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# Comparative study of isolated Guggulsterones as marker compounds from Guggulu, *Commiphora mukul* with Ayurvedic Guggulu containing formulations by HPTLC, an in-house quality control method

**[1]Selvakumar Duraipandi, [1,*]Vijaya Selvakumar**

[1]*School of Pharmacy, Taylor's University Lakeside, Subang Jaya, 47500, Malaysia*

**Abstract:** The guggulu containing polyherbal formulations in Ayurveda have been used for treating various inflammatory conditions. A simple HPTLC method has been developed to qualitatively analyze the formulations that claimed to have contained guggulu using the Guggulsterones isolated from the guggulu raw material as a marker. The isolation of Guggulsterones from the resinous gum obtained from the plant was used as a marker to determine the Guggulsterone content in the formulations. The study showed that all the preparations taken for analysis that claimed to contain Guggulsterones was originally having the contents but in variable amounts depending on the amount of resin taken for the preparation. Due to the fact that Guggulsterones are very expensive marker compounds if procured separately as Guggulsterone E and Z forms, this method can be used for routine qualitative analysis of presence of Guggulsterones as an in-house quality control method.

**Keywords:** Guggulsterones; HPTLC; Guggulu(Gulgulu); ayurveda

## 1. Introduction

Ayurveda, an indigenous system of medicine practiced for more than 3000 years in India, Sri Lanka and some other countries. Guggulu, a resin obtained from *Commiphora mukul,* a flowering plant from burseraceae and it commonly found in all the parts of India. It is an important component of many Ayurvedic preparations, Gulgulu in particular[1]. The Ayurvedic proprietary medicines Gulgulutiktakam Kashayam Tablets, Gulgulutiktakam Ghritam, Triphala Gulgulu Churna DS are gulgulu containing preparations used to treat various inflammatory conditions[1]. These are polyherbal formulations claimed to containing variable concentrations of guggulu in the final products. The disadvantage of these polyherbal formulations is the lack of appropriate method to analyze the contents qualitatively and quantitatively due to either disproportionate availability or very expensive of marker compounds. In the present study, Guggulsterones are isolated from resin obtained from *Commiphora mukul* and purified in the laboratory. Then the isolated Guggulsterone is qualitatively compared with commercial Ayurvedic formulations (Gulgulutiktakam Kashayam, Gulgulutiktakam Ghritam, Triphala Gulgulu Churna) by High Performance Thin Layer Chromatography (HPTLC).

## 2. Result and Discussion

Gulgulutiktakam Kashayam tablets contains 850mg of dried polyherbal extract per tablet(1g), Gulgulutiktakam Ghritam contains 1.5 g in each 10 mL; Triphala gulgulu tablet contains 425 mg of polyherbal extract per gram of the tablet. The study was related t o a method using laboratory produced marker of guggulsterones for the identification of the guggulsterones present the above preparations. Standard Guggulsterones were obtained from the raw guggulu by ethyl acetate extraction followed by alkalization and then extracted with petroleum ether. The guggulsterones obtained were a mixture of Guggulsterone E and Z rather than individual components.

The developed HPTLC plates visualized under UV cabinet at 254 nm and 366 nm and their corresponding fingerprint displays are shown in **Figure 1**. The spectral display showing the absorption of Rf 0.51 and 0.76 that is thought to be corresponding to guggulsterones (Since these spots fluoresce strongly under UV light) were shown in **Figure 2**. The **Figure 3** and **Figure 4** were showing peak display (densitometeric) of developed spots of test samples under UV light at 254 nm and 366 nm respectively. The calculated $R_f$ values and AU (Area Under the peak), a measure of peak intensity of spots were given in the **Table 1** and **Table 2** for the observations under UV light at 254 nm and 366 nm respectively.

The UV scan and the AU obtained for Rf values of 0.51 & 0.76 for both standard and test sample, which

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*corresponding author: VS Tel: +60102595347 ; E-mail: vijirx2008@gmail.com

corresponds to the fluorescent spots (Guggulsterones fluoresces brightly under 366nm in very small concentrations). The comparison of the concentration (AU) of all the samples resulted from the densitometric scanning have also showing the corresponding increase/decrease in the concentrations of the expected presence of guggulsterones in the formulations.

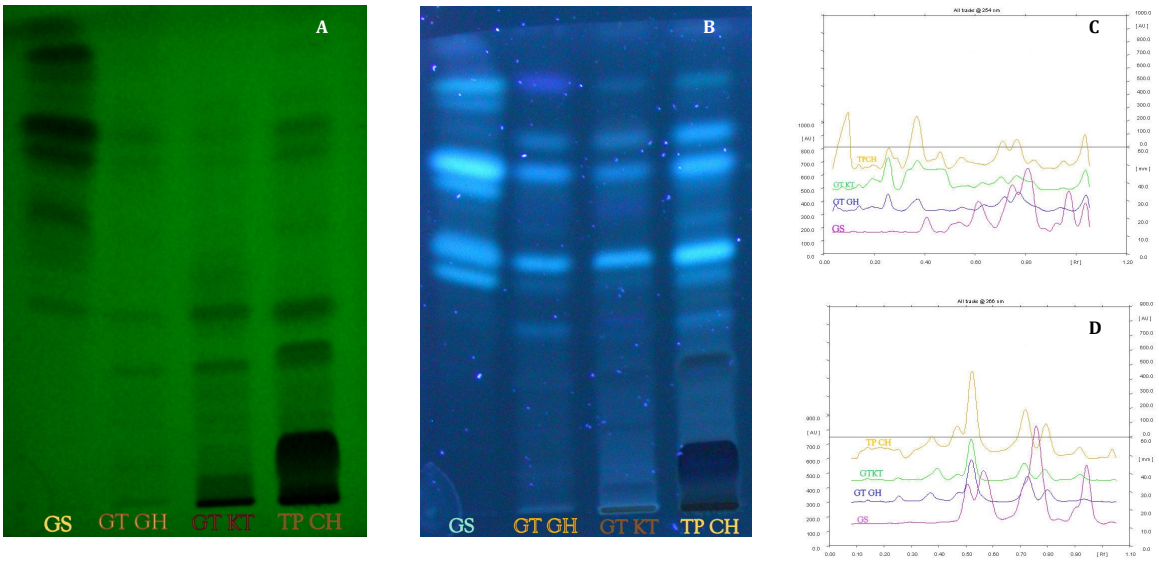


**Figure 1.** Visualization under UV light (A) @254nm (B) @366nm; 3D-display of Fingerprint (C) @254 nm (D) @366 nm

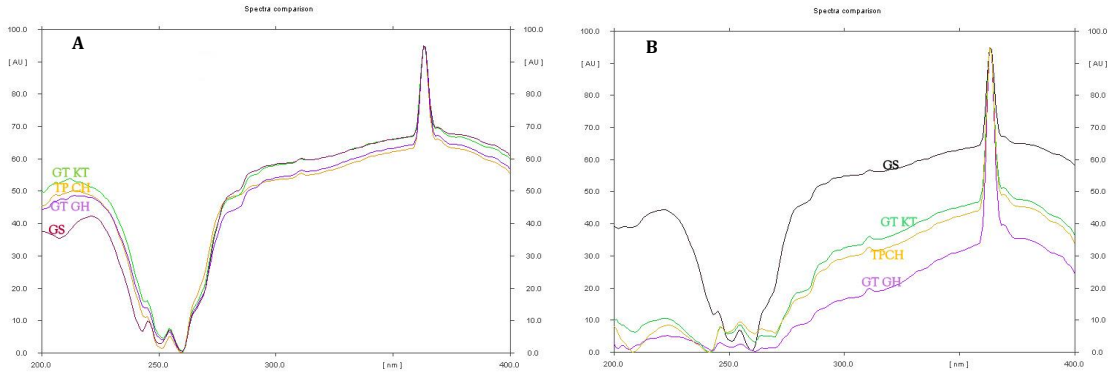


**Figure 2.** Spectral display @ 366 nm (A) $R_f$ = 0.51(B) $R_f$ = 0.76

**Table 1.** Showing the peaks of spots and their respective concentration (AU) observed under @254 nm

| Peaks | Rf value Tracks | | | | Area (AU) Tracks | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| 1 | | 0.05 | 0.08 | 0.10 | | 586.9 | 296.6 | 12035.6 |
| 2 | | 0.14 | 0.14 | 0.14 | | 593.8 | 635.1 | 270.1 |
| 3 | | 0.19 | 0.19 | 0.20 | | 1102.0 | 2787.7 | 486.0 |
| 4 | | 0.26 | 0.26 | 0.26 | | 2458.1 | 5536.4 | 4203.6 |
| 5 | | 0.37 | 0.37 | 0.37 | | 2791.6 | 10921.5 | 13205.8 |
| | 0.41 | | | | 2467.1 | | | |
| 6 | | 0.47 | 0.45 | 0.46 | | 273.7 | 5800.6 | 3676.4 |
| 7 | [a]0.53 | [a] 0.55 | [a] 0.57 | [a] 0.54 | [a] 3146.0 | [a] 873.7 | [a] 1015.1 | [a] 3441.7 |
| 8 | 0.61 | 0.63 | 0.63 | | 9706.1 | 1345.5 | 1512.3 | |
| 9 | | 0.72 | 0.70 | 0.71 | | 3681.4 | 3460.2 | 7459.3 |
| 10 | [a] 0.75 | [a] 0.77 | [a] 0.77 | [a] 0.77 | [a] 13695.9 | [a] 5945.8 | [a] 4470.5 | [a] 7067.4 |
| 11 | 0.81 | | 0.82 | 0.83 | 18370.0 | | 1171.4 | 1578.3 |
| 12 | 0.87 | | | | 459.3 | | | |
| 13 | [a] 0.92 | [a] 0.94 | [a] 0.95 | [a] 0.95 | [a] 1334.2 | [a] 692.0 | [a] 230.4 | [a] 1257.5 |
| | 0.97 | | | | 8533.3 | | | |

[a] The values shown in bold letters are the peaks of comparison between isolated markers from the guggulu and the test formulations

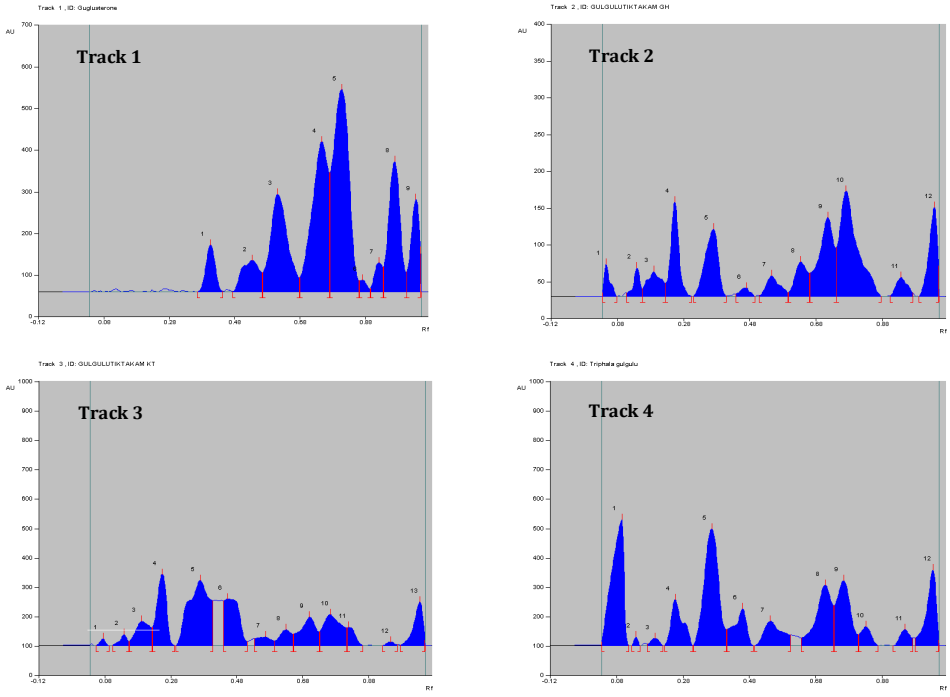


**Figure 3.** Peak display at 254 nm

It can be concluded that the levels of guggulsterones in the Ayurvedic preparations cannot be controlled but a uniform concentration can be maintained throughout a batch by adapting a method like this to use as an in-house method for the quality control check in Ayurvedic industries.

## 3. Experimental

**Materials and Methods:** All the solvents used in the extraction process were analytical grade. The solvents used in the HPTLC analysis were HPLC grade. The test samples are obtained commercially from the market and used for the study.

### 3.1. Sample Preparation, Test:

Powdered material of the test products of interest has been refluxed in 10 mL of methanol and filtered. The filtered extract was concentrated on a water bath to obtain 1 mL of extract and used for the analysis.

### 3.2. Extraction and Isolation of Guggulsterones.[2, 3, 4]

The resin taken and loaded in soxhlet extractor and extracted with ethyl acetate about five time the weight of gum. The temp is kept at 65-70 °C. The extracted fluid is taken for solvent recovery. The oleoresin (thick paste) obtained after solvent removal can be purified for enrichment of guggulsterones by solvent frication method. Take 2 g sample of guggulu extract in 250 mL round bottom flask, add 35 mL of 0.5 M alcoholic KOH and reflux for 90 min on a water bath. Transfer the content of flask to a separator rinse the flask with 50 mL lukewarm water. Extract while the liquid is warm by shaking vigorously with three successive quantities of 50 mL petroleum ether (60-80°). Combine the petroleum ether layers and wash with 20 mL water. Evaporate the petroleum ether and weigh the residue.

**Table 2.** Showing the peaks of spots and their respective concentration (AU) observed under @366 nm

| Peaks | Rf value Tracks | | | | Area (AU) Tracks | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| 1 | | 0.14 | 0.14 | 0.14 | | 179.6 | 262.5 | 2137.7 |
| 2 | | | | 0.19 | | | | 2384.4 |
| 3 | | | | 0.21 | | | | 1555.3 |
| 4 | | 0.26 | 0.25 | 0.25 | | 903.8 | 306.6 | 1208.4 |
| 5 | | 0.37 | 0.39 | 0.38 | | 2210.9 | 2573.5 | 8141.3 |
| 6 | | 0.47 | 0.47 | 0.47 | | 1518.5 | 981.4 | 7290.5 |
| 7 | [a] 0.51 | [a] 0.52 | [a] 0.52 | [a] 0.52 | [a] 7518.8 | [a] 8343.0 | [a] 6436.8 | [a] 18718.1 |
| 8 | 0.57 | | | | 14079.4 | | | |
| 9 | [a] 0.76 | [a] 0.73 | [a] 0.71 | [a] 0.72 | [a] 30288.2 | [a] 5123.2 | [a] 3588.6 | [a] 14757.1 |
| 10 | 0.84 | 0.80 | 0.79 | 0.79 | 808.5 | 2381.3 | 1907.6 | 7282.6 |
| 11 | [a] 0.94 | [a] 0.93 | [a] 0.92 | [a] 0.92 | [a] 12105.4 | [a] 843.6 | [a] 1138.2 | [a] 2249.5 |
| 12 | 0.98 | | | 0.98 | 234.8 | | | 189.4 |
| 13 | | | | | | | | 943.0 |

[a]The values shown in bold letters are the peaks of comparison between isolated markers from the guggulu and the test formulations

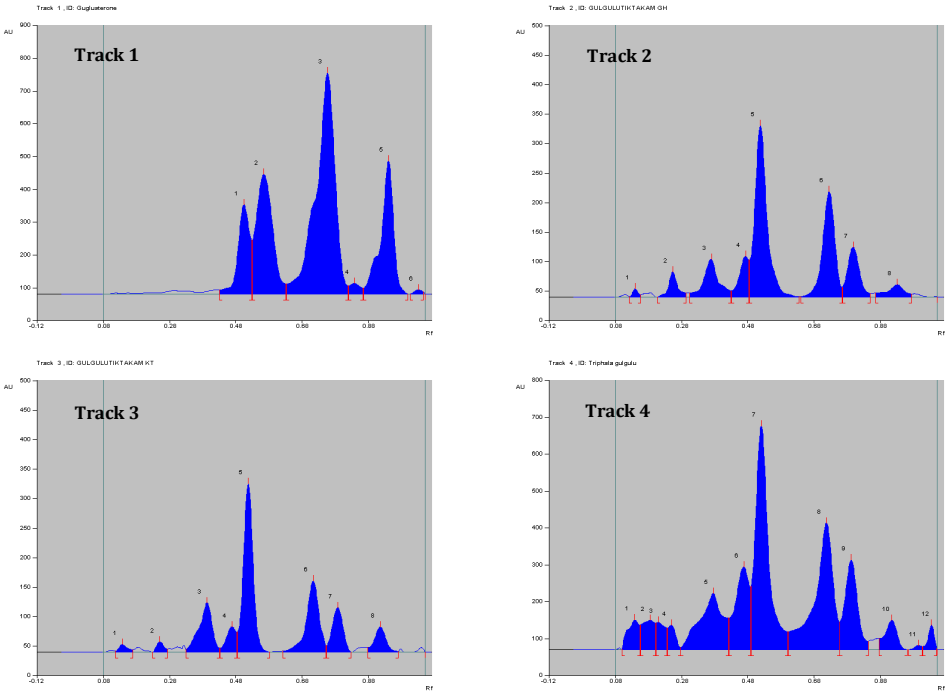


**Figure 4.** Peak display @ 366 nm

### 3.3. HPTLC Analysis[5-9]

The HPTLC analysis was carried out using the following conditions and the developed plates were visualized under UV light at 254 and 366 nm and densitometric scanning was performed to obtain the Rf Values and corresponding concentration of the spots (AU). The scanning was also performed by CAMAG densitometry scanner 3 between 200-400 nm to determine any additional existing spots. The spectral data was obtained by scanning the specific spots of Rf values of 0.51 and 0.72 under UV light at 366 nm.

### 3.4. HPTLC Conditions

Instrument used : CAMAG make HPTLC.
Software : winCATS 1.4.3
Sample Applicator : Linomat 5.
Detection : @254 nm & @366 nm in Densitometry TLC Scanner 3
STD Preparation : 1 mg of STD is dissolved in 0.25 mL water and 0.25 mL Ether
Stationary Phase : HPTLC plates silica gel 60 F 254.
Mobile Phase : Toluene: Ethyl acetate: Methanol (7:2:1)
Spectral Detection : Scanned at 200 nm to 400 nm
Sample Applied : 2µl sample is applied as 8mm band.
Track 1, **GS** : Guggulsterone Standard,
Track 2, **GT GH**: Gulgulutiktakam Ghritam
Track 3, **GT KT**: Gulgulutiktakam KashayamTablets
Track 4, **TP CH**: Triphala Gulgulu Churna

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