ORIGINAL ARTICLE

FUTURE JOURNAL OF PHARMACEUTICALS AND HEALTH SCIENCES

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# HPTLC Method in Determination of Guggulosterone Z from Leaf Extract of *Tribulus terrestris* Linn

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*Article History:*

Received on: 02 May 2022
Revised on: 17 May 2022
Accepted on: 18 May 2022

*Keywords:*

Guggulsterone Z, High-Performance Thin Layer Chromatography Technique, Tribulus terrestris Linn, Standardization, Phyto-chemicals

ABSTRACT

High Performance Thin Layer Chromatography (HPTLC) method is one of the best methods used in the analysis of herbal drugs. This technique is used to determine the quantity of bioactive compounds present in the extract. They have been utilized to analyze various types of natural products like alkaloids, flavonoids, steroids, terpenes, saponins, tannins etc. In this study, we have used HPTLC technique to estimate Guggulsterone Z from leaf extract of *Tribulus terrestris* Linn. In order to prepare the extracts, dry powder of *Tribulus terrestris* Linn leaves were extracted with solvent and these extracts were subjected to HPTLC analysis using mobile phase. The chromatographic plates silica gel 60F254 aluminium sheets of 20 cm x 10cm dimension were used for developing the samples. The plate was air-dried after completion of the run and observed under UV lamp. In the present research work, the results obtained determined the presence of Guggulsterone Z in *Tribulus terrestris* Linn by HPTLC method.

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eISSN: 2583-116X pISSN:

DOI: https://doi.org/10.26452/fjphs.v2i3.271

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## INTRODUCTION

High Performance Thin Layer Chromatography is a technique that allows for the separation of compounds in complex mixtures. HPTLC is based upon the principle of separating compounds using thin layers of stationary phase [1]. This method has been used for over 50 years but its popularity has increased greatly since the advent of modern high-speed computers and software. High Performance Thin Layer Chromatography (HPTLC) is a technique that uses thin layers of chromatographic material to separate compounds from complex mixtures. This method has been used to determine the chemical composition of many different types of samples including food products, pharmaceuticals, and even environmental pollutants [2]. HPTLC analysis can be performed using several different modes; these include normal phase, reverse phase, and polarimetric detection. In this we will discuss the importance of HPTLC, the various modes of operation, and how they are applied to analyze [3].

Herbal Analysis is the study of herbs and their active constituents. HPTLC (High Performance Thin Layer Chromatography) is a technique used to separate compounds based on their physical properties. This method is widely used in pharmaceutical research and quality control laboratories. Herbal products

are complex mixtures of compounds that have been used for centuries to treat various health conditions [2]. Herbs can be derived from either natural sources (plants) or synthetic sources (chemicals). Herbal products are usually classified into two broad categories: botanically-based and chemically-based. Botanical-based herbs contain active ingredients derived from plants while chemical-based products use chemicals synthesized to mimic the active components found in botanical-based herbs. There are several methods to determine the quality of a particular herb. One of these methods is High Performance Thin Layer Chromatography (HPTLC), which is a technique that separates different compounds based on their polarities and lipophilic properties. HPTLC is primarily used to separate and identify individual constituents of herbal extracts [4].

*Tribulus terrestris* Linn (also known as Triticum Vulgare) is a herbaceous perennial vine native to Europe. *Tribulus terrestris* Linn has been used traditionally in herbalism to treat infertility, impotence, male hypogonadism, and gynecological disorders. In traditional Chinese medicine, *Tribulus terrestris* Linn is used to enhance blood circulation and improve energy levels. *Tribulus terrestris* Linn contains saponins that are believed to have anti-inflammatory properties. *Tribulus terrestris* Linn is a herb that has been used for thousands of years for its medicinal properties [6]. *Tribulus terrestris* Linn is known to have many health benefits including improving fertility and sexual function, treating erectile dysfunction, increasing testosterone levels, reducing inflammation, and enhancing athletic performance. *Tribulus terrestris* Linn (also known as the greater fool's parsley) has been used throughout history to help treat various conditions including menopause, infertility, and sexual dysfunction. *Tribulus terrestris* Linn is a member of the pea family (Zygophyllaceae) that contains several compounds similar to phytoestrogens like daidzein and genistein. These compounds are responsible for many of the health benefits associated with this herb [5].

The following list includes some of the major health benefits of *Tribulus terrestris* Linn: Improves libido, Increases testosterone levels, Promotes fertility, Helps regulate menstrual cycle. The phytochemical screening test measures the total amount of chemical compounds present in a sample. A positive result indicates that the substance being tested may have some medicinal value. This can include both beneficial and harmful effects [6]. Phytochemicals are the chemicals responsible for the taste, smell, color, texture, and other properties of a food or beverage (e.g., tea). They are usually extracted from herbs, roots, seeds, bark, leaves, flowers, fruits, vegetables, nuts, grains, beans, and spices. Many of these substances are used in traditional medicine around the world. Some examples of common phytochemicals include tannins, saponins, alkaloids, terpenes, flavonoids, steroids, glycosides, and phenolic acids. Preliminary phytochemical analysis by High Performance Thin Layer Chromatography (HPTLC) can help identify potential active compounds present in extracts that may have beneficial effects on human health [7]. This method is highly sensitive and allows the detection of minute amounts of active constituents in crude extract samples. In this study, we report our preliminary findings on the qualitative determination of guggulsterones Z in *Tribulus terrestris* Linn using HPTLC fingerprinting. Herein, we evaluated the suitability of HPTLC-based fingerprinting for rapid quality control testing. Using TLC-silica gel plates, all solvents showed good separation between the target analytes and other interfering substances. Among these three solvent systems, ethyl acetate/n-butanol/acetic acid (5 + 5 + 1), n-hexane/acetone/ethyl acetate (4 + 6 + 2), and methanol/water (8 + 2) provided satisfactory separations. These results indicate that HPTLC fingerprinting has great potential for rapid qualitative screening of various herbal medicines containing extracts for the presence of bioactive compounds [8].

## MATERIALS AND METHODS

### Chemicals and Reagents

Guggulsterone Z (purity 99%), was procured from Sigma-Aldrich, New Delhi. All the chemicals, including solvents such as methanol were of analytical grade and were procured from E. Merck, India.

### Collection of Plant Material

*Tribulus terrestris* Linn. (Zygophyllaceae), was procured from the surroundings of Tirupathi and used for the present study. Leaves of *Tribulus terrestris* Linn (Zygophyllaceae) was collected from Tirumala hills, Tirupathi. The taxonomical identification and authentication of the plant was done by Director, National Institute of Herbal Medicine, Plant Anatomy Research Centre, Chennai.

### Extraction of *Tribulus terrestris* Linn

Leaves of *Tribulus terrestris* L. were collected, washed and dried at room temperature. After complete drying, it was powdered and passed through a 60 mesh sieve and stored in air tight container. Dried powdered drug was used to prepare extract. About 50 g of air dried powdered plant material was extracted with ethanol (60-80$^0$C), in a soxhlet appa-

ratus [9]. Extract was filtered, the solvent was evaporated and accurate weight of the extracts was taken [Figure 1].

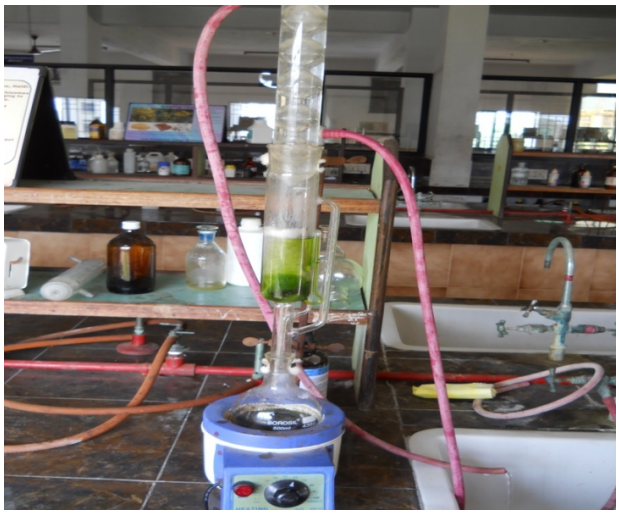

**Figure 1: Extraction of *Tribulus terrestris* L.**

### HPTLC Studies

In the present work, CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 with 12bit CCD camera for photo documentation, controlled by WinCATS Planar Chromatography Manager was used. All the solvents used were of HPTLC grade obtained from MERCK. All weighing were done on Precisa XB 12A digital balance.The HPTLC plates Si 60F254 Thin layer chromatography (20 cmX10 cm) were purchased from E. Merck (India) [10].

### Sample and Standard Preparation for HPTLC Finger Printing

### Preparation of *Triulus terrestris* Extract

Sample was prepared by making 200 mg of extract in 10ml of Methanol.

### Preparation of Guggulosterone Z solution

6.0 mg of Guggulsterone Z was weighed and dissolved in 50ml methanol

### Chamber Used for Mobile Phase

Camag twin trough chamber (20 x 10 cm)

### Chamber Saturation

Overnight saturation was done for all the HPTLC studies with Whatman filter paper lining.

### Stationary Phase

TLC aluminium sheet precoated with silica gel 60 $F_{254}$ (3x10 cm) was used as stationary phase, obtained from Merck [11].

## RESULTS AND DISCUSSION

The aim of this study is to determine the Guggulsterone Z content in *Tribulus terrestris* Linn leaf extract. HPTLC method is used for the determination of Guggulsterone Z in *Tribulus terrestris* Linn leaf extract. The HPTLC method was found to be accurate and reliable for determination of Guggulsterone Z in *Tribulus terrestris* Linn leaf extract. The HPTLC method was developed for the determination of guggulsterone Z from leaf extract of *Tribulus terrestris* Linn. The method is based on the use of a silica gel plate, an organic solvent with a low boiling point, and a specific reagent. The HPTLC plates are coated with silica gel which is then impregnated with an organic solvent such as chloroform, ethyl acetate or butanol. The plates are then developed in a solvent system that includes the specific reagent and visualized by exposure to ultraviolet light. Guggulsterone Z is a natural product isolated from the leaves of *Tribulus terrestris* Linn which has been used in traditional medicine and it is also sold as a dietary supplement. The HPTLC method (high-performance thin-layer chromatography) was developed to determine Guggulsterone Z in *Tribulus terrestris* Linn leaf extract. The method consists of a solvent system, mobile phase, developing agents, detector and receiver [Figure 2].

In this study, HPTLC was used as a means for qualitative and quantitative analysis of Guggulsterone Z in leaf extract. The HPTLC plates were developed with a mixture of chloroform-methanol-water (9:5:4) and the mobile phase consisted of methanol-acetic acid (1:1). The detection wavelength was set at 254 nm. The HPTLC method is based on the principle that the Guggulsterone Z elutes at a specific time from a thin layer chromatography plate. In this experiment, we will use a thin layer chromatography to determine the concentration of Guggulsterone Z in *Tribulus terrestris* Linn leaf extract [Figure 3]. The HPTLC method is based on the principle that Guggulsterone Z elutes at a specific time from a thin layer chromatography plate. The HPTLC method developed for the determination of guggulsterone Z from leaf extract of *Tribulus terrestris* Linn is based on the separation of the guggulsterone Z and its derivatives by a stationary phase composed of polyethylene glycol and a mobile phase consisting of methanol-water. The HPTLC method developed for the determination of guggulsterone Z from leaf extract of *Tribulus terrestris* Linn is based on the separation of the guggulsterone Z and its derivatives by a stationary phase composed of polyethylene glycol and a mobile phase consisting of methanol-water.

The HPTLC method was used to determine the gug-

gulsterone Z content from leaf extract of *Tribulus terrestris* Linn. The HPTLC method is a sensitive, rapid and reliable technique for the determination of guggulsterone Z in leaf extract of *Tribulus terrestris* Linn. It is a good alternative to other methods which are time-consuming and expensive. The retention time and peak area were found to be dependent on the concentration of Guggulsterone Z. Gugulosterones are the main active constituents of the plant, *Tribulus terrestris* Linn. These compounds have been reported to have a variety of biological activities including anti-inflammatory, anticancer and anti-diabetic properties. The HPTLC method for determination of Guggulsterone Z from leaf extract of *Tribulus terrestris* Linn is described in this paper. The HPTLC plates were prepared using different mobile phase compositions. The separation was carried out on a precoated silica gel plate, 20 x 20 cm, 0.25 mm thickness and eluted with different solvents [Figure 4].

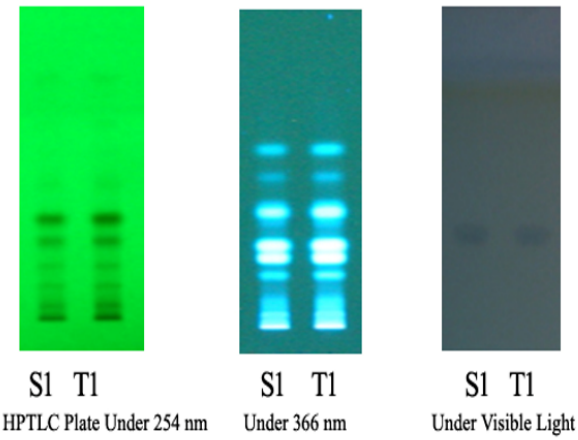


**Figure 2: UV Detection of HPTLC plates at 254, 366 and visible light**

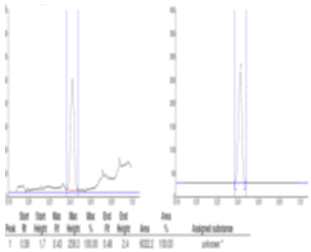

**Figure 3: HPTLC Chromatogram of Guggulosterone (Standard spot $S_1$)**

### HPTLC Fingerprinting of *Tribulus terrestris* Linn Extract

In precoated aluminium TLC plate 6$\mu$l and 9$\mu$l of *Tribulus terrestris* L. extract and standard guggulsterone Z were applied as 6mm band using Linomat V applicator with Hamilton syringe. Applied plate was developed in a twin trough chamber containing Hexane: Ethyl acetate (60:32) as mobile phase. The plate was developed for a migration distance of 75mm. It was then scanned under all the three wavelengths using Deuterium, Mercury and Tungsten lamps respectively and photo documented using Camag Reprostar 3. The Rf value and peak area of the extracts and standard were interpreted by using the software. *Tribulus terrestris* L. has shown 7 different well defined peaks indicating the presence of atleast seven different components in guggul extract. Guggulsterone Z the biomarker has shown the Rf value at 0.48 and the almost same Rf value ie 0.47 was revealed by *Tribulus terrestris* L. extract. The standard spot was not pronounced under 366nm and visible light. Sample and standard spot was very pronounced under 254nm.

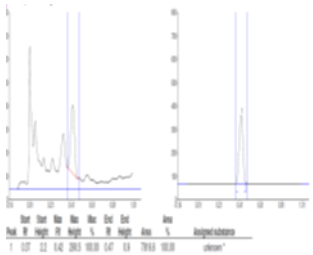

**Figure 4: HPTLC Chromatogram of ethanolic extract of *Tribulus terrestris* L**

## CONCLUSION

The HPTLC method in determining guggulosterone Z from *Tribulus terrestris* leaves extract is not a new technique, and has been used for many years. It is an established, reliable, and reproducible method which can be used to determine the concentration of guggulosterone Z in *Tribulus terrestris* leaves extract. This technique was used to detect guggulsterone Z in the *Tribulus terrestris* leaves extract. This study was conducted to investigate the effectiveness and safety of Hptlc method in determining guggulosterone Z from *Tribulus terrestris* leaves extract. The results indicated that guggulsterone Z can be extracted from the leaves of *Tribulus terrestris* by using a HPTLC method with a mixture of dichloromethane and methanol. The test results revealed that the HPTLC method is the most accurate and reliable way to determine guggulsterone Z from *Tribulus terrestris* leaves extract. The HPTLC method was found to be the most accurate and reliable way to determine guggulsterone Z from *Tribulus terrestris* leaves extract. The guggulsterone Z can be extracted from *Tribulus terrestris* leaves extract by following the proposed procedure and using the

HPTLC method. It is an established, reliable, and reproducible method which can be used to determine the concentration of guggulotine Z in *Tribulus terrestris* leaves extract.

## ACKNOWLEDGEMENTS

The authors are thankful to Dr. M. Balakrishnan, M.Pharm., Ph.D. Department of Pharmacognosy, Seshachala College of Pharmacy, Nagari road, Puttur-517 583, Chittoor (Dist), Andhra Pradesh, India Guided in research work.

### Funding Support

The author declares that no funding was supported for this study.

### Conflict of Interest

The author declares there was no conflict of interest for this study.

### Contribution of Authors

Authors declare that, the Research article by the names mentioned in the article and all the liabilities and claims related to the content of the article will be borne by the authors.

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**Cite this article:** Balakrishnan M, Melapudi Krishna Reddy Manisha, Monika P S. **HPTLC Method in Determination of Guggulosterone Z from Leaf Extract of *Tribulus terrestris* Linn**. Future J. Pharm. Health. Sci. 2022; 2(3): 125-129.