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Pharmacognostic and Comparative Quality Control Profiling of *Commiphora mukul*

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ABSTRACT
The present study was pointed to the pharmacognostic and comparative evaluation of shudha guggul which prepared by different methods. Guggul is yellowish gum-resin of the plant Commiphora mukul belongs to the Burseraceae family. The purification of guggul is done in Ayurveda for medicinal purposes. Shudha is the process of detoxification, effectiveness, relevance, and acceptability of guggul for medicinal purposes. The purification of guggul was performed using three different way of decoction techniques. Extractive values parameter such as Foreign Matter, Total Ash, Acid Insoluble Ash, Alcohol Soluble Extractive, Water Soluble Extractive and Loss on drying for the evaluation of shudha guggul. Qualitative and quantitative analyses were performed using HPLC and HPTLC methods. Further, investigate the percentage of GS-Z. With the help of an Inductive Coupled Plasma-Optical, Emission Spectrometer was determined the presence of heavy metal. Also, the microbial assay was performed. Analytical techniques were used to analyze results of SG exhibited Total Ash, Acid Insoluble Ash, Alcohol Soluble Extractive, Water Soluble Extractive and Loss on drying to 2.75% w/w, 0.21%w/w, 30.08%w/w, 60.59%w/w, and 2.67%w/w respectively. All the values of SG were different compare to raw guggul. These values of SG were compared with standard values and RG. HPTLC of each extract of SG shown 0.19 to 0.49 Rf value at 256 nm, 366 nm, and daylight out of which only one Rf will match with either wavelength. HPLC determine the concentration of GS-Z of TSG, VSG and GSG of 0.9%, 0.79% and 0.89% respectively. Microbial assay and heavy metal determination have not crossed the limits. The present study has shown that the different purification methods affect the physicochemical parameter concerning RG. Microbial growth and heavy metal impurities not found in SG. The Triphalakasaya method gives a better yield and extractive values as compared to Vasapatrakasaya and Gomutra Methods.
Keywords: Shuddha Guggul, Triphalakasaya, Vasapatrakasaya, Gomutra, HPTLC, HPLC, Densitometry

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INTRODUCTION

The herbal products are widely used as medicaments, their evaluation, and standardization is done by modern techniques. From the ancients, guggul is one of the herbal medicines, and vati, rasakriya, taila, lepa, dhupa were Ayurveda preparation of guggul [1, 2]. The properties of raw guggul like tikshna, ushna, and ruksha were harmful. Thus, the analytical techniques were adopted for the free from impurities and suitable for human consumption. It is the resinous bark of *Commiphora mukul* which is sticky. Hence, Chances of contamination may increase by soil, sand, and sticks [3, 5]. Guggul is a tiny grow plant at rocks tract with height 1-2 m and growing at the eastern region of India. By, making the incision to the bark of *Commiphora mukul* in summer, collected guggul exudate at approximate weight 250-300 grams [6]. Guggul has been used to treat various diseases arthritis, obesity, liver disorder, anemia, diabetes respectively. It is also used as a good binding agent in herbal formulations. The main phytoconstituent of guggul were gallic acid, quercetin, and guggulsterone E and Z. Therapeutically, these phytoconstituents have shown a wide range of pharmacological effects such as These phytochemicals have a wide range of pharmacological activities such as antioxidants, anti-obesity, hypolipidemic, anti-inflammatory, anti-oxidant, anti-fungal, anti-microbial, anti-tumor, cardio-protective respectively [4,7]. The analytical approaches have reproducible, automated, fast, cost-effective, and hyphenated to determine for the estimation of GS-Z and comparative evaluation of raw and purified guggul. The presents study investigates the standard process of purification and GS-Z concentration and its socio-economical importance [8].

MATERIAL AND METHODS

Material

All herbs and chemicals were used for the purification and evaluation studies of SG. RG, Amla, Behda, Hirda, Vasaka were obtained from Central India Herbs Pvt. Ltd. Gwalior. The analytical grade of reagents and chemicals were used to perform research work.

Macroscopic studies

Macroscopic and organoleptic studies were carried on whole materials. All samples were washed, air-dried in shade, and observed for color, odor, taste, and size characteristics [9].

Process of purification

A dried raw guggul frees from impurities and in small pieces. The pieces are bundled in a cloth and boiled for a few minutes in dola yantra with fluids such as Triphalakasaya, Vasapatrakasaya, and Gomutra. Further, macerated the guggul and filter off any remaining residue were discarded. Heated the filtered at low-temperature approx. 83^0 C to 89^0 C with constantly stirred to avoid stickiness and burning at the bottom of the flask. After completion of heating, the mass residue spread on the tray for the drying process at 40^0 C in a hot air oven. The final product of purified guggul was kept in an air-tight container for evaluation [10]

Method of Preparation of fluids used for guggul purification

Triphalakasaya (Decoction):

In this decoction process, take a coarse powdered of amala, behead, and hirda and mixed uniformly in equal quantity. The mixture was known as Triphala was transferred to an extraction vessel and mixed thoroughly. The mixture is allowed to stand for 12 hours. Then gentle heating was maintained till the drug-water mixture got reduce to half of its original volume. After this mixture was cooled at room temperature and bulk marc was allowed to settle down. Though, the residue of the mixture was wrapped in nylon cloth and washed with potable water. Further, repeated the procedure and get Triphalakasaya.

Vasapatrakasaya:

In the vasapatrakasaya procedure, taken leaves of adhatoda vasica were washed and cut into small pieces. Further, it is transferred in an extraction vessel along with portable water and mixed thoroughly. Gentle heating was carried and maintain the temperature till the concentration of drug water reduced to half of its original volume. The heating was stopped and the mixture was stood for cooling. The strained mixture then allows filtering through nylon cloth to get 'vasakasaya'.

Gomutra (Cow's urine):

In this procedure, collected fresh and uncontaminated urine samples were in a sterile container of fresh cow and immediately used for the guggul purification after filtration [11].

Qualitative and Quantitative Study

Determination of Foreign Matter

For the determination of the foreign matter, accurately weigh 100 gm. of sample and spread it as a thin layer. Inspected the foreign matter with help of the eye or by the microscopic lens at 6x. Separated the foreign matter and calculate its percentage.

Determination of Total ash

The total ash was calculated by incineration of sample in silica dish at 450 ^{o} c. Ashless filter paper was used for the collection of residue. Further, it gets incinerate and collects the total ash. Calculate its percentage.

Total Ash (% w/w) = W_3-W_1/ W_2-W_1 X 100
Where, W_1 = Weight of crucible (g), W_2 = Weight of crucible + test sample (g), W_3 = Weight of crucible + Residue (g)

Determination of acid-insoluble ash

An acid insoluble ash value was determined, by taking dilute hydrochloric acid and ash. Further, boil and collect the insoluble matter in a Gooch crucible or ashless filter paper and thoroughly wash with hot water and heated at a constant weight. Calculate its percentage concerning the air-dried compound.

Acid Insoluble Ash (% w/w) = W_3-W_1/ W_2-W_1 X 100
Where, W_1 = Weight of crucible (g), W_2 = Weight of crucible + test sample (g), W_3 = Weight of crucible + Residue (g)

Determination of Alcohol Soluble Extractive

The alcohol soluble extractive value was determined by maceration of an air-dried sample of guggul with little quantity of alcohol in the conical flask for one day. The process of maceration is carried by shaking the flask in between six hours and allow to stand for sixteen hours. Further, collect the filtered and proceed for the drying at 105 ^{o} c. weighing the extract and calculate the value.

Alcohol Soluble Extractive (% w/w) = (W3 – W1). (50) / (W2- W1). (25) X 100
Where, W_1 = Weight of Evaporating dish (g), W_2 = Weight of Evaporating dish + test sample (g) W_3 = Weight of Evaporating dish + Residue (g)

Determination of Water-Soluble Extractive

The water-soluble extractive value was estimated by the macerated coarse powder of guggul with a few volumes of chloroform for twenty-four hours. Further, the flask was shaken for six hours and allowed to

stand for sixteen hours. Filter off the solution and avoid the loss of solvent, evaporation process was performed and dried at 105 ° C.

Alcohol Soluble Extractive (% w/w) = (W3 – W1). (50) / (W2- W1). (25) X 100
Where, W_1 = Weight of Evaporating dish (g), W_2 = Weight of Evaporating dish + test sample (g) W_3 = Weight of Evaporating dish + Residue (g)

Determination of Moisture Content (LOD)

Weigh a glass stopper weighing bottle (A) that has dried and cooled in the desiccator. Transfer to the bottle 1 g of sample (B), cover it, and accurately weigh the bottle and the content. Distribute the sample as evenly as practicable. Place the loaded bottle on the oven at 105^0 C, remove the stopper and leave it also in the oven. Further, the completion of the drying process, the residue was kept at room temperature in a desiccator. Weigh the bottle and the content to a constant weight (C). Calculate the % w/w loss on drying.

% w/w LOD = (A + B) – C / (B) X 100
Where, A = Weight of weighing bottle (g), B = Weight of sample (g), C = weight of weighing bottle + Residue (g)

Determination of Volatile oil content

The procedure to determine volatile oil was carried by using a distillation flask. A suitable quantity of coarse powder was added with 75 mL of glycerin and 175 mL of water in a 1 L distillation flask. Also, added few pieces of porous earthenware and single filter paper with 15 cm in small strips with 7 to 12 mm wider. Equipped the distillation apparatus with a receiver, rubber tubes, condenser along with a tap of inlet and outlet water. The flask is rotated occasionally to wash down any material that adheres to its sides. After 3-4 hours of heating, the apparatus is allowed to cool for 10 min and the tap is opened and the tubes are lowered slowly for the oil to completely enters into the graduated part of the receiver, as the tap is closed and read the volume. Further, the procedure is continued until successive readings of the volatile oil do not differ [12, 13].

High Performance Thin Layer Chromatography (HPTLC)

The samples were applied using Linomat IV (CAMAG) semi-automatic applicator as a form of 7 mm long bands prewashed with methanol. 10 cm x 10 cm, 200 µm layered silica gel 60 F254 coated aluminum HPTLC plate (Merck) using the stream of neutral gas (Nitrogen) at a constant rate of 150 nl/s. Since the standardization of raw and purified guggul samples 20 and 40 µg/spot were applied to the plate at wavelength 256 nm, 366 nm, and daylight for the before and after derivatization. After the application of the sample, plates were developed in CAMAG Automatic Developing Chamber 2 with the mobile phase of Petroleum Ether: Ethyl Acetate (3:1) solution. The chamber was developed by saturated mobile 20 ml for 20 min, and few quantities of mobile phase were allowed to run in the chamber at 70 mm distance. on the plate at room temperature under a controlled moist state (55–75% with NaCl solution). The bands were scanned in CAMAG TLC scanner-3 (Wincat 1.4.1 software) [14]

High Performance Liquid chromatography (HPLC)

20 µg/ml Raw & Purified guggul were injected with help of a Rheodyne injector (20 µl) to estimate the concentration of GS-Z. HPLC analysis was performed on a rapid separation system equipped with LC-2010 pump (low-pressure gradient mode), C_{18} G5mµm column, degasser, and a UV/VIS detector. The separation of GS-Z was done using a mobile phase acetonitrile: water (70:30 v/v) was maintained at a constant flow rate (1.0 ml/min) and column temperature (25^0 C). The data of spectra were collected at detection wavelength 251 nm (LC- 2010 UV detector with Deuterium D2 lamp) and data acquirement was performed by LC- Solution software version 1.25 [15].

Microbial Analysis

Microbial assay carried out for the determination of microbial growth in raw and purified guggul [16]. There was done a disc plate method to determine total aerobic microbial and yeast & mold count and also particular test for specified microorganisms such as Escherichia coli, Salmonella, Pseudomonas aeruginosa, Staphylococcus aureus, etc.

Heavy Metal Determination

The presence of heavy metals above than limit may affect the normal functioning of the health [17]. So far, testing the presence and amount of heavy metals like lead, arsenic, and mercury is important. Inductive Coupled Plasma-Optical Emission Spectrometer was used to determine heavy metal in the raw and purified guggul.

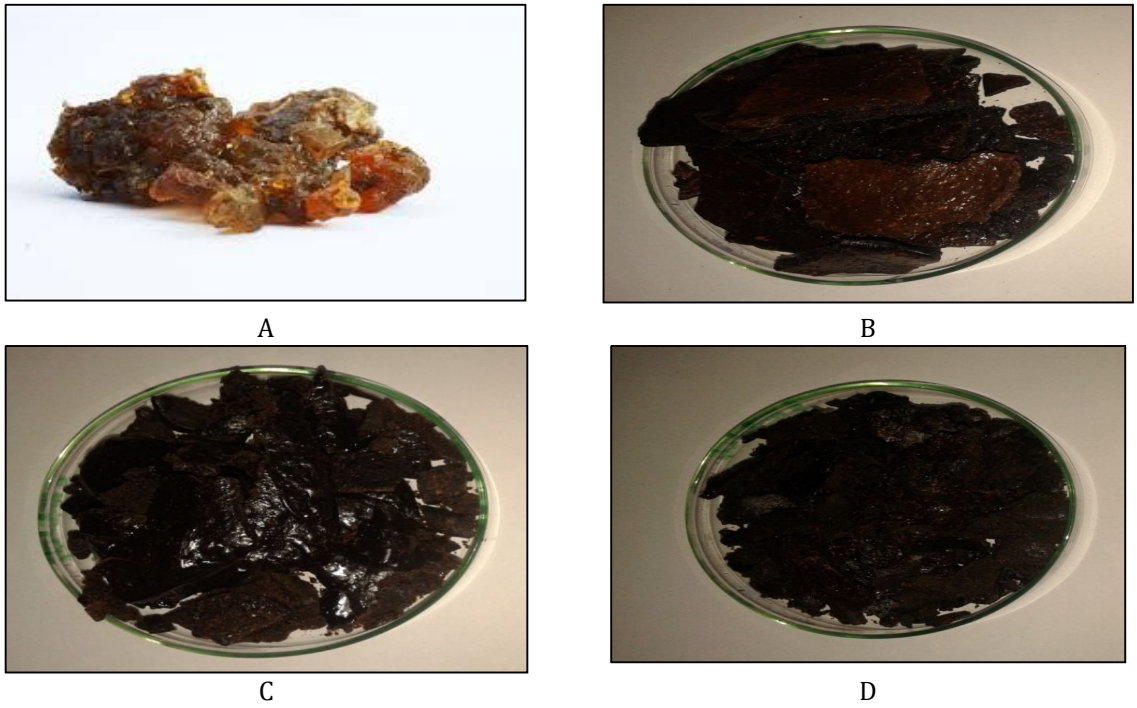
RESULT AND DISCUSSION

Macroscopic characters

The whole guggul was used to evaluate morphological parameters. The observations for color, odor, taste, and size were noted and are tabulated in **Table 1**. Similarly, before purification and after purification of SG were also evaluated organoleptically **[Figure 1].**

Table 1: Macroscopical / Organoleptic characteristics

Macroscopic characters	Before Purification	After Purification		
	RG A	TSG B	VSG C	GSG D
Colour	Multi-coloured	Brown coloured	Brown Coloured	Dark Brown Coloured
Odour	Agreeable, Aromatic, Balsamic	Aromatic	Aromatic	Aromatic
Taste	Characteristic bitter	Bitter & Astringent	Bitter & Astringent	Bitter & Astringent
Size	1.00 to 2.50 cm in diameter	1.00 to 2.20 cm in diameter	0.50 to 1.50 cm in diameter	1.00 to 2.00 cm in diameter



A B C D

Figure 1 Diagrammatic View of **A:** RG **B:** TSG **C:** VSG **D:** GSG

The result of the macroscopic evaluation, guggul before and after purification by Triphala, Vasaka, and Gomutra has excellent color, odor, and taste (Table 1) as compared to raw guggul.

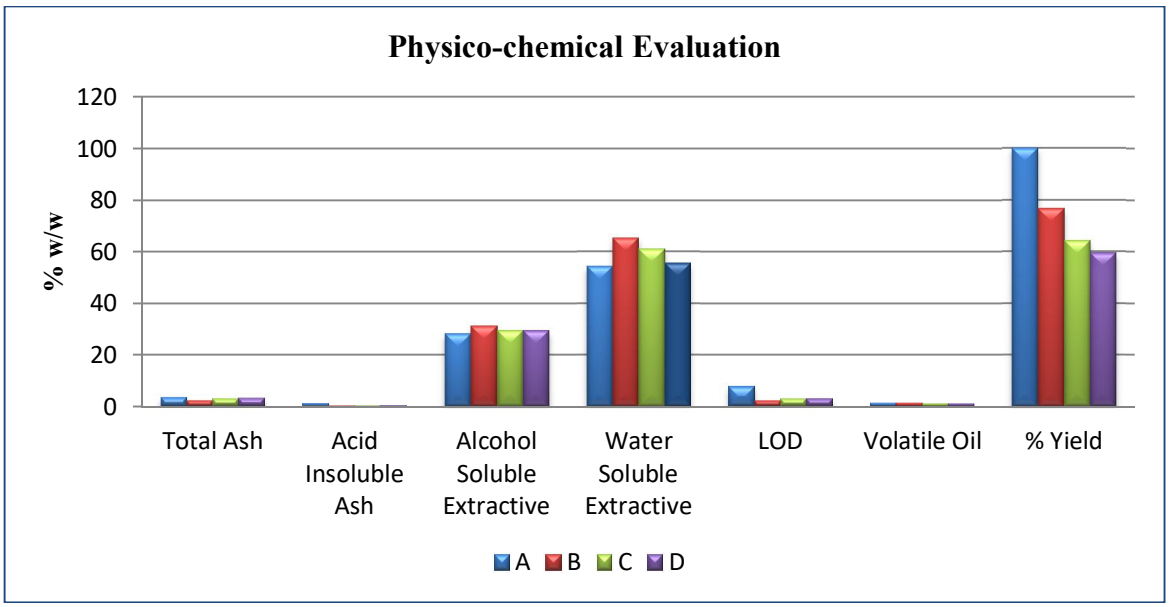
Physico-chemical Evaluation

Physico-chemical evaluation of raw guggul is compared the after purified guggul.

Table 2: Physico-chemical Evaluation of raw guggul and purified guggul

Physico-chemical Parameter	Before Purification	After Purification			Average purification (after)	Inference
	Standard	RG	TSG	VSG	GSG	
Foreign Matter %(w/w)	NMT 4%	3.41	***	***	***	***	Compliance
Total Ash % (w/w)	NMT 5%	3.38	2.21	2.89	3.17	2.75	Compliance
Acid Insoluble Ash % (w/w)	NMT 1%	0.89	0.12	0.23	0.29	0.21	Compliance
Alcohol Soluble Extractive %(w/w)	NLT 27%	28.22	31.24	29.58	29.44	30.08	Compliance
Water Soluble Extractive%(w/w)	NLT 53%	54.21	65.28	60.98	55.52	60.59	Compliance
LOD %(w/w)	NMT 8 %	7.58	2.13	2.89	3.01	2.67	Compliance
Volatile Oil %(v/w)	NLT 1 %	1.25	1.13	0.99	0.99	1.03	Compliance
% Yield	NA	100	76.53	64.28	59.48	66.76	Sufficient

NMT: Not More Than NLT: Not Less Than ***: Absent NA: Not Applicable



A: RG **B:** TSG **C:** VSG **D:** GSG

Figure 2 Comparison of analysis of Raw Guggul vs. Purified Guggul by Different Method

The physicochemical characteristic of raw guggul and after purification was compared. After purification guggul shows a good result as compared to raw guggul.

High Performance Thin Layer Chromatography (HPTLC)

Before Derivatization

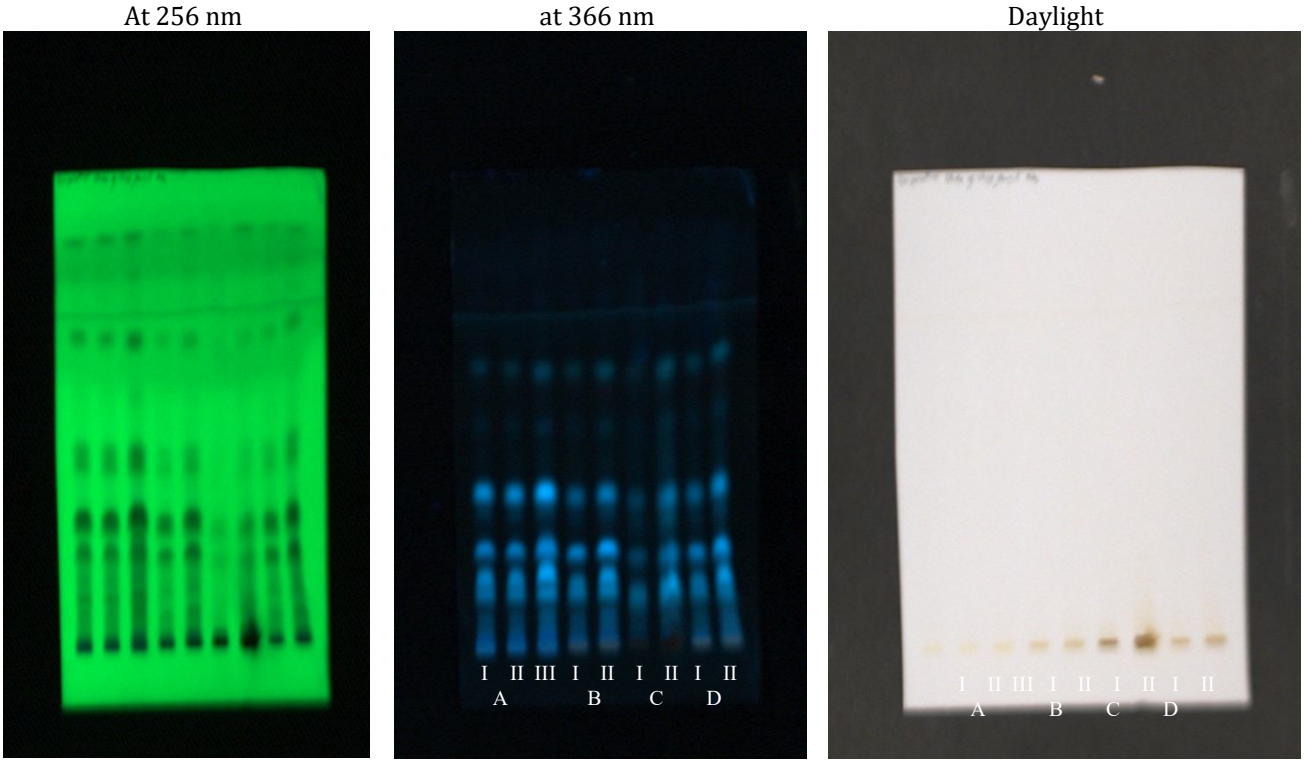


Figure 3: Represent HPTLC Plate (Before Derivatization)

After Derivatization:

At 256 nm | at 366 nm | Daylight

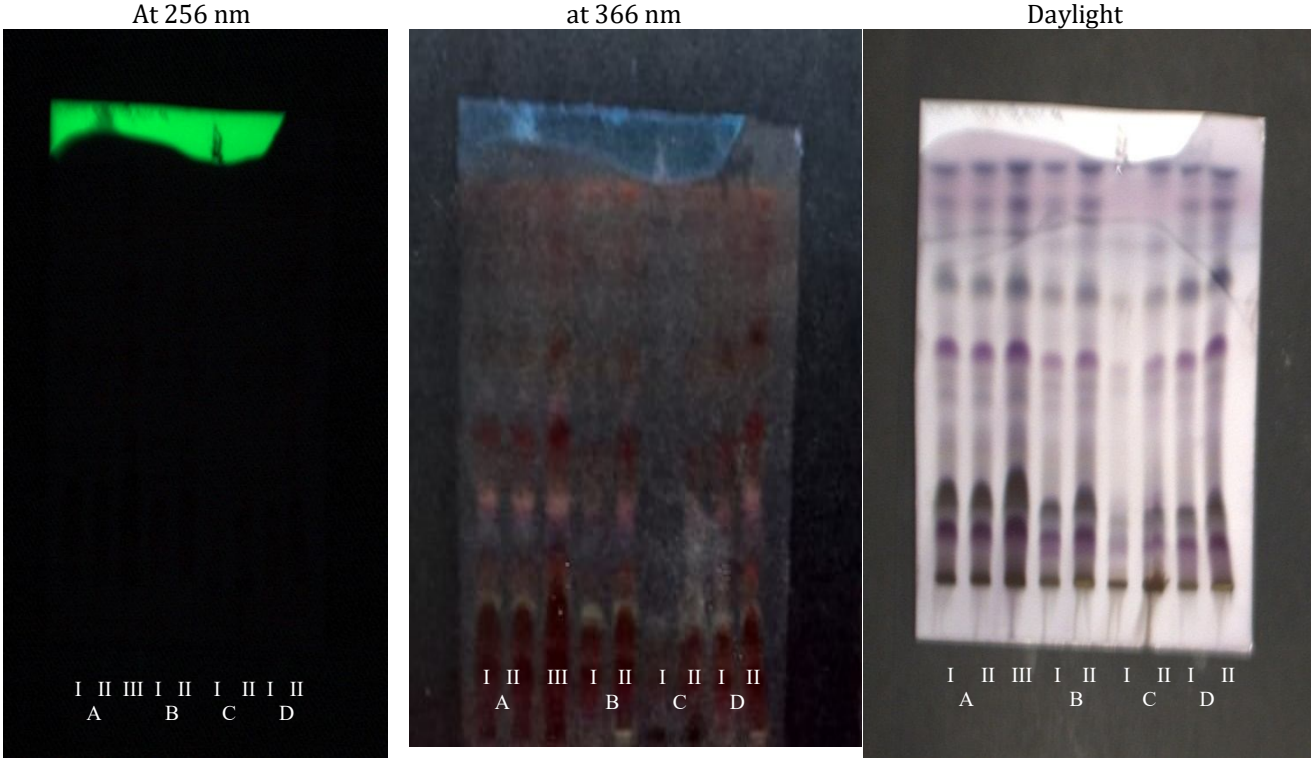


Fig 4 : Represent HPTLC Plate (After derivatization)

Densitogram diagram: (3D)

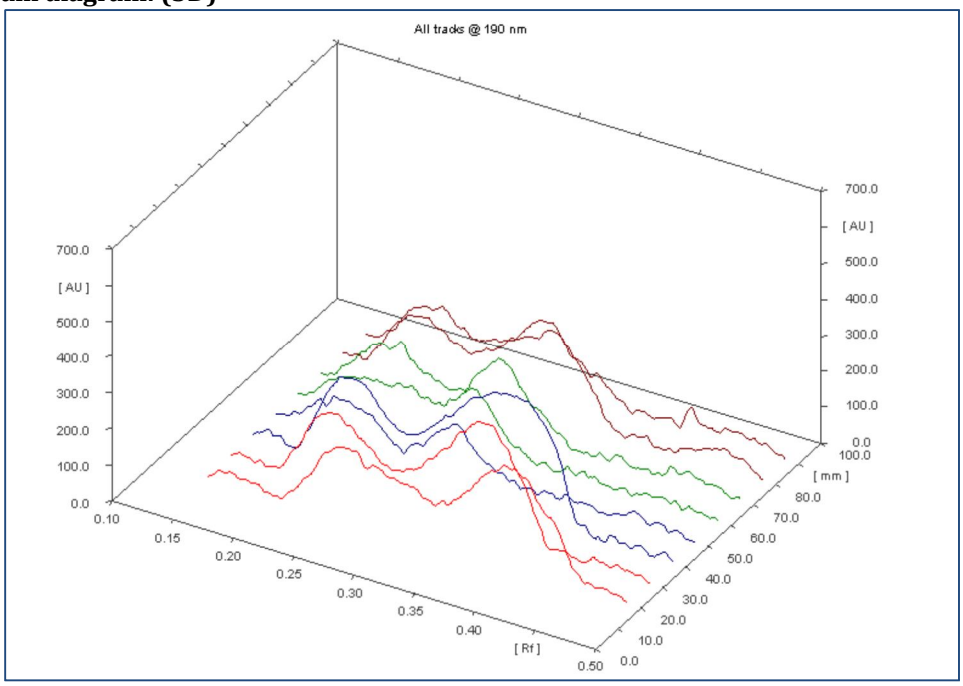


Figure 5: Densitogram of raw and Purified Gugggul (3D View)

Comparitive study of Guggul purification 02.cna* winCATS

Track	Scan	Integrate	Sample ID	Color
1	✓		raw guggul	
2	✓	✓	raw guggul	
3	✓	✓	raw guggul	
4	✓	✓	triphala guggul	
5	✓	✓	triphala guggul	
6	✓	✓	vasaka guggul	
7	✓	✓	vasaka guggul	
8	✓	✓	gomutra guggul	
9	✓	✓	gomutra guggul	

Figure 6 : Interpretation of Densitogram diagram

Table 10: R_f value and Calculated Amount of Phytoconstituents (HPTLC) of Extract

Comparitive study of Guggul purification 02.cna*

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
2	1	0.14 Rf	3.7 AU	0.16 Rf	29.1 AU	4.24 %	0.19 Rf	3.8 AU	550.9 AU	2.30 %
2	2	0.20 Rf	1.0 AU	0.26 Rf	91.9 AU	7.99 %	0.27 Rf	3.4 AU	5625.7 AU	3.48 %
2	3	0.28 Rf	57.0 AU	0.28 Rf	66.4 AU	4.26 %	0.32 Rf	7.6 AU	4227.0 AU	7.64 %
2	4	0.34 Rf	21.5 AU	0.39 Rf	76.0 AU	0.25 %	0.45 Rf	8.5 AU	3308.9 AU	5.55 %
2	5	0.46 Rf	16.3 AU	0.47 Rf	22.3 AU	3.25 %	0.49 Rf	2.1 AU	246.9 AU	1.03 %
3	1	0.18 Rf	6.5 AU	0.23 Rf	03.0 AU	5.76 %	0.27 Rf	2.9 AU	7562.1 AU	3.84 %
3	2	0.29 Rf	13.2 AU	0.35 Rf	00.4 AU	2.92 %	0.41 Rf	9.8 AU	4174.3 AU	3.43 %
3	3	0.41 Rf	14.5 AU	0.42 Rf	17.9 AU	3.15 %	0.43 Rf	0.0 AU	152.0 AU	0.68 %
3	4	0.44 Rf	1.9 AU	0.44 Rf	23.2 AU	4.09 %	0.45 Rf	0.9 AU	185.9 AU	0.83 %
3	5	0.46 Rf	9.2 AU	0.46 Rf	23.1 AU	4.07 %	0.49 Rf	0.2 AU	272.9 AU	1.22 %
4	1	0.15 Rf	10.2 AU	0.16 Rf	27.9 AU	4.25 %	0.17 Rf	2.6 AU	261.5 AU	0.86 %
4	2	0.18 Rf	4.5 AU	0.23 Rf	38.4 AU	6.23 %	0.26 Rf	3.1 AU	8618.8 AU	8.44 %
4	3	0.27 Rf	31.5 AU	0.35 Rf	26.6 AU	9.64 %	0.41 Rf	6.5 AU	1005.6 AU	9.32 %
4	4	0.43 Rf	7.6 AU	0.44 Rf	24.4 AU	3.70 %	0.45 Rf	4.7 AU	185.1 AU	0.61 %
4	5	0.45 Rf	4.1 AU	0.46 Rf	19.2 AU	2.92 %	0.47 Rf	0.9 AU	128.1 AU	0.42 %
4	6	0.48 Rf	4.1 AU	0.46 Rf	21.5 AU	3.26 %	0.48 Rf	0.9 AU	101.9 AU	0.34 %
5	1	0.16 Rf	21.8 AU	0.19 Rf	98.0 AU	9.73 %	0.24 Rf	7.1 AU	3394.5 AU	4.00 %
5	2	0.26 Rf	34.9 AU	0.29 Rf	24.8 AU	7.86 %	0.36 Rf	4.1 AU	3332.7 AU	3.19 %
5	3	0.36 Rf	0.0 AU	0.37 Rf	15.1 AU	4.58 %	0.37 Rf	3.8 AU	98.9 AU	1.28 %
5	4	0.38 Rf	4.6 AU	0.38 Rf	22.8 AU	6.91 %	0.39 Rf	2.7 AU	153.6 AU	1.99 %
5	5	0.40 Rf	14.6 AU	0.40 Rf	14.6 AU	4.44 %	0.41 Rf	2.3 AU	106.3 AU	1.38 %
5	6	0.42 Rf	0.3 AU	0.44 Rf	32.2 AU	9.76 %	0.45 Rf	3.3 AU	378.5 AU	4.91 %
5	7	0.45 Rf	10.6 AU	0.46 Rf	22.1 AU	6.72 %	0.48 Rf	0.0 AU	251.1 AU	3.25 %
6	1	0.15 Rf	14.3 AU	0.21 Rf	03.2 AU	2.48 %	0.22 Rf	7.3 AU	3165.6 AU	0.50 %
6	2	0.22 Rf	96.9 AU	0.24 Rf	15.7 AU	5.21 %	0.26 Rf	3.5 AU	2640.6 AU	5.44 %
6	3	0.27 Rf	11.9 AU	0.29 Rf	60.4 AU	4.95 %	0.32 Rf	9.0 AU	3692.6 AU	5.58 %
6	4	0.34 Rf	4.3 AU	0.35 Rf	19.6 AU	4.27 %	0.36 Rf	3.7 AU	101.0 AU	0.97 %
6	5	0.36 Rf	4.3 AU	0.38 Rf	26.0 AU	5.67 %	0.39 Rf	7.1 AU	352.3 AU	3.39 %
6	6	0.40 Rf	1.5 AU	0.41 Rf	12.8 AU	2.79 %	0.42 Rf	0.9 AU	68.8 AU	0.66 %
6	7	0.42 Rf	4.6 AU	0.44 Rf	21.3 AU	4.63 %	0.46 Rf	2.5 AU	358.0 AU	3.45 %
7	1	0.15 Rf	8.6 AU	0.19 Rf	38.0 AU	5.17 %	0.20 Rf	4.2 AU	2489.9 AU	9.80 %
7	2	0.20 Rf	26.7 AU	0.21 Rf	56.8 AU	8.61 %	0.25 Rf	1.4 AU	3209.8 AU	5.53 %
7	3	0.26 Rf	68.4 AU	0.29 Rf	90.6 AU	4.76 %	0.34 Rf	3.2 AU	5823.3 AU	6.32 %
7	4	0.36 Rf	5.0 AU	0.39 Rf	24.3 AU	4.43 %	0.40 Rf	2.2 AU	234.4 AU	1.86 %
7	5	0.43 Rf	4.5 AU	0.45 Rf	38.5 AU	7.02 %	0.48 Rf	2.0 AU	815.9 AU	6.49 %
8	1	0.16 Rf	3.9 AU	0.21 Rf	73.1 AU	4.92 %	0.23 Rf	5.3 AU	5687.3 AU	1.55 %
8	2	0.26 Rf	29.8 AU	0.32 Rf	42.0 AU	8.80 %	0.37 Rf	2.8 AU	0638.4 AU	9.02 %
8	3	0.38 Rf	0.0 AU	0.39 Rf	24.8 AU	5.00 %	0.40 Rf	3.8 AU	225.3 AU	1.25 %
8	4	0.42 Rf	2.9 AU	0.46 Rf	55.9 AU	1.27 %	0.49 Rf	1.4 AU	1475.0 AU	8.18 %
9	1	0.15 Rf	3.8 AU	0.20 Rf	39.1 AU	1.01 %	0.23 Rf	5.7 AU	4611.4 AU	4.33 %
9	2	0.25 Rf	92.0 AU	0.30 Rf	88.7 AU	2.05 %	0.35 Rf	4.2 AU	7328.3 AU	4.55 %
9	3	0.38 Rf	6.5 AU	0.40 Rf	19.5 AU	4.34 %	0.40 Rf	0.3 AU	145.4 AU	1.08 %
9	4	0.40 Rf	3.9 AU	0.41 Rf	64.1 AU	4.28 %	0.43 Rf	9.1 AU	663.8 AU	4.94 %
9	5	0.44 Rf	20.1 AU	0.44 Rf	37.3 AU	8.32 %	0.47 Rf	2.5 AU	684.7 AU	5.10 %

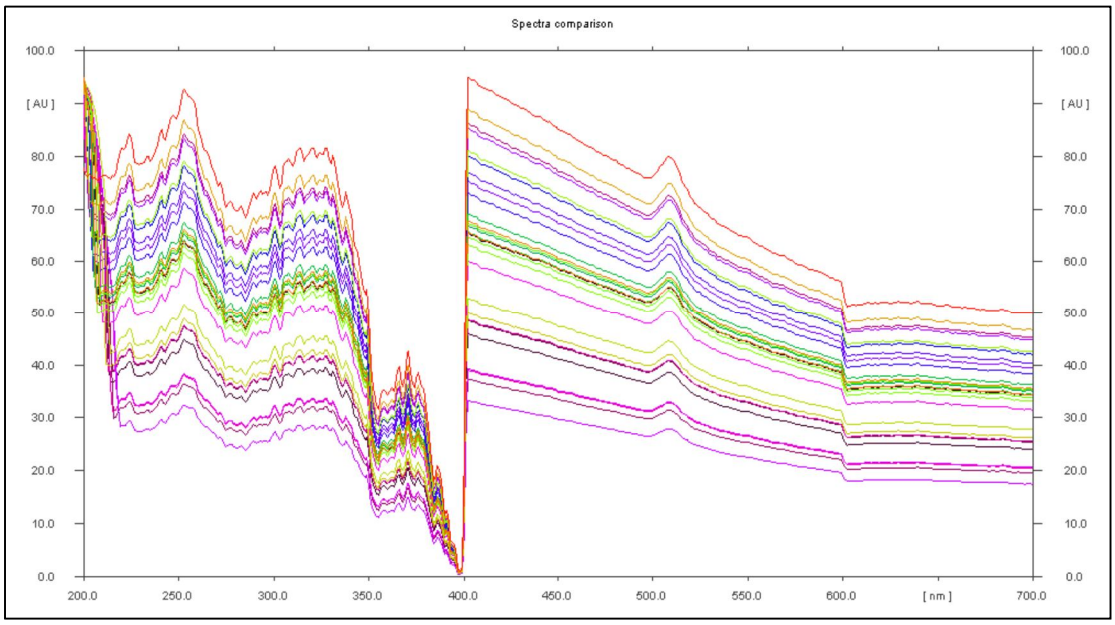


Figure 7 : Comparison Spectra of raw and Purified Guggul

Table 3 : Interpretation of Comparison Spectra

Comparitive study of Guggul purification 02.cna* winCAT

Track	Rf	Assigned Substance	Max. Signal	Display
2	0.16	AutoGenerated1	I Neg.abs. (-2910 AU)	
2	0.26	AutoGenerated4	I Neg.abs. (-2910 AU)	
2	0.28	AutoGenerated3	I Neg.abs. (-2910 AU)	
2	0.39	AutoGenerated2	I Neg.abs. (-2910 AU)	
2	0.47	AutoGenerated8	I Neg.abs. (-2910 AU)	
3	0.23	AutoGenerated4	I Neg.abs. (-2910 AU)	
3	0.35	AutoGenerated2	I Neg.abs. (-2910 AU)	
3	0.42	AutoGenerated13	I Neg.abs. (-2910 AU)	
3	0.44	AutoGenerated8	I Neg.abs. (-2910 AU)	
3	0.46	AutoGenerated10	I Neg.abs. (-2910 AU)	
4	0.16	AutoGenerated1	I Neg.abs. (-2910 AU)	
4	0.23	AutoGenerated4	I Neg.abs. (-2910 AU)	
4	0.35	AutoGenerated2	I Neg.abs. (-2910 AU)	
4	0.44	AutoGenerated8	I Neg.abs. (-2910 AU)	
4	0.46	AutoGenerated10	I Neg.abs. (-2910 AU)	
4	0.46	AutoGenerated10	I Neg.abs. (-2910 AU)	
5	0.19	AutoGenerated4	I Neg.abs. (-2910 AU)	
5	0.29	AutoGenerated3	I Neg.abs. (-2910 AU)	
5	0.37	AutoGenerated2	I Neg.abs. (-2910 AU)	
5	0.38	AutoGenerated11	I Neg.abs. (-2910 AU)	
5	0.40	AutoGenerated7	I Neg.abs. (-2910 AU)	
5	0.44	AutoGenerated8	I Neg.abs. (-2910 AU)	
5	0.46	AutoGenerated10	I Neg.abs. (-2910 AU)	
6	0.21	AutoGenerated4	I Neg.abs. (-2910 AU)	
6	0.24	AutoGenerated6	I Neg.abs. (-2910 AU)	
6	0.29	AutoGenerated3	I Neg.abs. (-2910 AU)	
6	0.35	AutoGenerated12	I Neg.abs. (-2910 AU)	
6	0.38	AutoGenerated2	I Neg.abs. (-2910 AU)	
6	0.41	AutoGenerated7	I Neg.abs. (-2910 AU)	
6	0.44	AutoGenerated8	I Neg.abs. (-2910 AU)	
7	0.19	AutoGenerated5	I Neg.abs. (-2910 AU)	
7	0.21	AutoGenerated4	I Neg.abs. (-2910 AU)	
7	0.29	AutoGenerated3	I Neg.abs. (-2910 AU)	
7	0.39	AutoGenerated2	I Neg.abs. (-2910 AU)	
7	0.45	AutoGenerated8	I Neg.abs. (-2910 AU)	
8	0.21	AutoGenerated4	I Neg.abs. (-2910 AU)	
8	0.32	AutoGenerated3	I Neg.abs. (-2910 AU)	
8	0.39	AutoGenerated2	I Neg.abs. (-2910 AU)	
8	0.46	AutoGenerated8	I Neg.abs. (-2910 AU)	
9	0.20	AutoGenerated4	I Neg.abs. (-2910 AU)	
9	0.30	AutoGenerated3	I Neg.abs. (-2910 AU)	
9	0.40	AutoGenerated2	I Neg.abs. (-2910 AU)	
9	0.41	AutoGenerated7	I Neg.abs. (-2910 AU)	
9	0.44	AutoGenerated8	I Neg.abs. (-2910 AU)	

HPTLC study standardized the raw and purified guggul at Rf value 0.19 to 0.49 at detection wavelength 256 nm, 366 nm, and daylight out, of which only one Rf will match with either wavelength depicted in Table 3. The band was scanned in CAMAG TLC scanner-3 (Wincat 1.4.1 software) which indicated the zone of raw and purified guggul whereas, shows the same distance traveled as compared with a raw and purified guggul. There were no significant changes observed in comparison with the extract which depicted Fig 6 & 7.

High Performance Liquid Chromatography (HPLC)
Estimation of GS-Z by HPLC

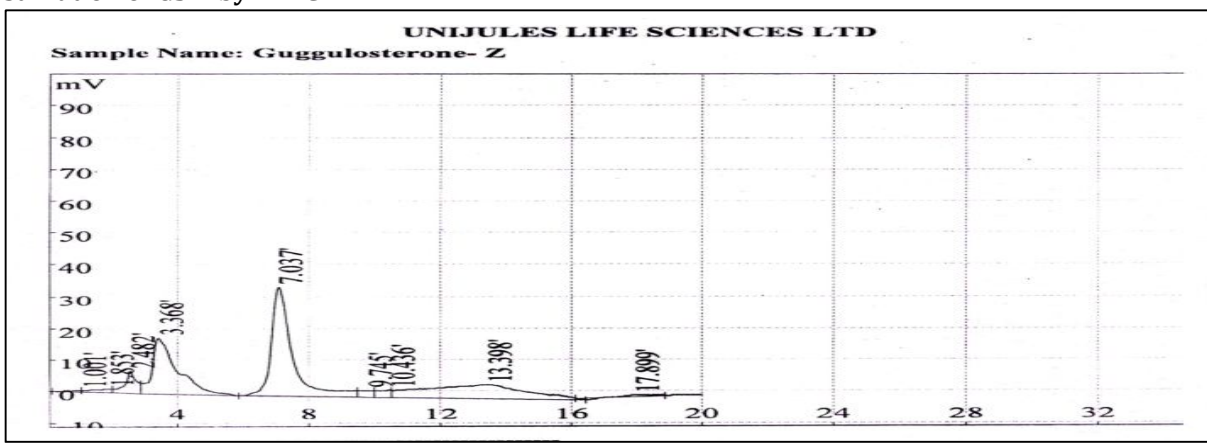


Figure 8 : HPLC Chromatogram of RG

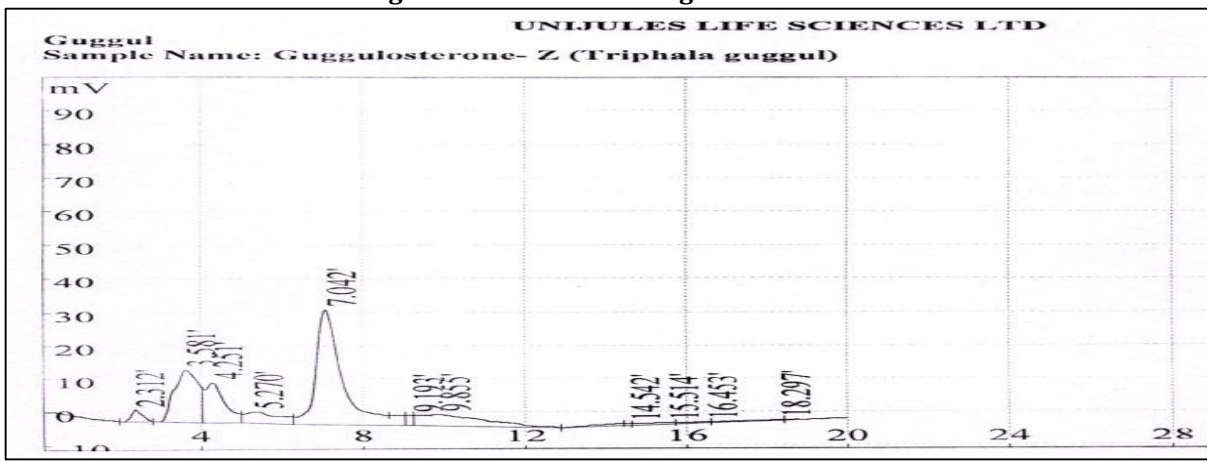


Figure 9 : HPLC Chromatogram of TSG

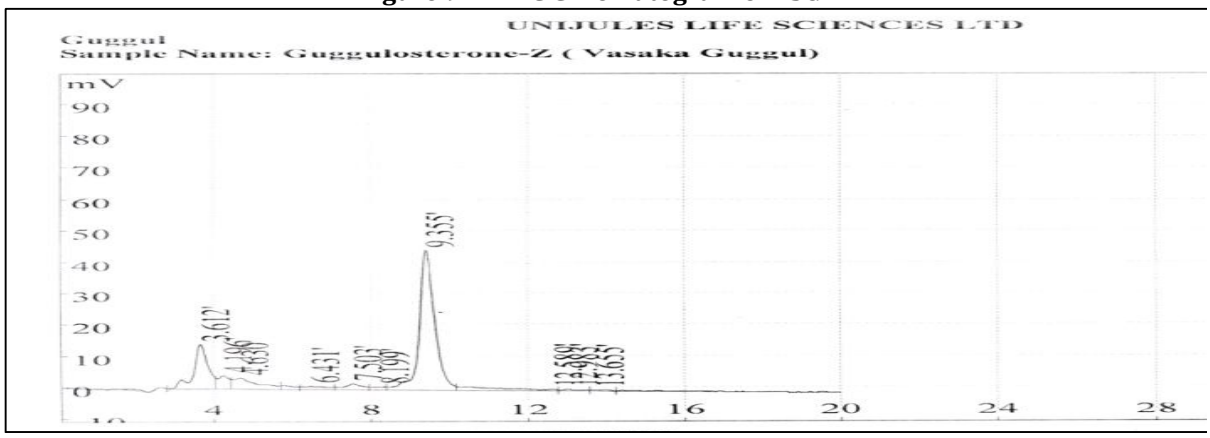


Figure 10: HPLC Chromatogram of VSG

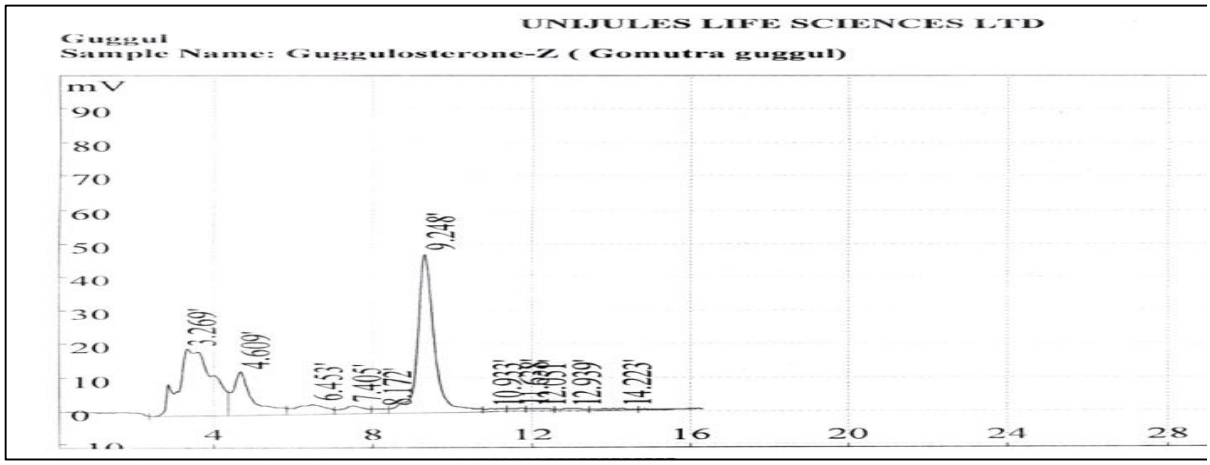
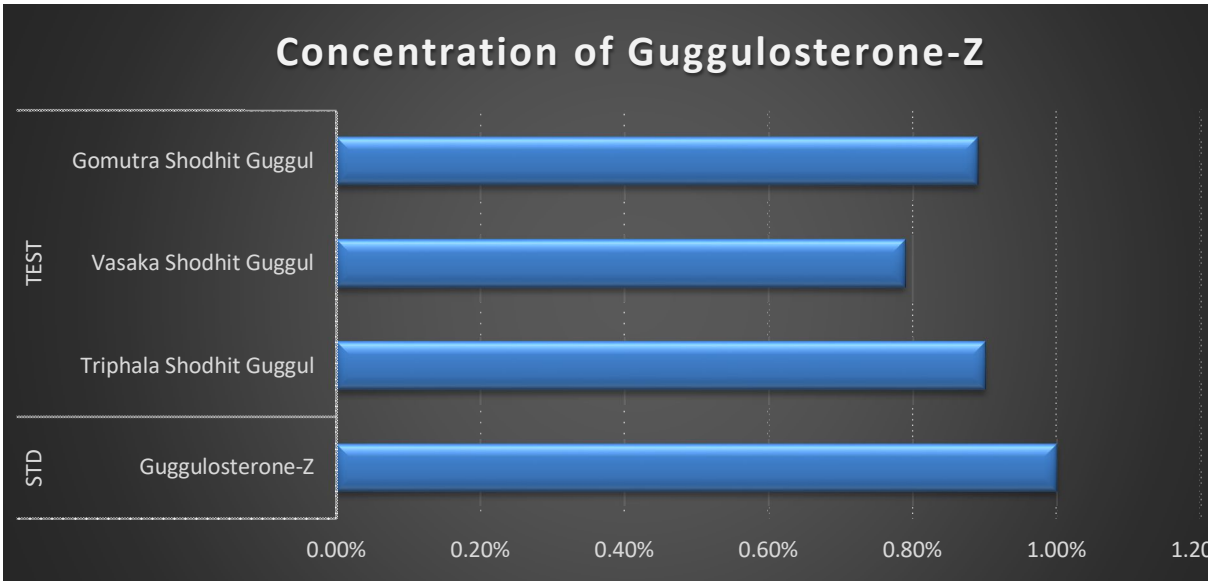


Figure 11: HPLC Chromatogram of GSG

Table 4: Concentration of GS –Z after Purification

Concentration of GS –Z	STD	After Purification (Test)			After Purification Average	Inference
	GS-Z	TSG	VSG	GSG		
GS-Z %	1-2%	0.9%	0.79%	0.89%	0.86 %	Compliance



STD: Standard **Test: After Purification**

Figure 12 : Comparison of standard GS-Z and test GS-Z

HPLC method is precise and suitable for the estimation of the concentration of GS-Z. In this method, the separation of GS-Z was done using a mobile phase acetonitrile: water (70:30 v/v) was maintained at a constant flow rate (1.0 ml/min) and column temperature (25^0 C). The data of spectra were collected at detection wavelength 251 nm (LC- 2010 UV detector with Deuterium D2 lamp). As shown in Fig 8, 9, 10 & 11, the chromatogram has a sharp Gaussian peak of GS-Z of RG, TSG, VSG & GSG was obtained at retention times 7.037, 7.042, 9.355 & 9.248 respectively. From Table 4, indicated that percentage concentration of GS-Z in TSG, VSG & GSG were 0.9%, 0.79% & 0.89% respectively. Figure no. 8, 9, 10, 11 shows the no degradation observed in the raw and purified guggul and which is confirmed by the same distance traveled by all the bands and thus the nearly same Retention time.

Microbial Analysis

Table 5: Microbial analysis of Raw & Purified Guggul

Test	Guggul Sample (cfu/g)					Inference
	RG	TSG	VSG	GSG	Limits	
Total Aerobic Microbial/ Viable Count	$84.5x10^1$	$61x10^1$	$63.5x10^1$	$68x10^1$	NMT- 10^5	Passed
Total Yeast and Mould Count	$14x10^1$	$11x10^1$	$11.5x10^1$	$10x10^1$	NMT- 10^3	Passed
Staphylococcus aureus/g	***	***	***	***	***	NA
Salmonela sp./g	***	***	***	***	***	NA
Pseudomonas aeruginosa/g	***	***	***	***	***	NA
Escherichia coli	***	***	***	***	***	NA

NMT: Not More Than ***: Absent NA: Not Applicable

Microbial analyses of raw and purified guggul were carried for different microbes. There were not crossed the limit and all the guggul samples passed the test.

Test for Heavy Metals

Table 6: Heavy Metal Analysis of raw & Purified Guggul

Test	Result (in ppm)				Limits	Inference
	RG	TSG	VSG	GSG		
Arsenic	0.06	###	0.02	###	NMT 3.0 ppm	Compliance
Lead	###	###	###	###	NMT 10 ppm	Compliance
Cadmium	###	###	2	###	NMT 0.3 ppm	Non Compliance
Mercury	###	###	###	###	NMT 1.0 ppm	Compliance

###: Not detected

All guggul sample was performed the heavy metal test such as arsenic, lead, cadmium, and mercury. Only VSG has shown a higher amount of cadmium, but the remaining RG, TSG & GSG were compliances with the heavy metal test.

CONCLUSION

The pharmacognostic and physicochemical evaluation of RG was obtainable but yet not enough evidence to evaluate on three SG. According to WHO guidelines, the present research work was performed to standardized shudha guggul within the parameters. In this study, the raw guggul was purified using Triphala, Vasaka, and Gomutra. Also, investigate physicochemical parameters of different SG to analyze their quality, safety, and standardization for their safe use. Further, scrutinize raw and purified guggul by using chromatography techniques which shows excellent separation and percentile of guggulsterone and also microbial growth and heavy metal impurities not seen in SG. This study suggests that the TSG gives a better yield and extractive values than VSG and GSG.

ABBREVIATION

SG: Shodhit Guggul RG: Raw Guggul TSG: Triphala Shodhit Guggul VSG: Vasaka Shodhit Guggul GSG: Gomutra Shodhit Guggul GS: Guggulsterone

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CONFLICT OF INTEREST

The authors stated No Conflict of Interest for the Publication of this research article in the Journal.

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