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QUALITATIVE EVALUATION AND IMPACT OF VISHESH SHODHANA PROCESS ON GUGGUL (*COMMIPHORA MUKUL*)

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Keywords:

High Performance Thin Layer Chromatography (HPTLC), Guggulsterone (E and Z), Tinosporaside, Diosgenin, Ellagic acid, Gallic acid

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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". Ashuddha guggulu 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 processes *i.e.* Samanya shodhana and Vishesh shodhana on properties of guggul by employing various physicochemical and chromatographic methods. **Method:** Physicochemical screening was done by evaluating ash, Acid Insoluble Ash (AIA), Loss on Drying (LOD), Water Soluble Extractive (WSE), Alcohol Soluble Extractive (ASE) and Ethyl acetate Soluble Extractive (EASE). Chromatographic analysis was performed to estimate guggulsterone (E and Z) content and to confirm the presence of ellagic acid and gallic acid, tinosporaside and diosgenin after shodhana with Triphala kwath, Gulvel kwath and Dashmool kwath respectively, using High Performance Thin Layer Chromatography (HPTLC). **Result:** The Physico-chemical studies showed decrease in LOD, Ash and AIA content and increase in extractive values such as ASE and EASE of guggul after shodhana process. The HPTLC analysis showed no significant change in guggulsterone (E and Z) content in guggul after shodhana process. The peak of ellagic acid and gallic acid, tinosporaside and diosgenin was observed in Triphala shodhit Guggul, Gulvel shodhit Guggul and Dashmool Shodhit Guggul respectively. **Conclusion:** This study helps to understand the effect of Vishesh shodhana on the efficacy of drug. In this study, we established qualitative profile of Vishesh Shodhit Guggul in terms of physicochemical parameters and phytochemical content by HPTLC.

INTRODUCTION: Guggul is an oleo gum resin, obtained from exudates of stem and branches of *Commiphora mukul* [1, 2]. It is an important medicinal plant widely used in Ayurveda since ancient time for various therapeutic uses. Guggul has natural cholesterol lowering substance [3]. Guggulsterone (E and Z) the active chemical constituents of guggul, have been reported to influence numerous natural processes like tumour cell proliferation and apoptosis [4].

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There are higher chances of contamination by sand, soil, sticks *etc*. as guggul resin is sticky in nature [5]. Raw guggul has more Tikshna, Ushna and Ruksha properties which if used as such, it may cause harmful effects such as skin rashes, diarrhoea, mild nausea, irregular menstruation, headache [6]. Hence to eliminate physical and chemical impurities of drug and to control toxic effect and then enhanced the therapeutic properties of drug, guggul must be detoxified or purified using different shodhan dravyas before using into formulation.

The term "Shodhana" in Sanskrit means Purification. Shodhana process is used to remove or decrease the concentration of toxic compounds and sometimes enhances the potency of drugs. Shodhana vidhi undergoes various processes like cleaning, de-husking, sorting, sifting, distillation, filtering,

straining, *etc* [7]. Shodhana process has two major types; Samanya shodhana means General Purification while Vishesh shodhana means specific purification. Shodhana dravyas plays very important role in the therapeutic effect of drugs. For the vishesh shodhana of guggul, heating and dissolving procedure is followed using different vishesh dravyas (liquids) like Triphala kwath (Decoction of Triphala), Gulvel kwath (Decoction of Gulvel), Dashamool kwath (Decoction of Dashamool) [8, 9, 10, 11, 12]. Triphala acts as digestive tonic, stabilizes blood sugar and a good source of Vitamin C and antioxidants [13].

Gulvel is used for hepatitis, diabetes, high cholesterol, upset stomach, rheumatoid arthritis (RA), lymphoma and other cancers and to boost immune system [14]. Because of its various medicinal properties, Gulvel is also known as 'Amrita' [15]. Dashamool is Combination of Kshudra Pancha Mool (Salvan, Pithvan, Ringani big, Kate Ringani and Gokharu) and Mahat Pancha Mool (Bilva, Tetumool, Shivanmool, Airanmool and Padal). Dashmoola helps to balance Vata, pitta and Kapha. It is excellent medicine for treating vata vyadhi and inflammatory condition [16].

METHOD AND MATERIALS: Analytical study of Ayurvedic drugs has become the need of present hour. Data generated by the analytical study of any standard drug suggest the quality of that drug and specific therapeutic effects. For the present study the sample of Ashodhit guggul was collected from approved vendor of Shree Dhootapapeshwar Ltd. This sample was taken for further shodhana processes *i.e*. Samanya shodhana (Samanya shodhit Guggul), and Vishesh shodhana (Triphala shodhit Guggul, Gulvel shodhit Guggul, Dashamool shodhit Guggul) as per the text references at manufacturing unit of Shree Dhootapapeshwar Ltd., [7, 8, 9, 19, 11].

After shodhana process these samples were analysed for physicochemical parameters and Chromatographic evaluations to compare the effect of different shodhana dravyas on the properties of Guggul. All chemicals and reagents (Toluene, Acetone, Methanol, Dil. HCl, *etc*.) used in analysis were of analytical reagent grade of Merck. Reference standards (RS) guggulsterone E (purity 99.60%; CAS No, 39025-24-6), guggulsterone Z (purity 97.60%; CAS no. 39025-23-5), ellagic acid (purity 99.8%; CAS no. 476-66-4), gallic acid (purity 98.5%; CAS no. 149-91-7), diosgenin (purity 97.3%; CAS no. 512-04-9) and tinosporaside (extracted at SDL by prep TLC) were used for the Chromatographic study.

Organoleptic Evaluation: Various parameters such as colour and texture of the samples of Ashodhit guggul and all Shodhit gugguls were observed and recorded.

Physicochemical Screening: Physicochemical parameters, such as Loss on drying (LOD), Ash, Acid insoluble ash (AIA), Water soluble extractive (WSE), Alcohol soluble extractive (ASE) and Ethyl acetate soluble extractive (EASE) checked for all the batches of Shodhit guggul and Ashodhit guggul as per The Ayurvedic Pharmacopoeia of India (API) [17].

Chromatographic Analysis: Camag HPTLC Instrument with Linomat 5, TLC Scanner 4 and Wincat Software was used for chromatographic analysis of Ashodhit guggul and Shodhit guggul. Twin trough chamber was used for development of HPTLC plate. Photo documentation cabinet fitted with High Resolution camera was used for capturing images at different wavelengths. Densitometer TLC Scanner 4 equipped with D_2, Hg and W lamp was used to obtain densitogram for quantitative determination of compound. The optimised solvent systems were used for estimation of each marker compound *i.e* guggulsterone (E and Z), ellagic acid, gallic acid, tinosporaside and diosgenin, which give good resolution **Table 1**.

RESULTS AND DISCUSSION: Physicochemical characterization of Ashodhit guggul and Shodhit guggul was carried out according to methods described in API. The results of the Physico-chemical studies of Ashodit and shodhit guggul are tabulated in **Table 2**. The percentage of Ash and Acid Insoluble Ash (AIA) content decreased after shodhan processes of guggul which shows the purity of drug increased after shodhan vidhi. Ash content is the residue (Inorganic salts of carbonates, phosphates, silicates of sodium, potassium, calcium and magnesium) remain after incineration and the Acid-Insoluble Ash content is the amount of ash insoluble to diluted hydrochloric acid.

TABLE 1: HPTLC SYSTEM CONFIGURATIONS

Phytoconstituents / Marker compounds	Solvent systems	Detection	Lamp used	R_f value
Guggulsterone E	Toluene : Acetone (9 : 1 v/v)	at 252 nm	D_2	0.23 ±0.04
Guggulsterone Z	Toluene : Acetone (9 : 1 v/v)	at 252 nm	D_2	0.29±0.04
Ellagic acid	Chloroform : Ethyl acetate : Formic acid (5 : 4 : 1.6 v/v/v)	at 278 nm	D_2	0.24 ± 0.04
Gallic caid	Chloroform : Ethyl acetate : Formic acid (5 : 4 : 1.6 v/v/v)	at 290 nm	D_2	0.34 ±0.04
Tinosporaside	Tolune : Acetone : Water (5 : 15 : 1 v/v/v)	at 220 nm	D_2	0.43 ±0.04
Diosgenin	Toluene : Ethyl acetate : Formic acid (7 : 2 : 1 v/v/v)	at 430 nm	W	0.42 ±0.04

TABLE 2: RESULTS OF ORGANOLEPTIC AND PHYSICOCHEMICAL ANALYSIS

Parameters	Ashodhit Guggul	Samanya Shodhit Guggul	Triphala Shodhit Guggul	Gulvel Shodhit Guggul	Dashmool Shodhit Guggul
Colour	Brown	Light Brown	Light Brown	Brown	Brown
pH	4.72	5.47	4.34	5.45	5.34
LOD (%)	12.19	2.76	7.88	2.69	9.23
Ash (%)	11.9	9.66	6.63	6.42	7.70
AIA (%)	7.85	4.02	2.73	2.78	3.90
WSE (%)	39.21	30.39	32.68	24.3	30.95
ASE (%)	23.55	28	27.13	31.53	24.03
EASE (%)	12.51	22.98	19.97	27.85	17.56

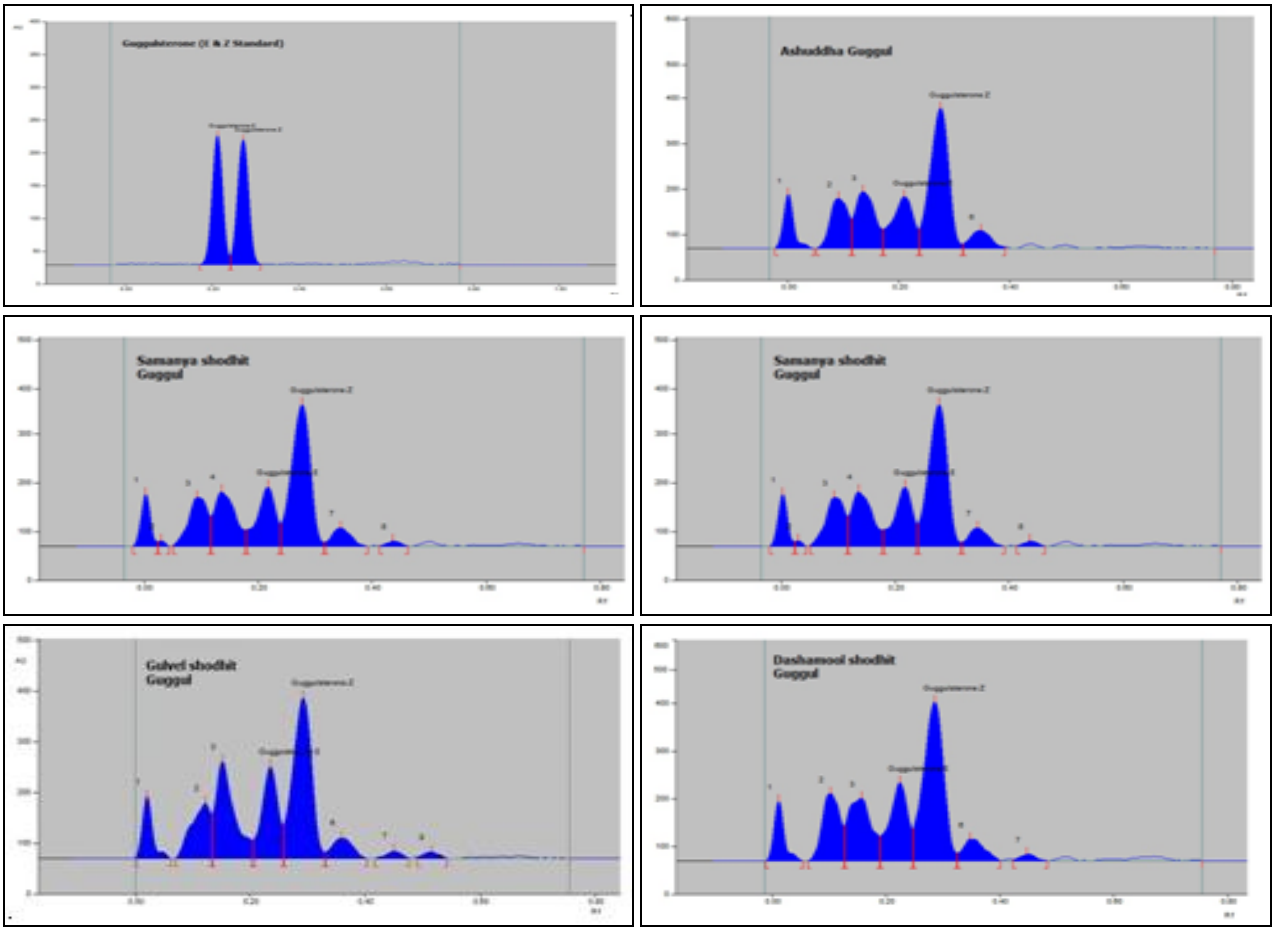

FIG. 1: PEAK DISPLAY OF GUGGULSTERONE (E AND Z) IN REFERENCE STANDARD, ASHODHIT GUGGUL AND SHODHIT GUGGUL

The extractive values such as ASE and EASE increased after Shodhan processes which indicates improved solubility of alcohol and ethyl acetate soluble contents in guggul. HPTLC analysis confirms the presence of Guggulsterone E and Guggulsterone Z at R_f 0.23 ± 0.04 and 0.29 ± 0.04 respectively **Fig. 1.** It shows there is no significant change in total guggulsterone (E and Z) content after Shodhan process of guggul **Table 3**. The peak of tinosporaside at R_f 0.43 ± 0.04; diosgenin at R_f 0.42 ± 0.04; ellagic acid at R_f 0.24 ± 0.04 and gallic acid at R_f 0.34 ± 0.04 confirms the vishesh shodhana of guggul with Gulvel kwath, Dashmool kwath, and Triphala kwath, respectively **Fig. 2**, **3** and **4**.

TABLE 3: RESULTS OF TOTAL GUGGULSTERONE CONTENT IN ASHODHIT AND SHODHIT GUGGUL

S. no	Samples	Total guggulsterone (E and Z) content
1	Ashodhit Guggul	1.60 %
2	Samanya Shodhit Guggul	1.42 %
3	Triphala Shodhit Guggul	1.35 %
4	Gulvel Shodhit Guggul	1.52 %
5	Dashmool Shodhit Guggul	1.55 %

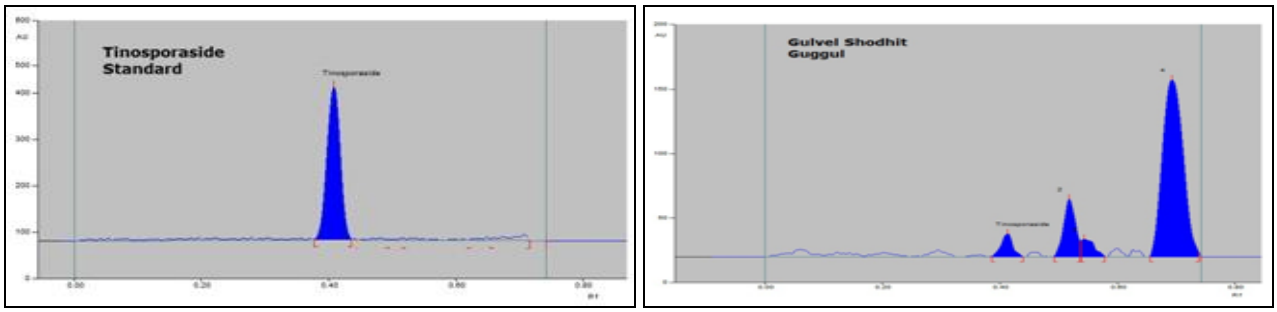

FIG. 2: PEAK DISPLAY OF TINOSPORASIDE IN REFERENCE STANDARD AND GULVEL SHODHIT GUGGUL

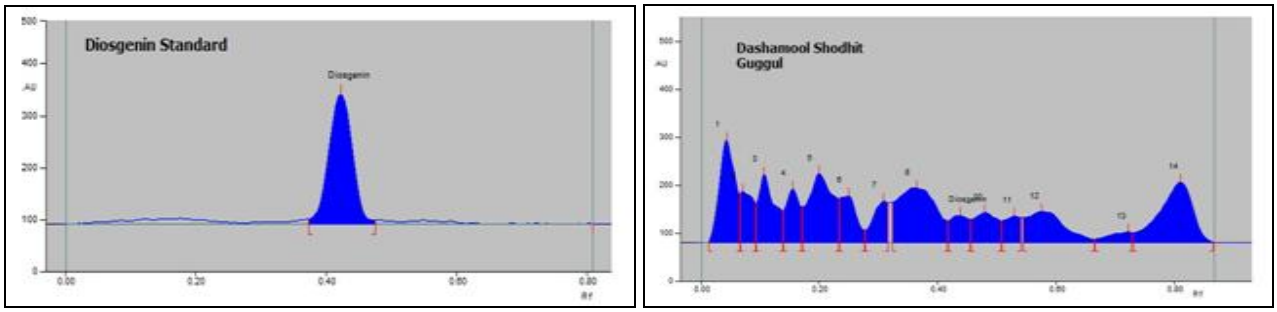

FIG. 3: PEAK DISPLAY OF DIOSGENIN IN REFERENCE STANDARD AND DASHAMOOL SHODHIT GUGGUL

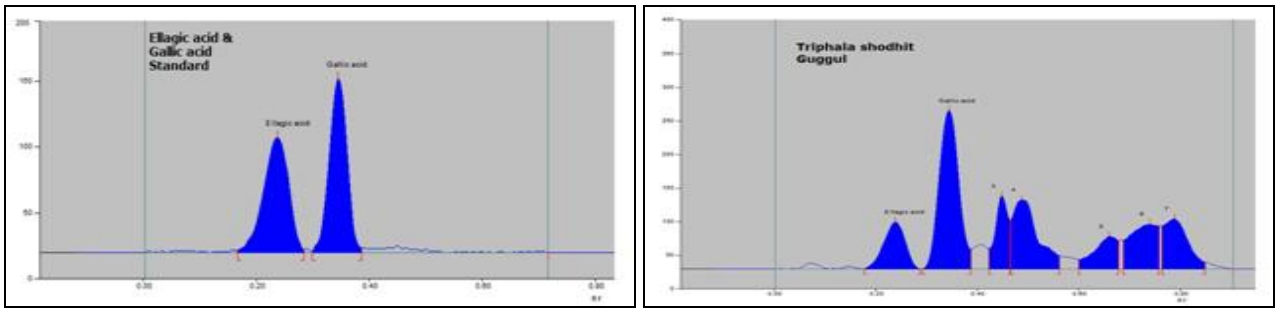

FIG. 4: PEAK DISPLAY OF ELLAGIC ACID AND GALLIC ACID IN REFERENCE STANDARD AND TRIPHALA SHODHIT GUGGUL

CONCLUSION: From the Physicochemical analysis, we observed unique characteristics of Vishesha Shodhana process when compared with the Samanya shodhana process of guggul. The extractive values get increased and Ash as well as AIA values get decreased after Vishesh shodhana as compared to Samanya shodhana. HPTLC studies confirmed the presence of Vishesha dravyas in guggul after Vishesh shodhana. Samanya shodhana helps to remove impurities of Ashuddha guggul whereas Vishesha shodhana removes impurity as well as improves the efficacy, as the Vishesh dravyas such as Triphala, Gulvel and Dashamool has various medicinal properties. So, this study help in setting modern phytochemical profile of Vishesh Shodhana process and establishing link of

Vishesh Shodhana vidhi. Thus application of such modern analytical tools is required to explore and understand importance of Ayurvedic concept which can be offered to modern practitioners in scientific language.

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CONFLICT OF INTEREST: Nil

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