

## Method development for isolation and purification of Z-Guggulsterone, Dihydroguggulsterone, and Progesterone from guggul resin using RP-HPLC

Rohit Singh,<sup>a,b,c</sup> Astbhuja Mishra,<sup>a,b</sup> Ramanand Prajapati,<sup>a,d</sup> and T. Narender<sup>a,b</sup>

<sup>a</sup>Medicinal & Process Chemistry, CSIR-Central Drug Research Institute, Lucknow (U.P)-226031, India

<sup>b</sup>Academy of Scientific & Innovative Research (AcSIR), Ghaziabad (U.P)-201002, India

<sup>c</sup>Department of Pharmaceutical Chemistry, College of Pharmacy, JSS Academy of Technical Education, C-1/A, Sector-62, Noida, (U.P)-201309, India

<sup>d</sup>Chemistry Innovation Research Center, Jubilant Biosys Ltd, Greater Noida, Uttar Pradesh-201310, India

Email: [t\\_narendra@cdri.res.in](mailto:t_narendra@cdri.res.in)

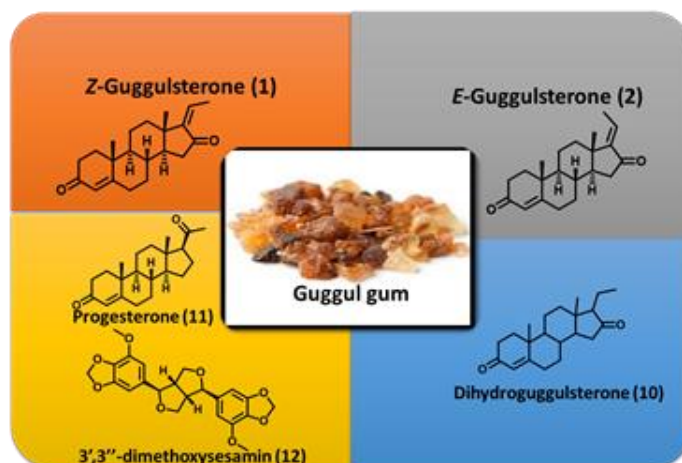
Received 10-10-2022

Accepted Manuscript 06-19-2023

Published online 07-11-2023

### Abstract

Over thousands of years, oleogum resin (also known as guggul) from the *Commiphora mukul* tree, found in India, Bangladesh, and Pakistan, has been used to treat hypercholesterolemia, atherosclerosis, rheumatism, and obesity. The bioactive ingredient responsible for guggul's medicinal properties has been discovered as guggulsterone isolated from guggul. In the present work, *Commiphora mukul* components were obtained using bioactivity-guided fractionation. *C. mukul* exudates were extracted with ethyl alcohol, and the extract was subjected to column chromatography. Z-guggulsterone, dihydroguggulsterone, and progesterone were isolated by semi-preparative RP-HPLC. On the contrary, we isolated a mixture of E-guggulsterone and 3',3''-dimethoxysesamin from the fraction comprising the main components of different Sterones. <sup>1</sup>H, <sup>13</sup>C NMR, and LC-MS confirmed the compound's structure.



**Keywords:** Guggul, Guggulsterone, oleogum resin, HPLC, *Commiphora mukul*

Arkivoc 2023 (vi) 202211902

DOI: <https://doi.org/10.24820/ark.5550190.p011.902>

Page 1 of 12

©AUTHOR(S)

## Introduction

Plants have long been used as a source of medicine, spanning back to prehistoric times. Plants have been used to fight illness and maintain health by communities worldwide for ages as they grow naturally around us. According to the World Health Organization (WHO), traditional medicine is used by up to 80% of the world's population for primary health care.<sup>1</sup> Oleogum resin (also known as guggul) from the *Commiphora mukul* tree belongs to the family Burseraceae, found in India, Bangladesh, and Pakistan. Guggul has a long history dating back to 1700 BC.<sup>2</sup> According to the Sushrut Samhita, an ancient medical and surgical literature, guggul has been used to cure a variety of ailments, including hypercholesterolemia, atherosclerosis, rheumatism, obesity, Internal tumors, malignant sores, liver malfunction, intestinal worms, leucoderma, sinus, and edema when taken orally.<sup>3,4</sup> Guggul was initially introduced to the scientific community in 1966 by G. V. Satyavati, an Indian medical researcher.<sup>5</sup> In recent years, significant progress has been made in unraveling the molecular mechanisms accountable for the various pharmacological actions of guggul.

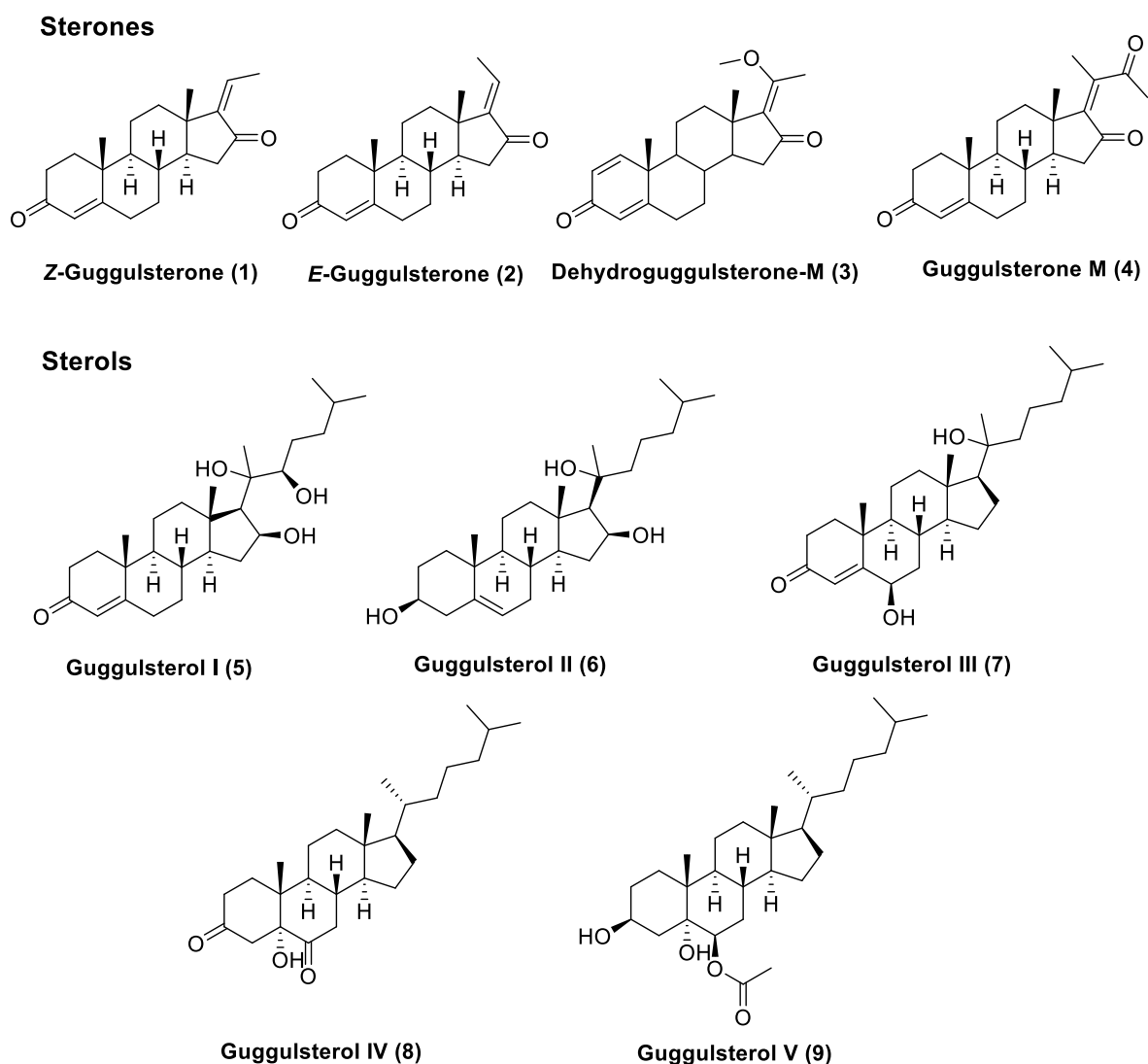
Guggul is oleogum resin in its dried form, typically extracted from the bark of the guggul plant. It comprises a complex mixture of compounds, including higher alcohols, various plant sterols, esters, and steroid hormones, which constitute around 6.1%, the gum around 29.3%, and resin 61%.<sup>6</sup> The critical bioactive ingredient for guggul's medicinal benefits has been discovered as guggulsterone extracted from guggul. The gum resin has been used to isolate numerous steroidal components.<sup>7</sup> Guggulsterone, on the other hand, comes in steroidal, interconvertible isomeric forms *E* and *Z* based on the two different three-dimensional configurations of CH<sub>3</sub> at position C<sub>20</sub>. These two isomeric forms are the result of the inhibited rotation about the carbon-carbon double bonds at positions C<sub>17</sub> and C<sub>20</sub>.<sup>8</sup> *E*-guggulsterone (2) (Figure 1), *Z*-guggulsterone (1) (Figure 1), dehydroguggulsterone-M (3) (Figure 1), Guggulsterone M (4) (Figure 1), guggulsterol-I (5) (Figure 1), guggulsterol-II (6) (Figure 1), guggulsterol-III (7) (Figure 1), guggulsterol-IV (8) (Figure 1), guggulsterol-V (9) are the major constituents isolated from the oleogum resin.<sup>9,10</sup> 4-pregnene-3,16-dione, a steroid similar to progesterone, has also been isolated. It also contains ferrulates, lignans, and flavanones, among several other components.<sup>11</sup>

The exposure to light, temperature, packing, length of storage, climatic circumstances under which the plants are cultivated, and the method of harvesting all affect how much Guggulsterone *E* & *Z* are present.<sup>12,13</sup> Since the pharmaceutical and perfume industries are becoming increasingly interested in this wonder plant and placing enormous pressure on its natural wild populations, the genetic diversity of this species is in jeopardy. Therefore, it is crucial to accurately and consistently quantify the bioactive components in *C. mukul* natural populations in order to identify them.

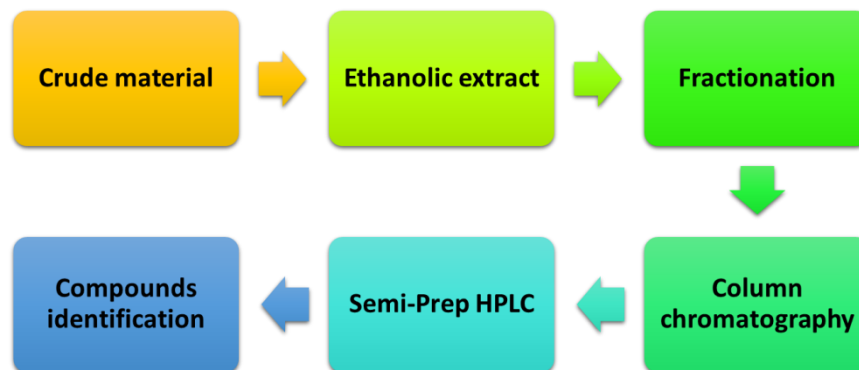
Several analytical techniques are listed in the literature for quantifying the markers mentioned above, including liquid chromatography, high performance thin-layer chromatography (HPTLC), and Liquid chromatography–mass spectrometry (LCMS). Recently Kulhari *et al.* used HPTLC to evaluate the amount of guggulsterone in 11 samples of *C. wightii*.<sup>14</sup> To quantify *E* and *Z* stereoisomers in *C. mukul* oleogum resin exudates, Mesorb *et al.* validated a gradient High performance liquid chromatography (HPLC) approach.<sup>15</sup> Using HPLC, Soni *et al.* estimated the amount of guggulsterone isomers in guggul resin.<sup>16</sup> Guggulsterone values were determined using reverse phase HPLC (RP-HPLC) by Dass and Ramawat in *C. wightii* cell and callus cultures.<sup>17</sup> After administering a single dose (50 mg/kg), Verma *et al.* and Singh *et al.* simultaneously quantified the two isomeric forms of guggulsterone by HPLC in the serum of rats.<sup>18,19</sup> The concentration of *E* (Rf 0.38) and *Z* (Rf 0.46) guggulsterone in pharmaceutical dose forms was evaluated by Agrawal *et al.*<sup>20</sup> *E* and *Z* guggulsterones are the only recognized marker compounds for the qualitative and quantitative analysis of raw guggul and its completed products that have been employed in previously developed HPLC and Ultra Performance Liquid

Chromatography (UPLC) techniques for guggul.<sup>21,22</sup> For the simultaneous estimation of *E*- and *Z*-guggulsterone isomers (an antihyperlipidemic medication) in rabbit plasma, Bhatta *et al.* devised a sensitive and focused liquid chromatography/tandem mass spectrometric technique.<sup>23</sup>

Guggul has a complex combination of secondary metabolites, as previously stated. As a result, there is a need to have systematic and dependable access to as many of these compounds as feasible, in pure form, to enable further exploration of their biological activities. We've devised a reproducible technique for isolating, purifying, and identifying the critical guggul ingredients to achieve this goal. Our method adapts current technology to traditional phytochemical methods of medicinal plant analysis at its basis. A qualitative HPLC method designed and optimized to lead the isolation/purification process from start to finish is a crucial component of the approach. In the current study, a novel LC-MS approach for confirming guggulsterone (*E* & *Z*), dihydroguggulsterone, progesterone, and an HPLC method quantifying guggulsterone (*E* & *Z*), dihydroguggulsterone, and progesterone have been established. Figure 2 depicts a schematic illustration of this method.



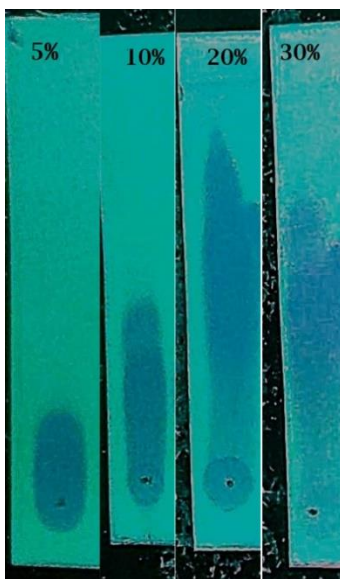
**Figure 1.** Major chemical constituents of *Commiphora mukul* (guggul).



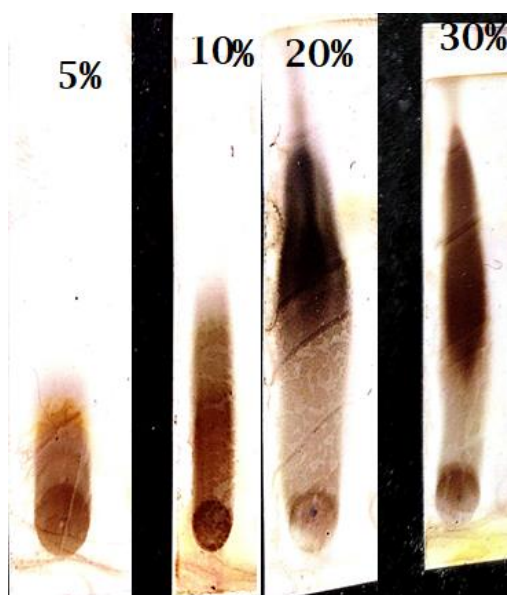
**Figure 2.** Schematic diagram of the workflow of the HPLC-guided isolation of guggulsterone and related compounds from *Commiphora mukul*.

## Results and Discussion

Since sample preparation is a critical element in the development of analytical techniques, an extraction protocol was validated during the method development process before the tuning of chromatographic parameters. Samples of gum resin from *C. mukul* were extracted using various solvents, temperatures, and extraction times. Under the optimal extraction methodology, the gum resin samples were extracted four times using ethanol at room temperature, with a 24-hour interval between each extraction. The ethanolic fraction was thus chosen for further isolation procedures. Repeated column chromatography of an aliquot of the ethanolic fraction was run using various solvent mixtures with varying compositions. This resulted in 6 sub-fractions whose thin layer chromatography (TLC) and HPLC fingerprints was aligned to generate a 'roadmap' to aid subsequent HPLC purification of the principal constituent(s) present in the fraction. Of the six significant subfractions, 30% ethyl acetate (EtOAc)-hexane fraction demonstrates the presence of major constituents. The 30% ethyl acetate-hexane enriched fraction was then run using the different solvent systems to standardize its TLC pattern. We decided that EtOAc: Hexane was the best suitable among the various solvent systems. Then we finally ran the TLC in 4 varying concentrations of EtOAc: Hexane (5%, 10%, 20%, and 30%) solvent systems. TLC visualization was carried out under ultraviolet (UV) lighting, and 10% sulphuric acid in methanol was sprayed on the TLC plate for charring.



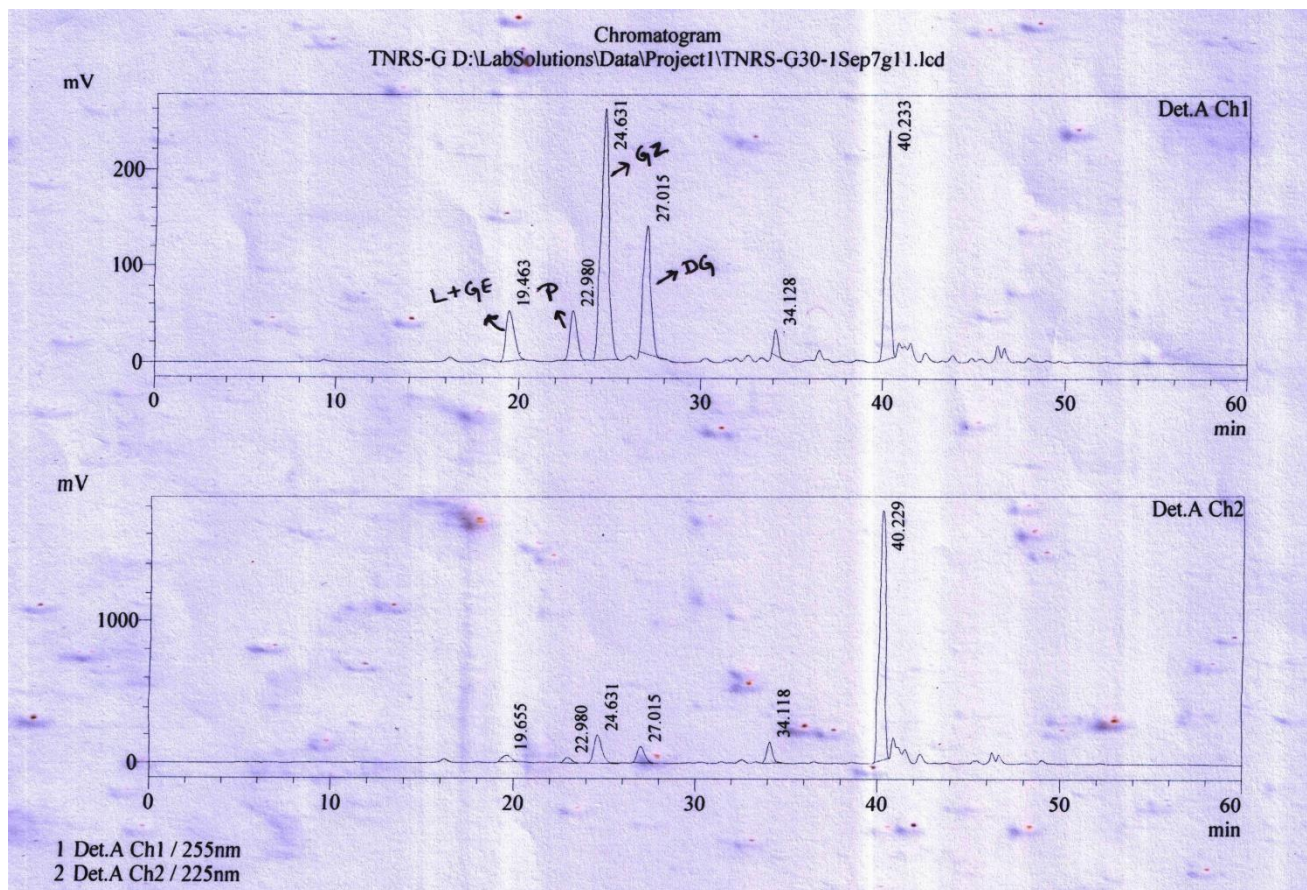
**Figure 3.** TLC fingerprints of the enriched fraction of *Commiphora mukul* in 5, 10, 20, and 30% EtOAc: Hexane solvent system (UV visualization).



**Figure 4.** TLC fingerprints of the enriched fraction of *Commiphora mukul* in 5, 10, 20, and 30% EtOAc: Hexane solvent system (10% sulphuric acid in methanol).

The HPLC process was optimized to produce a precise, reliable, and rapid method for evaluating *E*- and *Z*-guggulsterones and other related substances. Various solvent systems, C18 columns, and column temperatures have been adjusted to choose the best chromatographic conditions. Different solvent systems were used to run the standard guggulsterones mixture (which contains *E*- and *Z*-isomers), Dihydroguggulsterone, Progesterone, and 3',3''-dimethoxysesamin. At first, variable ratios of water-acetonitrile (both in 0.1% formic acid) were tested to enhance resolution and reduce analysis time, and roughly 30 different gradient systems were evaluated. We employed a binary gradient system for the mobile phase containing eluents A and B (0.03% and 0.1% formic acid in the water) and acetonitrile (0.05% and 0.2% formic acid,

respectively). Still, we were unable to separate the individual components from the mixture. Also, we attempted to optimize the elution procedure utilizing mobile phase, a binary gradient system made up of eluents A and B (0.03%, 0.1% trifluoroacetic acid in the water, and 0.05%, 0.2% trifluoroacetic acid in acetonitrile, respectively); however, the appropriate separation was not successful. In addition to the previously mentioned parameters, we ran the experiment with mobile phase, a binary gradient system of eluents A (0.03%, 0.1% acetic acid in water) and B (0.05%, 0.2% acetic acid in acetonitrile). Still, we were unable to get the expected results. Using a methanol-water system was also considered employing the above acidic conditions in varying compositions using formic acid, trifluoroacetic acid, and acetic acid. Still, it was not helpful because of excessive pressure. In contrast to the acidic acetonitrile-water system, it was found that integrating different gradient systems of acetonitrile and water without formic acid produced sharp, symmetrical peaks with better resolution. To get the best resolution, different run times were also tried. At first, we tried a 10-minute run time, but no elution was detected. Then, we increased the run time to 25 minutes, but the proper separation was still not obtained. Next, we increased the run time to 40 minutes, but some peaks were merging during this time, which prevented the pure compound's isolation. Finally, we increased the run time to 60 minutes and obtained proper separation. The gradient program: 0.0–20% B from 0.01–5 min, 20–40% B from 5–15 min, 40–60% B from 15–30 min, 60–90% B from 30–45 min, 90–100% B from 45–60 min, nevertheless, utilizing this gradient program, proper elution and separation were not accomplished. To achieve the best separation and elution, we altered the gradient program to read 0.8–60% B from 0.01–10 min, 60–90% B from 10–20 min, 90–90% B from 20–50 min, 90–45% B from 50–55 min, 45% B from 55–60 min. The experiments mentioned above were carried out at various column temperatures ranging from 35 °C to 40 °C utilizing two different types of reversed-phase C18 columns, including Phenomenex Luna Omega Polar C18 (150 x 4.6 mm; 5 µm) and Phenomenex Luna Omega PS C18 (150 x 4.6 mm; 5 µm). The Luna Omega Polar C18 (150 x 4.6mm; 5 µm) column operating at 35 °C produced better results. HPLC fingerprinting is a crucial part of the “roadmap” because it provides an initial general profile of the extract used to separate specific compounds from their appropriate sub-fractions and confirm their identity after separation. The peak at retention time 19.463 was identified as *E*-guggulsterone and 3',3''-dimethoxysesamin mixture, the peak at retention time 22.980 were identified as progesterone, the peak at retention time 24.631 was identified as *Z*-guggulsterone and the peak at retention time 27.015 was identified as dihydroguggulsterone with the help of the chromatograms.



**Figure 5.** HPLC profiles of enriched fractions at two different wavelengths, i.e., 225 and 255 nm (Note- L+GE: 3',3''-dimethoxysesamin and *E*-Guggulsterone isomer; P: Progesterone; GZ: *Z*-Guggulsterone isomer; DG: Dihydroguggulsterone).

## Conclusions

To summarize, we developed a reproducible HPLC-guided isolation/purification technique that is quick and easier to use by individuals with less experience to address this difficulty. The process was applied to guggul resin, a substance that contains a complex profile of several secondary metabolites that may have therapeutic significance. Three different compounds were isolated in varied quantities: *Z*-guggulsterone, Dihydroguggulsterone, and Progesterone; *E*-guggulsterone and 3',3''-dimethoxysesamin were isolated as a mixture from this plant and are being reported. In our lab, the isolated and purified chemicals are employed as biomarkers for creating quantitative analytical standards and leads for biological assessment. The reported method has also been employed with various medicinal plants under study in our lab.

## Experimental Section

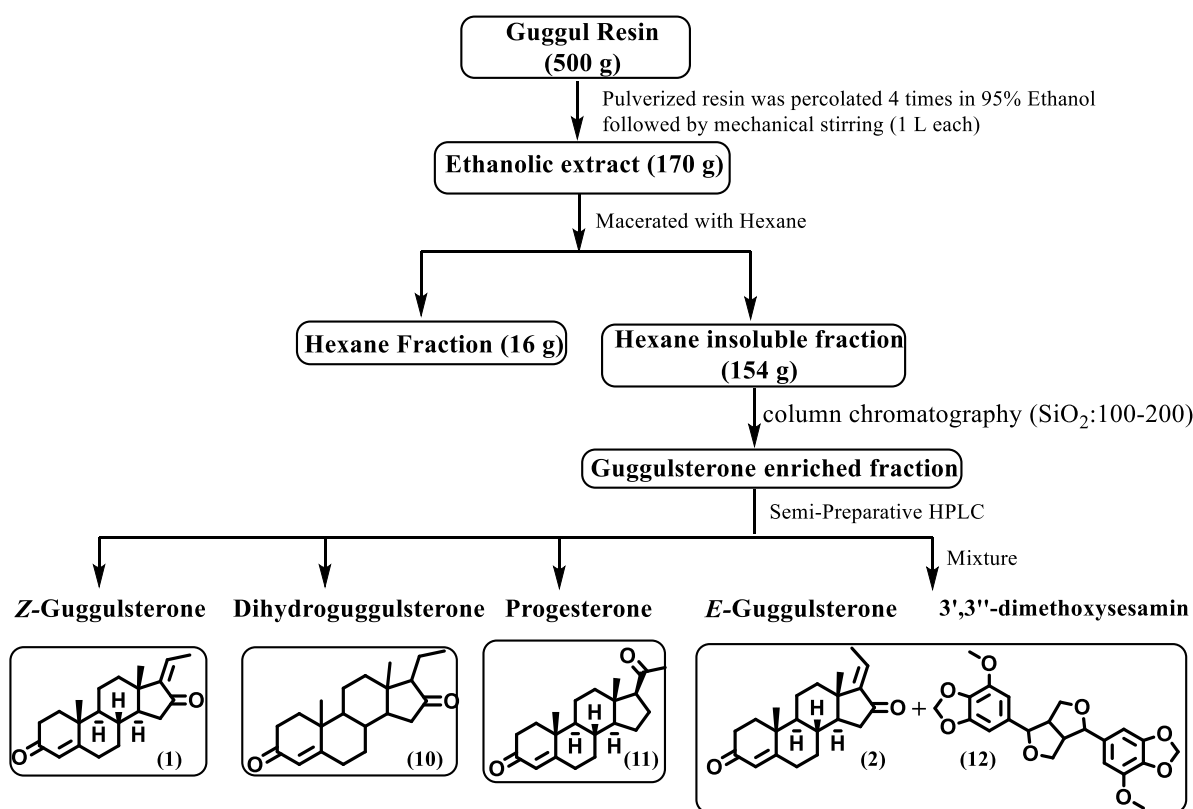
**General.** Acetonitrile, methanol, and Milli Q water, all organic solvents of HPLC grade, were purchased from Merck Pvt. Ltd. in Mumbai, India. Silica gel 60 GF254 (Merck) was used to make plates for TLC, which were then activated by drying for two hours at 100 °C. Silica gel (100-200 mesh) was used for chromatography, and ethyl

acetate, methanol, and hexane combinations were used as eluents. Under UV light, visualization was carried out. Deuterated chloroform ( $\text{CDCl}_3$ ) was used to run  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra on the Bruker Advance DPX 500MHz and 100MHz. Chemical shifts are quantified using internal standards Tetramethylsilane (TMS) and are expressed as ppm values relative to  $\text{CDCl}_3$ . LC-MS experiments were performed on Waters triple quadrupole mass spectrometer coupled with an H-class alliance HPLC system via interfaced with an electrospray ionization source (Waters Co., Milford, MA, USA). The Sunfire C18 (250 x 4.6 mm, 5 $\mu\text{m}$ ) column was used for chromatographic separation. High-purity  $\text{N}_2$  was used as the nebulizing and drying gas. Positive and negative ionization modes were used to detect MS with a full scan ( $m/z$  100-1000). OpenLynx Software was used to collect and compare the data.

Semi-preparative HPLC (Shimadzu) was used with a Photodiode Array Detector (PDA). To acquire the chromatogram, the PDA was configured by optimizing wavelength to produce the best response for all samples at 255nm and 225 nm.

The resin (guggul resin) of *Commiphora mukul* was procured from a local market in Lucknow, U.P., India, and authenticated by the CSIR-Central Drug Research Institute Botany Division. A glass percolator containing 500 g of pulverized resin and 1 L of 95 percent ethanol was left to stand for 24 hours while being periodically stirred by a mechanical stirrer at room temperature. These steps were repeated four times after the percolates had been collected. An extract was prepared by evaporating the combined percolates at 50 °C and reducing pressure. The weight of the extract was found to be 170 g.

For the isolation of the compounds, the mentioned below protocol was used (Scheme 1):



**Scheme 1.** Flow chart for the isolation of Compounds from guggul resin.



2 g of the hexane insoluble fraction was taken, and the slurry was prepared using 5 g of 100-200 mesh size silica. For column chromatography, the column dimension used was 45 x 3.2 cm. 100 g of silica (100-200 mesh) was used for column chromatography. Wet packing of the column chromatography was done using the suspension of silica 100-200 with 5% ethyl acetate in hexane in regular mode. The column was run successively with 10 %, 20 %, 30 %, and 40% ethyl acetate in hexane (1 L each). Finally, the column was eluted with pure methanol. The total weight of the different fractions obtained is indicated below:

- ✓ **10 % Ethyl acetate-hexane (1 L) - 189 mg**
- ✓ **20 % Ethyl acetate-hexane (1 L) - 220 mg**
- ✓ **30 % Ethyl acetate-hexane (1 L) - 484 mg**
- ✓ **30 % Ethyl acetate-hexane (1 L) - 490 mg**
- ✓ **40 % Ethyl acetate-hexane (1 L) - 229 mg**
- ✓ **100 % Methanol (500 ml) – 300 mg**

The NMR and LC-MS data of all the fractions were recorded. The data obtained were compared with reported *E*- and *Z*- Guggulsterones data. Among all 6 fractions, the fraction obtained by initially eluting the column with 30% ethyl acetate-hexane confirmed the presence of the desired guggulsterones and some other similar moieties. So, the fraction was used for further purification of compounds by HPLC. The production of the standardized fraction was reproducible in the second batch also.

The separation of different components from the enhanced fraction was accomplished using semi-preparative HPLC. The Luna Omega Polar C18 (150 x 4.6mm; 5  $\mu$ m) column operating at 35 °C was used for RP-HPLC. The 30% EtOAc: Hexane enriched fraction (100 mg) was accurately weighed, dissolved with 10 mL acetonitrile, and sonicated for 20 min. Before HPLC analysis, the enriched fraction was filtered with a 0.45- $\mu$ m nylon syringe filter. From the above stock solution, 20 $\mu$ L was repeatedly injected into the semi-preparative HPLC system. From the above-enriched fraction, the significant constituents isolated were *Z*- Guggulsterone (28 mg; RT: 24.768), Dihydroguggulsterone (18 mg; RT: 27.141), Progesterone (16 mg; RT: 22.721), and the mixture of *E*-Guggulsterone and 3',3''-dimethoxysesamin (23 mg; RT: 19.532 and 24.747) through semi-preparative RP-HPLC. The separation process to get pure *E*-Guggulsterone with semi-preparative HPLC is still underway.

The NMR spectral data of all the three pure compounds, i.e., *Z*-Guggulsterone,<sup>24,25</sup> Dihydroguggulsterone,<sup>24</sup> Progesterone (a hormone)<sup>26</sup> and a mixture of *E*-Guggulsterone,<sup>24,25</sup> and 3',3''-dimethoxysesamin,<sup>24</sup> in CDCl<sub>3</sub> combined with HPLC and LC-MS data were analyzed to confirm the purity and authenticity of the compounds. The HPLC Conditions used for the isolation of guggulsterones and other related compounds are mentioned below:

**Run Time: 60 minutes**

**Wavelength: 225 & 255 nm**

**Flow rate: 0.8 ml/min**

**Solvent system: Binary gradient (A: Water, B: Acetonitrile)**

S. No	Time (min)	Function	Solvent A (%)	Solvent B (%)
1	0.01	Start	99.2	0.8
2	0.02	Run	45	55
3	10	Run	40	60
4	20	Run	10	90
5	50	Run	10	90
6	55	Run	55	45
7	60	Stop	55	45

The isolated compounds are characterized by  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ , and LC-MS techniques. Physical data of the isolated compounds are:

**Z-Guggulsterone (1).** Light yellow crystalline powder; Mp 187-189 °C;  $^1\text{H-NMR}$  (501 MHz,  $\text{CDCl}_3$ )  $\delta$  5.72 (m, 2H), 2.42 (m, 2H), 2.32 (m, 1H), 2.23 (m, 1H), 2.06 (m, 4H), 1.86 (m, 2H), 1.72 (m, 3H), 1.57 (m, 3H), 1.39 (m, 2H), 1.23 (s, 3H), 1.10 (m, 2H), 0.97 (s, 3H).  $^{13}\text{C-NMR}$  (126 MHz,  $\text{CDCl}_3$ )  $\delta$  207.82, 199.27, 170.32, 147.89, 130.57, 124.21, 53.68, 49.07, 43.07, 39.33, 38.76, 35.57, 35.51, 34.68, 33.95, 32.65, 31.85, 20.72, 19.59, 17.40, 14.11 ppm. LC-MS  $m/z$   $[\text{M}+\text{H}]^+$  313.2, found 313.4, calcd for  $\text{C}_{21}\text{H}_{29}\text{O}_2$ .

**Dihydroguggulsterone (10).** White powder; Mp 181-183 °C;  $^1\text{H NMR}$  (501 MHz,  $\text{CDCl}_3$ )  $\delta$  5.75 (s, 1H), 2.42 (m, 3H), 2.29 (m, 2H), 2.01 (m, 1H), 1.96 (d,  $J$  12 Hz, 1H), 1.82 (m, 2H), 1.74 (m, 1H), 1.69 (m, 3H), 1.62 (br, 2H), 1.52 (m, 1H), 1.47 (m, 1H), 1.40 (m, 1H), 1.22 (d,  $J$  1.5 Hz, 3H), 1.12 (m, 2H), 1.04 (m, 3H), 0.75 (s, 3H).  $^{13}\text{C NMR}$  (126 MHz,  $\text{CDCl}_3$ )  $\delta$  218.71, 199.35, 170.36, 124.21, 65.22, 53.78, 50.04, 41.97, 38.74, 38.41, 37.94, 35.59, 34.73, 33.96, 32.67, 32.19, 20.57, 17.68, 17.43, 13.44 ppm. LC-MS  $m/z$   $[\text{M}+\text{H}]^+$  315.2, found 315.4, calcd for  $\text{C}_{21}\text{H}_{31}\text{O}_2$ .

**Progesterone (11).** White crystalline Powder; Mp 128-132 °C;  $^1\text{H NMR}$  (501 MHz,  $\text{CDCl}_3$ )  $\delta$  5.74 (s, 1H), 2.53 (t,  $J$  9.0 Hz, 1H), 2.38 (m, 3H), 2.28 (m, 1H), 2.19 (m, 1H), 2.12 (s, 3H), 2.05 (m, 2H), 1.86 (m, 1H), 1.69 (m, 5H), 1.46 (t,  $J$  10.8 Hz, 2H), 1.27 (m, 2H), 1.19 (s, 3H), 1.03 (m, 2H), 0.67 (s, 3H).  $^{13}\text{C NMR}$  (101 MHz,  $\text{CDCl}_3$ )  $\delta$  209.22, 199.37, 170.88, 123.88, 63.45, 55.98, 53.60, 43.87, 38.62, 38.52, 35.67, 35.50, 33.89, 32.72, 31.85, 31.43, 24.31, 22.79, 20.97, 17.32, 13.28 ppm. LC-MS  $m/z$   $[\text{M}+\text{H}]^+$  315.2, found 315.4, calcd for  $\text{C}_{21}\text{H}_{31}\text{O}_2$ .

## Acknowledgments

The authors are thankful to the Director, CSIR-CDRI, for constant encouragement for the program on natural products of biological importance. This work was supported by the Council of Scientific and Industrial Research, New Delhi. The authors acknowledge SAIF-CDRI, Lucknow, for providing spectral data. Rohit Singh gratefully acknowledges the College of Pharmacy, Jagadguru Sri Shivarathreeshwara (JSS) Academy of Technical Education, Noida, Uttar Pradesh, India. This Manuscript has CSIR-CDRI Communication Number 10618

## Supplementary Material

Characterization data, including copies of  $^1\text{H}$ ,  $^{13}\text{C}$  NMR spectra, HPLC Chromatogram, and LC-MS spectra associated with this paper, can be found in the online version.

## References

1. Ekor, M., *Front. Pharmacol.* **2014**, *4*, 177-177.  
<https://doi.org/10.3389/fphar.2013.00177>
2. Shishodia, S.; Harikumar, K. B.; Dass, S.; Ramawat, K. G.; Aggarwal, B. B., *Anticancer Res.* **2008**, *28*, 3647-3664.
3. Deng, R., *Cardiovasc. Drug Rev.* **2007**, *25*, 375-390.  
<https://doi.org/10.1111/j.1527-3466.2007.00023.x>
4. Saxena, G.; Singh, S. P.; Pal, R.; Singh, S.; Pratap, R.; Nath, C., *Pharmacol. Biochem. Behav.* **2007**, *86*, 797-805.  
<https://doi.org/10.1016/j.pbb.2007.03.010>
5. Satyavati, G. V.; Dwarakanath, C.; Tripathi, S. N., *Indian J. Med. Res.* **1969**, *57*, 1950-1962.
6. Kulhari, A.; Sheorayan, A.; Chaudhury, A.; Sarkar, S.; Kalia, R. K., *Physiol. Mol. Biol. Plants.* **2015**, *21*, 71-81.  
<https://doi.org/10.1007/s12298-014-0271-1>
7. Sarup, P.; Bala, S.; Kamboj, S., *Scientifica (Cairo)* **2015**, *2015*, 138039-138039.  
<https://doi.org/10.1155/2015/138039>
8. Agrawal, H.; Kaul, N.; Paradkar, A. R.; Mahadik, K. R., *J. Pharm. Biomed. Anal.* **2004**, *36*, 23-31.  
<https://doi.org/10.1016/j.jpba.2004.04.014>
9. Patil, V. D.; Nayak, U. R.; Dev, S., *Tetrahedron* **1972**, *28*, 2341-2352.  
[https://doi.org/10.1016/S0040-4020\(01\)93577-X](https://doi.org/10.1016/S0040-4020(01)93577-X)
10. Bajaj, A. G.; Dev, S., *Tetrahedron* **1982**, *38*, 2949-2954.  
[https://doi.org/10.1016/0040-4020\(82\)85024-2](https://doi.org/10.1016/0040-4020(82)85024-2)
11. Zhu, N.; Rafi, M. M.; DiPaola, R. S.; Xin, J.; Chin, C.-K.; Badmaev, V.; Ghai, G.; Rosen, R. T.; Ho, C.-T., *Phytochemistry* **2001**, *56*, 723-727.  
[https://doi.org/10.1016/S0031-9422\(00\)00485-4](https://doi.org/10.1016/S0031-9422(00)00485-4)
12. Patel, M.; Carol, M.; Suhagia, B.; Acharya, S., *J. Nat. Remedies* **2017**, *17*, 96-113.  
<https://doi.org/10.18311/jnr/2017/18928>
13. Singh, S.; Manika, N.; Rk, V.; Bagchi, G. D., *Int. J. Phytomedicine* **2013**, *5*, 62-68.  
<https://doi.org/10.5138/ijpm.v5i1.844>
14. Kulhari, A.; Sheorayan, A.; Saxena, N.; Mohan, C.; Mangal, M.; Chaudhury, A.; Dhawan, A. K.; Kalia, R. K., *Genet. Resour. Crop Evol.* **2013**, *60*, 1173-1180.  
<https://doi.org/10.1007/s10722-012-9947-y>
15. Mesrob, B.; Nesbitt, C.; Misra, R.; Pandey, R. C., *J. Chromatogr. B. Biomed. Sci. Appl.* **1998**, *720*, 189-196.  
[https://doi.org/10.1016/S0378-4347\(98\)00433-2](https://doi.org/10.1016/S0378-4347(98)00433-2)
16. Soni, V.; Swarnkar, P. L.; Tyagi, V.; Pareek, L. K., *S. Afr. J. Bot.* **2010**, *76*, 421-424.  
<https://doi.org/10.1016/j.sajb.2009.10.004>
17. Dass, S.; Ramawat, K., *Nat. prod. radiance* **2009**, *8*, 532-536.
18. Verma, N.; Singh, S. K.; Gupta, R. C., *J. Chromatogr. B. Biomed. Sci. Appl.* **1998**, *708*, 243-248.  
[https://doi.org/10.1016/S0378-4347\(97\)00626-9](https://doi.org/10.1016/S0378-4347(97)00626-9)

19. Singh, S. K.; Verma, N.; Gupta, R. C., *J. Chromatogr. B. Biomed. Sci. Appl.* **1995**, *670*, 173-176.  
[https://doi.org/10.1016/0378-4347\(95\)00149-D](https://doi.org/10.1016/0378-4347(95)00149-D)
20. Agrawal, H.; Kaul, N.; Paradkar, A. R.; Mahadik, K. R., *J. Pharm. Biomed. Anal.* **2004**, *36*, 33-41.  
<https://doi.org/10.1016/j.jpba.2004.04.014>
21. Nagarajan, M.; Waszkuc, T. W.; Sun, J., *J. AOAC Int.* **2001**, *84*, 24-28.  
<https://doi.org/10.1093/jaoac/84.1.24>
22. Haque, I.; Kumar, M.; Mukhopadhyay, K., *Chromatographia* **2009**, *70*, 1613-1619.  
<https://doi.org/10.1365/s10337-009-1347-x>
23. Bhatta, R. S.; Kumar, D.; Chhonker, Y. S.; Jain, G. K., *Biomed. Chromatogr.* **2011**, *25*, 1054-1060.  
<https://doi.org/10.1002/bmc.1574>
24. Francis, J. A.; Raja, S. N.; Nair, M. G. *Chem. Biodivers.* **2004**, *1*, 1842-1853,  
<https://doi.org/10.1002/cbdv.200490138>.
25. Ham, J.; Chin, J.; Kang, H., *Molecules* **2011**, *16*, 4165-4171.  
<https://doi.org/10.3390/molecules16054165>
26. Budweg, S.; Junge, K.; Beller, M., *Chem. Commun.* **2019**, *55*, 14143-14146.  
<https://doi.org/10.1039/C9CC07337J>

This paper is an open access article distributed under the terms of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>)