

# HPLC Method for Chemical Fingerprinting of Guggul (*Commiphora wightii*) – Quantification of E- and Z-Guggulsterones and Detection of Possible Adulterants

## Authors

Rida Ahmed<sup>1,3,5</sup>, Yan-Hong Wang<sup>1</sup>, Zulfiqar Ali<sup>1</sup>, Troy J. Smillie<sup>1</sup>, Ikhlas A. Khan<sup>1,2,4</sup>

## Affiliations

The affiliations are listed at the end of the article

## Key words

- *Commiphora wightii*
- Burseraceae
- guggul
- HPLC
- quantitative analysis

## Abstract

A reversed-phase high-performance liquid chromatography method was developed for the chemical fingerprinting of *Commiphora wightii* gum resin (guggul). This method was also used for the quantification of E- and Z-guggulsterones in different *C. wightii* gum resin samples. The analysis was conducted on a high-performance liquid chromatography system with a reversed-phase column using a gradient elution comprised of

water and acetonitrile with 0.1% acetic acid, and a run time of 50 min at 38 °C. The calibration curve of E- and Z-guggulsterones showed good linearity with a standard correlation coefficient > 0.999, which is within the established range (0.5–250 µg/mL). Twelve marker compounds were selected and successfully analyzed by this method in 22 *C. wightii* gum resin samples along with 9 gum resin samples of possible adulterant species.

## Introduction

*Commiphora wightii* (Arn.) Bhandari (Burseraceae), syn. *Commiphora mukul* (Hook.) Engl., is an important medicinal plant which grows in the arid regions of Pakistan and India. Due to its significant pharmacological properties, the gum resin of *C. wightii* (guggul) has been extensively used in Asian indigenous medicines. Scientific studies have proven the significance of guggul as a hypolipidemic and hypoglycemic agent [1,2]. Several dietary supplements claiming to contain guggul extracts are commercially available in dosage forms of tablets and capsules. The demand of guggul has been increasing with the increasing trend of its use in botanicals in Europe, Japan, and North America.

*C. wightii* has been declared as a red-listed endangered plant species in Rajasthan state (India) because of its poor growth and unsustainable harvesting practices [1]. Currently, the world demand for guggul is largely met from Pakistan, with a reported serious source depletion. The supply of authentic guggul cannot meet the demand owing to its poor growth. This leads to the possibility of adulteration, either deliberately or accidentally. The probability of deliberate adulteration is more since there is gap in demand and supply. The availability of gum resins from different species of

*Commiphora* or other commonly available plants species in both Pakistan and India gives a further edge to this issue. As it is difficult to identify gum resins obtained from different plant species on the basis of physical characteristics, there is a more likelihood of adulteration.

During a detailed phytochemical investigation of guggul in our previous study [3], some compounds were found which were never reported from the *Commiphora* genus. Literature review of those compounds indicated that they have been previously isolated from the gum resin of *Mangifera indica* L. (Anacardiaceae; mango gum), a commonly available gum resin in both Pakistan and India. This led us to develop the chemical fingerprinting method of guggul by using HPLC to evaluate the authenticity of its raw material and finished products.

Guggul contains different classes of compounds including triterpenes, steroids, and lignans. Several scientific studies have shown that the pharmacological properties associated with guggul are due to the E- and Z-guggulsterones, while other compounds may synergistically enhance its overall activity [4–6]. Previously developed HPLC and UPLC methods for guggul have only used E- and Z-guggulsterones as standard marker compounds for the qualitative and quantitative analysis of raw guggul and its finished products [7–9].

received January 27, 2015  
revised Sept. 21, 2015  
accepted October 8, 2015

## Bibliography

DOI <http://dx.doi.org/10.1055/s-0035-1558211>  
Published online December 15, 2015  
Planta Med 2016; 82: 356–361  
© Georg Thieme Verlag KG  
Stuttgart · New York ·  
ISSN 0032-0943

## Correspondence

**Dr. Rida Ahmed**  
Department of Basic Sciences  
DHA Suffa University  
DG-78, Off Kh-e-Tufail, Phase VII  
(Ext.), DHA Karachi  
Karachi 75500  
Pakistan  
Phone: + 92 21 35 24 48 51 54  
Fax: + 92 21 35 24 48 56  
ridahej@hotmail.com

Interestingly, E- and Z-guggulsterones are not specific to *C. wightii*. These compounds have been found from the gum resins of *Ailanthus grandis* Prain (Simaroubaceae). Z-guggulsterone has also been identified from *Ailanthus malabarica* DC. as a major compound [10, 11]. Therefore, the authentication of raw material and/or finished pharmaceutical preparations of *C. wightii* gum resin needs the chemical profile of other secondary metabolites which are specific to *C. wightii* along with E- and Z-guggulsterones. In order to unambiguously identify the source of guggul, a new chemical fingerprinting method was developed by using different classes of standard compounds, including triterpenes, steroids, and lignans. This method assesses the quality of guggul based on both qualitative and quantitative aspects and can differentiate between gum resins of *C. wightii* and its closely related plant species that are commonly available in Pakistan and India. This chemical fingerprinting method is unique, as so far no such chemical fingerprinting approach has been reported for guggul.

## Results and Discussion

During the method development, an extraction protocol was validated before the optimization of chromatographic conditions because sample preparation is a crucial step in the development of analytical procedures. The extraction method of gum resin samples of *C. wightii* was evaluated for different solvents, temperatures, and extraction times. Methanol, acetonitrile, and acetone have been tested for the extraction of *C. wightii* gum resin (NCNPR #4998). The liquid chromatography with ultraviolet detection (LC-UV) analysis of different samples prepared using methanol, acetone, and acetonitrile showed similar results for methanol and acetonitrile, whereas acetone was found inefficient. Finally, methanol was selected because it is cost effective compared to acetonitrile. For the optimization of temperature, samples were extracted using methanol at ambient temperature and 55 °C during sonication, respectively. LC-UV results revealed no significant difference for the main components at two different temperatures. Therefore, extraction was carried out at the ambient temperature (~27 °C). Extraction time was tested for 15 min, 20 min, and 30 min, respectively, under sonication, but LC-UV results showed no major difference for different sonication times. Twenty minutes were finally used as the sonication time. The gum resin samples were extracted four times under the optimized extraction protocol using methanol at the ambient temperature, and each extraction time was set for 20 min. LC-UV analysis was carried out at 243 nm and 205 nm, owing to the detection of compounds 1–7 and 8–12, respectively.

In the developed method, 12 standard compounds were selected for chemical fingerprinting analysis of *C. wightii* gum resin (Fig. 1). These standard compounds were isolated from a commercial *C. wightii* gum resin during the phytochemical investigation of guggul in our previous study [3]. The mixture of 12 standard compounds (standard mixture-12) was prepared after mixing 50 µL of 1.0 mg/mL of each standard compound. Different solvent system, C-18 columns, and column temperatures have been optimized for choosing the best chromatographic conditions. Acetonitrile/water and acetonitrile/methanol/water were used in different ratios along with various reversed-phase C18 columns, including Phenomenex Synergi Hydro-RP (150 mm × 4.6 mm i.d.; 4 µm), Phenomenex Synergi Fusion-RP (150 mm × 4.6 mm i.d.; 4 µm), and Phenomenex Synergi Polar-RP (150 mm × 4.6 mm i.d.; 4 µm), under numerous temperatures ranging from 35 °C to 45 °C.

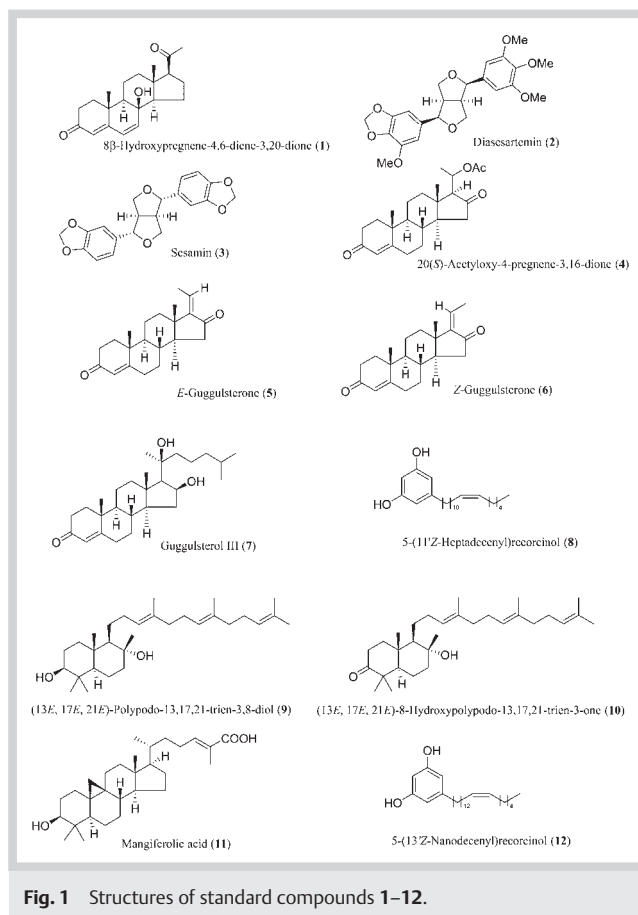
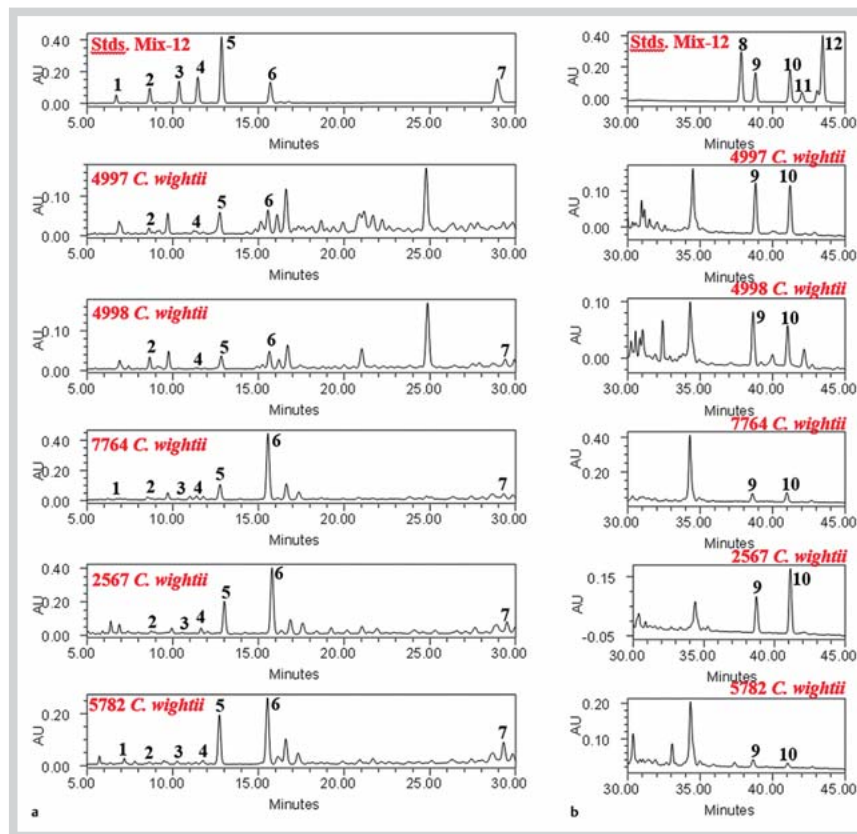


Fig. 1 Structures of standard compounds 1–12.

The best results were obtained with the Phenomenex Synergi Hydro-RP (150 mm × 4.6 mm i.d.; 4 µm) column at 38 °C. The mobile phase was composed of water with 0.1% acetic acid (A) and acetonitrile containing 0.1% acetic acid (B) with a gradient elution from 47% B to 100% B in 46 min. Acetic acid was used to facilitate the peak separation (see details in Material and Methods). Standard mixture-12 and the gum resin sample (NCNPR #4998) were analyzed during the optimization of chromatographic conditions. Twenty-two samples of *C. wightii* gum resin were analyzed by using a newly developed chemical fingerprinting method. LC-UV data showed some similarities and differences among all the guggul samples. Fig. 2a, b represents the chemical profiles of five samples of guggul including three authentic (NCNPR #4997, #2567, #7764) and two commercial samples (NCNPR #4998, #5782) both from Pakistan and India. LC-UV profiles of NCNPR #5782 from Pakistan and NCNPR #7764 from India showed the presence of nine standard compounds, whereas NCNPR #4997, #4998, and #2576 revealed the presence of eight standard compounds. The analysis of all twenty-two guggul samples revealed the presence of four marker compounds including E-guggulsterone (5) and Z-guggulsterone (6) along with the two major triterpenes (13E,17E,21E)-ploypodo-13,17,21-trien-3,8-diol (9) and (13E,17E,21E)-8-hydroxypolypodo-13,17,21-trien-3-one (10), whereas compounds 8, 11, and 12 were absent in twenty-one guggul samples and found only in one commercial guggul sample, which was used in our previous study [3]. The absence of compounds 8, 11, and 12 in all of the samples except one guggul sample indicated that these compounds do not belong to the gum resin of *C. wightii* and can be the result of possible adulteration.



**Fig. 2** a HPLC-UV chromatograms of the standards and extracts of *C. wightii* at 243 nm (RTs between 5 and 30 min). b HPLC-UV chromatograms of the standards and extracts of *C. wightii* at 205 nm (RTs between 30 and 45 min). (Color figure available online only.)

To identify the possible adulterants, gum resins of *Acacia nilotica* (L.) Willd. ex Del. (Fabaceae), *Melia azadirachta* L. (Meliaceae), *M. indica*, and *Commiphora myrrha* (Nees) Engl. were obtained from different sources in Pakistan, India, and the USA. These gum resins are commonly available in both Pakistan and India. Nine samples were analyzed and compared with standard mixture-12 for the detection of any potential adulteration in the commercial guggul sample.

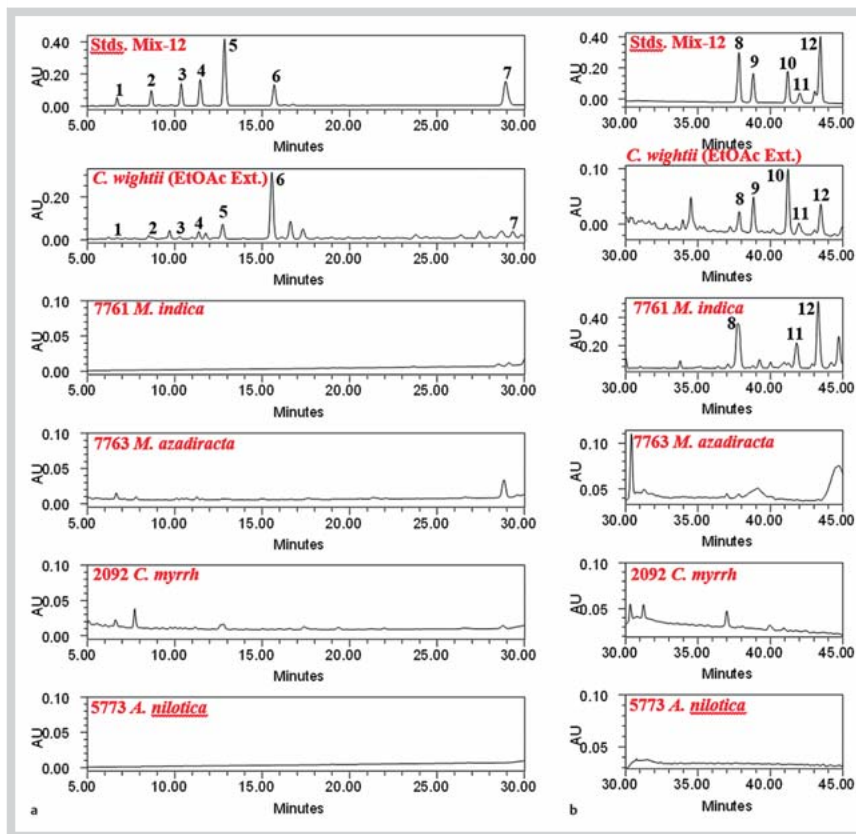
● **Fig. 3 a, b** represents the LC-UV data of commercially available *C. wightii* gum resin sample from Pakistan (EtOAc extract, which was used in our previous phytochemical study, [3]) and four possible adulterant gum resin samples (NCNPR #2092, #7761, #7763, and #5773). The LC-UV data of commercially available *C. wightii* gum resin sample from Pakistan (EtOAc extract) showed the presence of all the 12 standard compounds (1–12). LC-UV profiles of the gum resins of *A. nilotica*, *M. azadirachta*, and *C. myrrha* did not show the presence of any of the standard compounds. Only the LC-UV profile of NCNPR #7761, which is an authentic sample of *M. indica*, showed the presence of standard compounds 8, 11, and 12, which confirms the adulteration of *M. indica* in one of the commercially available *C. wightii* gum resin samples (EtOAc extract). Selected parts of the LC-UV chromatograms are presented (retention times between 5–30 min at 243 nm and 30–45 min at 205 nm) in ● **Figs. 2** and **3**.

This method was also validated for the standard parameters such as specificity, accuracy, precision, linearity, limit of detection (LOD), and limit of quantification (LOQ). All standard compounds kept in a  $-20^{\circ}\text{C}$  refrigerator were found to be stable for up to 30 days, and no changes were observed with UV spectra, peak areas, and the appearance of any extra peaks. The specificity of the HPLC method was determined by injecting the individual standard compounds, wherein no interference was observed for any

of the components. The precision of the developed procedure was assessed by carrying out three independent assays on three days. Three *C. wightii* gum resin samples (NCNPR #4998) were extracted under the extraction protocol on three different days and injected in duplicate. High reproducibility was obtained in results after multiple injections with a low standard error. The RSD of assay results obtained in interday and intraday studies is listed in ● **Table 1**. The RSD was less than 2% with a maximum 1.49% in the interday assays for E- and Z-guggulsterones, which indicated the good precision of the developed method.

Known quantities of each E- and Z-guggulsterone, i.e., 500  $\mu\text{g}$ , were spiked in the *C. wightii* gum (NCNPR #5781) resin sample to measure the accuracy of the assay method. 500  $\mu\text{L}$  of both E- and Z-guggulsterones (1 mg/mL) were spiked in two different samples of *C. wightii* gum resin, weighing 102.3 mg and 102.0 mg, respectively. Spiked samples were dried under vacuum and extracted by using the standard extraction protocol. The extracted samples were injected and the data was analyzed to find out the percentage of recovery of the two ketosteroids. The percentage of recovery of E- and Z-guggulsterones was found to be 96.5% and 100.3%, respectively.

Linear calibration plots for the related substances were obtained at ten different concentration levels (between 0.5–250  $\mu\text{g}/\text{mL}$ ) in duplicate. Linear regression analysis of the calibration plot of E- and Z-guggulsterones yielded equations  $Y = (4.55e + 004) X + (2.30e + 004)$  and  $Y = (4.13e + 004) X + (1.46e + 004)$ , respectively, and the calibration data indicated the linearity ( $r^2 > 0.999$ ) of the detector response for both guggulsterones by the LC-UV method. LOD and LOQ were determined by injecting a series of dilute solutions with known concentrations. LOD and LOQ were defined as the signal-to-noise ratio equal to 3 and 10, respectively. The



**Fig. 3** a HPLC-UV chromatograms of the standards, EtOAc extract of *C. wightii*, and possible adulterants at 243 nm (RTs between 5 and 30 min). b HPLC-UV chromatograms of the standards, EtOAc extract of *C. wightii*, and possible adulterants at 205 nm (RTs between 35 and 45 min). (Color figure available online only.)

LOD and LOQ for E- and Z-guggulsterones were found to be 0.1 µg/mL and 0.5 µg/mL, respectively.

The chemical fingerprinting method developed in this study has been validated for the quantification of E- and Z-guggulsterones in all of the *C. wightii* gum resin samples. The percentage content of E- and Z-guggulsterones of all of the twenty-two samples of guggul is shown in Table 2. The amount of E- and Z-guggulsterones ranges from 0.051% to 0.867% and 0.063% to 4.623%, respectively. There was a considerable variation in the amount of both guggulsterones in all of the *C. wightii* gum resin samples. The overall amount of Z-guggulsterone was found to be more compared to E-guggulsterone in most of the *C. wightii* gum resin samples. This can be because of several factors, such as habitat, soil conditions, weather, time of collection, and age of the plant at the time of harvesting.

The newly developed HPLC chemical fingerprinting method was applied for both qualitative and quantitative analysis of the gum resin of *C. wightii*. Twelve standard compounds have been successfully identified by using this method in all samples, which include twenty-two *C. wightii* gum resin samples and nine samples of possible adulterant gum resins. The quantitative analysis of E- and Z-guggulsterones showed variations in the amount of guggulsterones in several samples. Chemical fingerprinting studies showed that out of the twelve standard compounds, nine compounds were specific to *C. wightii* gum resin, whereas three compounds, 8, 11, and 12, belonged to *M. indica*. This confirmed the adulteration of *M. indica* gum resin in one of the commercial *C. wightii* gum resin samples. The results of the analysis revealed that the newly developed HPLC chemical fingerprinting method can be effectively used for the quality control and authentication of herbal raw materials and/or finished pharmaceutical preparations of guggul.

**Table 1** Intraday and interday precision data of E- and Z-guggulsterone content (% , mg/100 mg sample) in NCNPR #4998.

Analyte	Intraday (n = 3)			Interday (n = 9)
	Day 1	Day 2	Day 3	
E-guggulsterone	0.907 (0.21)	0.928 (0.78)	0.911 (1.49)	0.916 (1.35)
Z-guggulsterone	1.176 (0.23)	1.206 (0.75)	1.195 (1.22)	1.192 (1.30)

% Relative standard deviation given in parenthesis

## Materials and Methods

### Instrumentation and chromatographic conditions

Samples were analyzed on a Waters Alliance 2695 HPLC system (Waters Corp.), 6000 A pumps with quaternary solvent manager, U6 K injector, 680 automated gradient controller, and 2996 photodiode array detector connected to a computerized data station using Waters Empower 2 software. A Synergi-hydro column (150 mm × 4.6 mm; 4 µm) from Phenomenex (Torrance) was used as the stationary phase. The column temperature was maintained at 38 °C. An LC-18 (2 cm) guard column (Phenomenex, Torrance) was used prior to the Synergi-hydro column. A binary mobile phase system comprised of water (A) and acetonitrile (B), both with 0.1% acetic acid, were used for the study. A flow rate of 1.0 mL/min was maintained during the analysis by using the following gradient elution: 53% A/47% B to 28% A/72% B in 27 min, continually increase B to 81% B in 1 min, then to 93% B and 100% B in next 17 min and 1 min, respectively. After each run, washing for 5 min with 100% acetonitrile was carried out along with an equilibration period of 15 min. The injection vol-

**Table 2** E- and Z-guggulsterones % of content in different *C. wightii* gum resins and related genera samples.

Sample code	Weight of the sample (mg)	% Content (mg/weight of the product)	
		E-guggulsterone	Z-guggulsterone
4997	102.3	0.144	0.128
4998	102.2	0.091	0.211
4999	100.2	0.134	0.292
2567	100.5	0.455	1.162
5772	101.7	0.051	0.074
5774	100.9	0.558	2.352
5775	101.7	0.091	2.090
5776	100.9	0.198	1.471
5777	104.8	0.143	0.168
5778	104.8	0.224	0.592
5779	104.8	0.244	1.962
5780	104.7	0.378	0.568
5781	100.2	0.362	0.903
5782	102.7	0.446	0.710
5783	101.3	0.236	0.331
5784	100.9	0.797	2.411
5785	101	0.074	0.063
5786	101.1	0.288	0.907
3639	104.4	0.455	3.173
4985	104	0.238	0.409
7764	103.5	0.241	1.219
<i>C. wightii</i> EtOAc extract	1 mg/mL	0.867	4.623
2092 <i>C. myrrha</i>	104.4	ND	ND
3550 <i>C. myrrha</i>	102.2	ND	ND
5000 <i>M. indica</i>	103.3	ND	ND
7761 <i>M. indica</i>	102.5	ND	ND
7762 <i>M. indica</i>	104	ND	ND
6517 <i>M. indica</i>	104.7	ND	ND
7763 <i>M. azadiracta</i>	104.7	ND	ND
7760 <i>A. nilotica</i>	101.1	ND	ND
5773 <i>A. nilotica</i>	101.2	ND	ND

ND: not detected

ume was 10  $\mu$ L. Peaks were detected at UV wavelengths of 205 and 243 nm and were assigned by spiking the samples with standard compounds, comparison of UV spectra, and from the retention times.

### Chemicals

Twelve standard compounds, 8 $\beta$ -hydroxypregnene-4,6-diene-3,20-dione (**1**), diasasartemin (**2**), sesamin (**3**), 20(S)-acetyloxy-4-pregnene-3,16-dione (**4**), E-guggulsterone (**5**), Z-guggulsterone (**6**), guggulsterol III (**7**), 5-(11'Z-heptadecenyl)resorcinol (**8**), (13E,17E,21E)-polypodo-13,17,21-trien-3,8-diol (**9**), (13E,17E,21E)-8-hydroxypolypodo-13,17,21-trien-3-one (**10**), mangiferolic acid (**11**), and 5-(13'Z-nanodecenyl)resorcinol (**12**) (● Fig. 1), were isolated from commercially available *C. wightii* gum resin at NCNPR. The identity and purity of all of the standard compounds were confirmed by chromatographic (TLC, HPLC) methods and by analysis and comparison of the spectroscopic data (IR, 1D- and 2D-NMR, HR-ESI-MS) with the published data [3]. The following % purities were calculated for compounds **1–12**: 99.65%, 98.29%, 96.41%, 85.01%, 93.18%, 92.13%, 98.78%, 98.86%, 91.48%, 95.71%, 94.93%, and 96.19%, respectively. Acetonitrile, methanol, and acetic acid were of HPLC grade and purchased from Fisher Scientific.

Different gum resin samples of *C. wightii*, *C. myrrha*, *A. nilotica*, *M. indica*, and *A. indica* were collected from different authentic and commercial sources in Pakistan and India. Some of the commercial samples were also obtained from USA. All of the samples of *C. wightii* were gum resin agglomerates. Out of twenty-two samples, one sample was the ethyl acetate extract of the *C. wightii* gum resin (EtOAc extract). Different *C. wightii* samples and related plant samples were assigned the following code numbers: *C. wightii* (NCNPR #4997), *C. wightii* (NCNPR #4998), *C. wightii* (NCNPR #4999), *C. wightii* (NCNPR #7764), *C. wightii* (NCNPR #4985), *M. indica* (NCNPR #5000), *M. indica* (NCNPR #7761), *M. indica* (NCNPR #7762), *M. indica* (NCNPR #6517), *M. azadiracta* (NCNPR #7763), and *A. nilotica* (NCNPR #7760). All were obtained from India. *C. wightii* (NCNPR #2567), *C. wightii* (NCNPR #5772), *C. wightii* (NCNPR #5774), *C. wightii* (NCNPR #5775), *C. wightii* (NCNPR #5776), *C. wightii* (NCNPR #5777), *C. wightii* (NCNPR #5778), *C. wightii* (NCNPR #5780), *C. wightii* (NCNPR #5781), *C. wightii* (NCNPR #5782), *C. wightii* (NCNPR #5783), *C. wightii* (NCNPR #5784), *C. wightii* (NCNPR #5785), *C. wightii* (NCNPR #5786), *C. wightii* (EtOAc extract), and *A. nilotica* (NCNPR #5773). These were obtained from Pakistan. *C. myrrha* (NCNPR #2092) was procured from William Ware House. *C. myrrha* (NCNPR #3550) was purchased from Frontier Natural Products Corporation.

Voucher specimens of all of the samples were deposited at the National Center for Natural Product Research (NCNPR), University of Mississippi, Mississippi, USA.

### Sample preparation

Homogenized gum resin sample (100 mg) was accurately weighed and mixed with 2.5 mL MeOH, and sonicated for 20 min. It was then centrifuged for 10 min at 3000 rpm. The supernatant was transferred into a 10.0-mL volumetric flask. The procedure was repeated three times, and all extracts were combined. The final volume was adjusted with MeOH to 10.0 mL. The extract was thoroughly mixed and filtered with a 0.45- $\mu$ m nylon syringe filter prior to HPLC analysis. The first filtrate, 1 mL, was discarded and the remaining filtrate was collected in an LC vial for further analysis.

All of the powdered gum resin samples were extracted on the basis of this protocol except one *C. wightii* gum resin sample; this sample was previously extracted in ethyl acetate (EtOAc extract) and used in our previous phytochemical study [3]. The EtOAc extract of the gum resin was taken (1 mg), diluted 10 times, and then subjected to the above procedure to make up the final volume in 10 mL of methanol solution.

### Preparation of standard solution

Stock solutions of E- and Z-guggulsterones were prepared in methanol at a concentration of 1.0 mg/mL. Ten different concentration levels were used to record the calibration curve. The range of the calibration curve was 0.5–250  $\mu$ g/mL for HPLC-UV analysis. Stock solution of the individual standard was prepared at a concentration of 1.0 mg/mL in methanol. 50  $\mu$ L of each standard solution was mixed to prepare a standard mixture of all of the twelve standard compounds for chemical fingerprinting analysis.

### Validation procedure

The newly developed HPLC method was validated in terms of specificity, precision, accuracy, and linearity according to ICH guidelines [12]. The assay method precision was measured by interday and intraday studies. Known amounts of the E- and Z-gug-

guglsterones, each 500 µg, were spiked to evaluate the accuracy of the assay method. LOD and LOQ were determined by injecting a series of dilute solutions with known concentrations. LOD and LOQ were defined as the signal-to-noise ratio equal to 3 and 10, respectively.

### Acknowledgements

This research was co-funded by the Pak-USAID project (Linkages of Centers for Chemical Sciences, Grant No. 1-5/ILS-US/HEC/2004), and the United States Food and Drug Administration (FDA) Specific Cooperative Research Agreement Number 5 U01FD002071-08. The authors are grateful to Dr. Vaishali C. Joshi, a plant taxonomist, for identification of the plant samples.

### Conflict of Interest

There is no conflict of interest to disclose.

### Affiliations

- <sup>1</sup> National Center for Natural Products Research, University of Mississippi, University, MS, USA
- <sup>2</sup> Division of Pharmacognosy, Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS, USA
- <sup>3</sup> H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan
- <sup>4</sup> Department of Chemistry, College of Science, King Saud University, Riyadh, Saudi Arabia
- <sup>5</sup> Department of Basic Sciences, DHA Suffa University, Karachi, Pakistan

### References

- 1 *Soni V.* IUCN final project report of *in-situ* conservation of *Commiphora wightii* a red-listed medicinal plant species of Rajasthan State, India. 2008: 1-39. Available at: [https://cmsdata.iucn.org/downloads/final\\_project\\_report\\_dr\\_vineet\\_soni.pdf](https://cmsdata.iucn.org/downloads/final_project_report_dr_vineet_soni.pdf). Accessed November 30, 2015.
- 2 *Ichikawa H, Aggarwal BB.* Guggulsterone inhibits osteoclastogenesis induced by receptor activator of nuclear factor-kappaB ligand and by tumor cells by suppressing nuclear factor-kappaB activation. *Clin Cancer Res* 2006; 12: 662-668
- 3 *Ahmed R, Ali Z, Wu Y, Kulkarni S, Avery MA, Choudhary MI, Khan IA.* Chemical characterization of a commercial *Commiphora wightii* resin sample and chemical profiling to assess for authenticity. *Planta Med* 2011; 77: 945-950
- 4 *Zhu N, Rafi MM, DiPaola RS, Xin J, Chin CK, Badmaev V, Ghai G, Rosen RT, Ho CT.* Bioactive constituents from gum guggul (*Commiphora wightii*). *Phytochemistry* 2001; 56: 723-727
- 5 *Meselhy MR.* Inhibition of LPS-induced NO production by the oleogum resin of *Commiphora wightii* and its constituents. *Phytochemistry* 2003; 62: 213-218
- 6 *Francis JA, Raja SN, Nair MG.* Bioactive terpenoids and guggulsteroids from *Commiphora mukul* gum resin of potential anti-inflammatory interest. *Chem Biodivers* 2004; 1: 1842-1853
- 7 *Mesrob B, Nesbitt C, Misra R, Pandey RC.* High-performance liquid chromatographic method for fingerprinting and quantitative determination of E- and Z-guggulsterones in *Commiphora mukul* resin and its products. *J Chromatogr B Biomed Sci Appl* 1998; 720: 189-196
- 8 *Nagarajan M, Waszkuc TW, Sun J.* Simultaneous determination of E- and Z-guggulsterones in dietary supplements containing *Commiphora mukul* extract (guggulipid) by liquid chromatography. *J AOAC Int* 2001; 84: 24-28
- 9 *Haque I, Kumar M, Mukhopadhyay K.* A rapid and simple UPLC-MS-MS based simultaneous determination of the medicinally important E- and Z-guggulsterone from oleogum-resins of naturally occurring *Commiphora wightii* plants. *Chromatographia* 2009; 70: 1613-1619
- 10 *Hung T, Stuppner H, Ellmerer-Mueller EP, Scholz D, Eigner D, Manandhar MP.* Steroids and terpenoids from the gum resin of *Ailanthus grandis*. *Phytochemistry* 1995; 39: 1403-1409
- 11 *Hung T, Sturm S, Stuppner H.* TLC and HPLC/MS analysis of the gum resin of *Ailanthus grandis* and related species. *Sci Pharm* 1996; 64: 471-478
- 12 *European Medicines Agency.* ICH Topic Q2 (R1) Validation of Analytical Procedures: Text and Methodology. London: EMEA; 1995