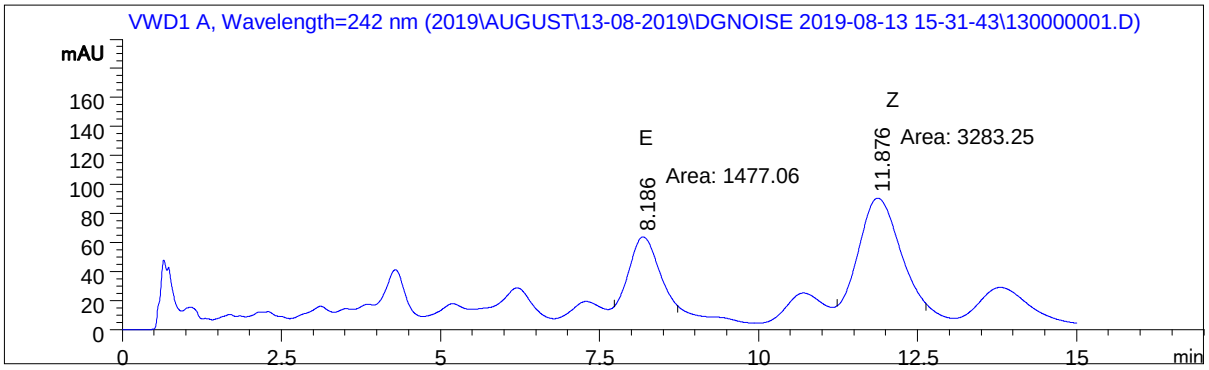
Graph No: 2 Showing Chromatogram of sample *Guduchi Kwatha Shodhitha Guggulu*(GKSG



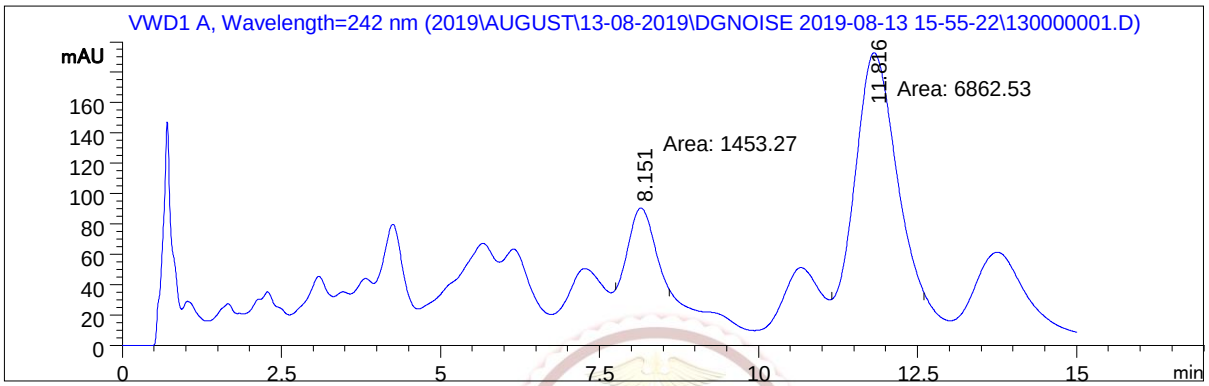
Graph No: 3 Showing Chromatogram of *Triphala Kwatha Shodhitha Guggulu* (TKSG)



Graph No: 4 showing Chromatogram of *Gomuthra Shodhitha Guggulu* (GMSG)



Graph No: 5 showing Chromatogram of *Nirgundi swarasa* with *Haridra Choorna Shodhitha guggulu* (NHSG)



DISCUSSION

Classics advocates to use purified *Guggulu* in therapeutics. Adverse effects are associated with crude gum *Guggulu.* Studies also reported gastric irritancy found to be reduced with purified *Guggulu*. Pharmacological action is found to be increased after *Shodhana* of *Guggulu*.

Among different types of *Guggulu shodhana*, four methods of *Guggulu Shodhana* were adopted in this present study. Often, crude *Guggulu* is advocated to dissolve in specified liquids and filter trough cotton fabric in *Shodhana* process.

For the present study, common process of *Guggulu Shodhana,* which is in practice at commercial level was adopted, instead of *Pottali, Shodhana* method as per the opinion of the experts of subject. There are a lot of problem with *Pottali Shodhana* method, first of all the problem is with the quantity of *Shodhana* media as mentioned that make the *Pottali* of *Ashuddha Guggulu* and immersed completely in to liquid, then heat it. Water-soluble part of *Guggulu* passes from *Pottali* to the liquid, but side by side because of evaporation, quantity of liquid media gradually decreases. As a result one stage will come when *Pottali* will not be remained immersed completely in to the liquid, so more *Shodhana* media or water will be needed for the complete dipping of *Pottali* into the liquid. Because of this phenomenon any standard quantity of *Shodhana* media cannot be standardized for the *Shodhana* process. Climatic conditions also affect the evaporation of any liquid so again it becomes difficult to bring out any kind of similarity in quantity of *Shodhana* media. Finally all liquid soluble part should pass from *Pottali* to liquid media then continuous heating of this *Guggulu* containing liquid will give the *Ghana* of *Shuddha Guggulu*. By this *Pottali Shodhana* method, *Shodhana* of *Guggulu* cannot be performed on large scale and the quantity of *Shodhana* media cannot be standardized. The present procedure adapted for *Shodhana* of *Guggulu* therefore appears to be a sensible way of purifying *Guggulu*, because in the process toxic insoluble part is removed only soluble part of *Guggulu* is taken.

Pharmaceutical Study

In all *Shodhana* process 250g of raw *Guggulu* was used. After finishing of *Shodhana* process the yield of *Shodhita Guggulu* was more in *Thriphala kwatha Shodhita guggulu* (TKSG) =198G (79.6%). Even though highest quantity of *Shudha guggulu* was obtained by *Thriphala Kwatha Shodhana* method*,* this may be due to the tannins and other fibrous particles present along with *Thriphala kwatha* which was used as the *Shodhana* media had to stick with the gummy *Shodhita Guggulu* during the procedure of purification. Gummy nature of *Shodhita Guggulu* also noted more in TKSG. Second largest yield of *Shodhita Guggulu* is got after *Nirgundi Swarasa* with *Haridra Choorna Shodhita*

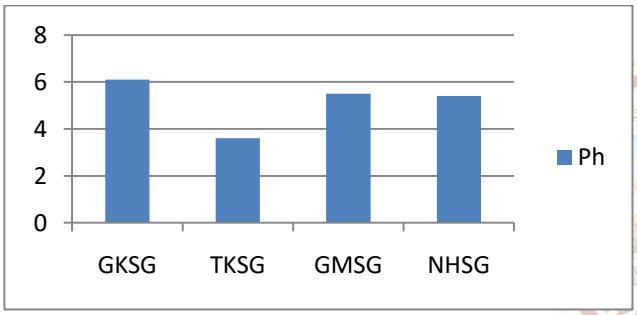
guggulu (NHSG) which was 179G (71.6%). Here the *Swarasa* of *Nirgundi* leaves may also be dried and mixed with the *Shodhita Guggulu* during the procedure.

Thirdly placed yield was got in *Guduchi kwatha Shodhita Guggulu* (GKSG). Total 172gm (68.8%) was of *Shudha Guggulu*. This may be due to the viscosity of *Guduchi Kwatha* was much lesser than *Thriphala Kwatha* and the least yield of *Shodhita Guggulu* got by *Gomutra Shodhana* (GMSG). Total 156g (62.4%) was the yield. The reason for this may be *Gomutra* is a clear liquid with very less residual particles than other three *Shodhana* media. Most of the pharmaceutical companies are using *Thriphala Kwatha* for the *Shodhana* of *Guggulu.* Many reasons are there behind this. One among them may the largest yield getting by the *Thriphala Kwatha Shodhana* method.

Analytical Study

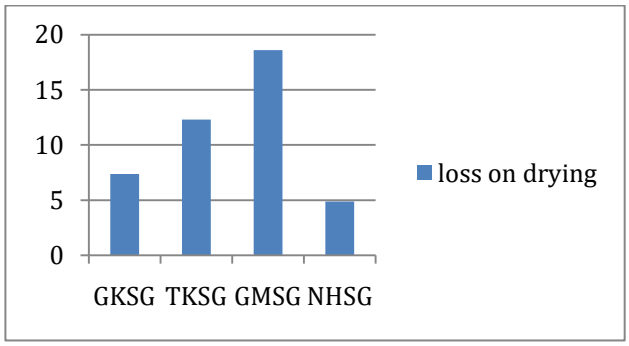
The comparative analytical profile of *Shodhitha Guggulu* was recorded as below:

pH value



pH value was found to be more in GKSG sample allowed by GMSG. Thirdly, NHSG pH value was least in TKSG sample, which may be due to the acidic nature of the *Thriphala* fruits which are used as *Shodhana* media.

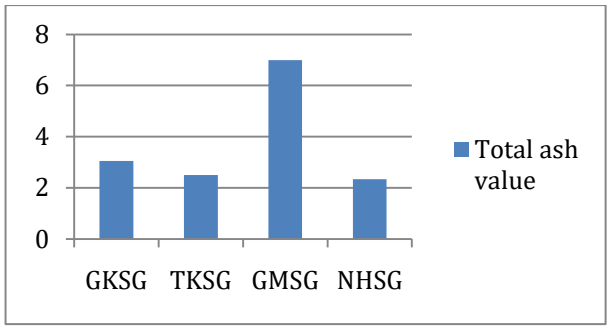
Loss on drying at 105°C



In loss on drying study, the value got more in GMSG sample, followed TKSG sample.

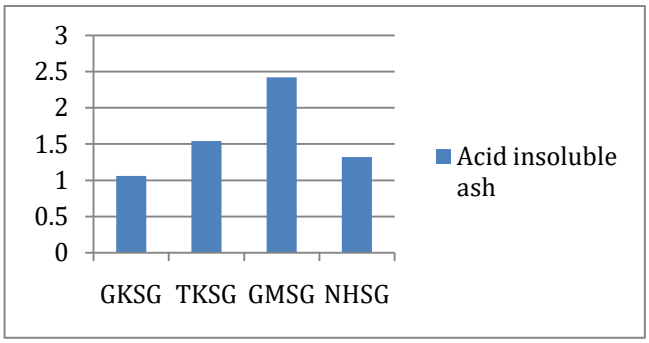
Thirdly placed value in GKSG sample and the least value got in NHSG sample.

Total Ash value



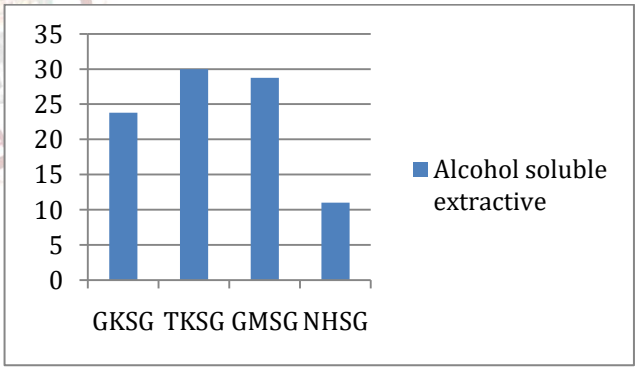
The ash value of sample GMSG < GKSG< TKSG< NHSG

Acid Insoluble Ash



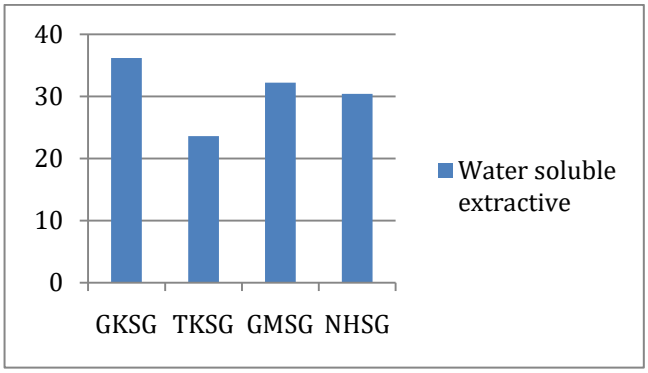
After finishing all the four samples of *Shodhita Guggulu*, acid insoluble ash was found more in GMSG < TKSG < NHSG < GKSG sample.

Alcohol Soluble Extractive



In case of alcohol soluble extractive value the most value was got in TKSG sample, followed by GMSG, GKSG, and least value got in NHSG sample.

Water Soluble Extractive



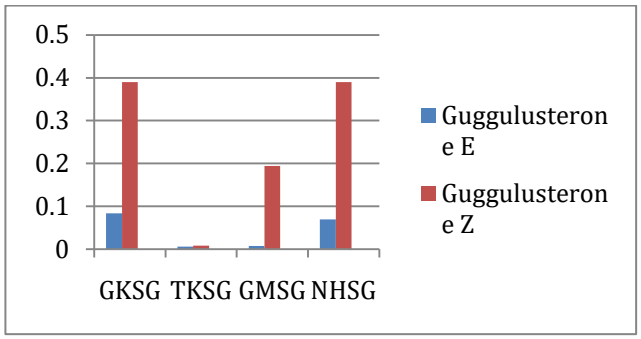
Water soluble extractive value is highest in GKSG sample

Second largest in GMSG sample

Thirdly placed value got in NHSG sample

And the least value was in TKSG sample.

Comparative analytical parameters of four *Shodhitha Guggulu* by HPLC with special reference to Guggulsterone isomers (E & Z)



Different *Shodhana* methods of *Guggulu* reflected a change in the phytochemical contents present in it. The results of HPLC study of *Shodhitha guggulu* to estimate Guggulsterone isomers E & Z showed that

A- Guggulsterone E was present highest in GKSG sample.

B- Guggulsterone E was secondly largest in NHSG sample**.**

C- Thirdly placed value in GMSG sample and

D- Least value of Guggulsterone E is noted in TKSG sample.

In the case of Guggulsterone Z,

A- The value got largest in both sample GKSG and NHSG.

B- Secondly largest in sample GMSG and C- Least value of Guggulsterone Z is noted in TKSG sample. There are many *Guggulu Shodhana* methods advocated in classics. Here, in this present study only four methods of *Guggulu Shodhana* were conducted. So scope for further studies regarding guggulsterone isomers are also there for other *Shodhana* methods when we consider the therapeutic efficacy of *Guggulu* with reference to quantitative estimation of guggulsterone isomers, GKSG method may the best to adopt for pharmaceutical procedures. Further supportive clinical studies also need to be conducted.

CONCLUSION

To evaluate the impact of *Shodhana Samskara* on raw *Guggulu*, heating and dissolving in four different *Shodhana* media is followed in the current study. The yield of *Shodhita Guggulu* was more in *Thriphala Kwatha Shodhita Guggulu* (TKSG) =198g (79.6%) followed by *Nirgundi Swarasa* with *Haridra Choorna Shodhita Guggulu* (NHSG) which was 179g (71.6%), and then followed by *Guduchi kwatha Shodhita Guggulu* (GKSG) which was 68.8%, And the least yield of *Shodhita Guggulu* got by *Gomutra Shodhana* (GMSG). Total 156g of *Shodhita Guggulu* yielded by this study was about 62.4%. The analytical profiles of *Shuddha Guggulu* were recorded. The value of pH got from the highest to lowest is in the samples GKSG (6.1), GMSG (5.5), NHSG (5.4), and TKSG (3.6) respectively. Then the LOD also recorded and found from the highest to lowest as GMSG (18.6), TKSG (12.3), GKSG (7.38), NHSG (4.86) respectively.

In the case of total ash value highest to lowest value is in samples GMSG (7%), GKSG (3.05%), TKSG (2.5%) & NHSG (2.34%) respectively. Acid insoluble ash of samples from highest to lowest is GMSG (2.42%), TKSG (1.54%), NHSG (1.32%) & GKSG (1.06%) respectively.

For the value of alcohol soluble extractive the analytical value got highest to lowest was TKSG (30%), GMSG (28.73%), GKSG (23.79%) & NHSG (11%). For the value of water soluble extractive GKSG (36.15), GMSG (32.18), NHSG (30.38) & TKSG (23.6) are placed respectively in highest to lowest order. In the comparative analytical parameters of four *Shodhitha guggulu* by HPLC with special reference to guggulsterone isomer E was found highest to lowest in GKSG (0.084%), NHSG (0.0697%), GMSG (0.0074%) & TKSG (0.006%) respectively.

The analytical study of Guggulsterone isomer Z was found highest in both sample GKSG (0.39%) & NHSG (0.39%). And the second highest value got in GMSG (0.194%), and the least value of Guggulsterone Z was noted in TKSG (0.008%).

Many active principles are found in *Guggulu* including Guggulsterone isomers E & Z. From the current study it was concluded that the therapeutic efficacy of *Shodhitha Guggulu* prepared by the four *Shodhana* media in the study with special reference to Guggulsterone isomer E is more in *Guduchi kwatha Shodhitha Guggulu* and with respect to Guggulsterone isomer Z both *Guduchi kwatha Shodhitha guggulu* and *Nirgundi swarasa* with *Haridra choorna Shodhitha guggulu* are found to be the best.

ACKNOWLDGEMENT

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2. Dr. Sangeetha K.S . Final year PG Scholar, Dept of RS & BK. KVG Ayurveda Medical College and Hospital, Sullia.
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Cite this article as:
K.G Purushotham, K Shruthan, Gopakumaran K, Harshitha, G.B Rohith Krishnan. A comparative Pharmaceutical and Analytical Study of Shodhita Guggulu in Different Shodhana Media w.s.r. to Estimation of Guggulsterone Isomers (E&Z). AYUSHDHARA, 2022;9(2):1-11.
https://doi.org/10.47070/ayushdhara.v9i2.920
Source of support: Nil, Conflict of interest: None Declared

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