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HPTLC Method in Determination of Lupeol from Bark Extract of *Crataeva religiosa Hook and Frost*

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ABSTRACT

HPTLC (high performance thin-layer chromatographic) is a new method which has been developed for the determination of lupeol in petroleum ether extract from bark of *Crateava religiosa*. This method is simple, precise, rapid and selective. It can be applied to determine lupeol in petroleum ether extract from bark of *Crateava religiosa*. This method is suitable for routine analysis and can be used by anyone with minimal training. As per International Conference on Harmonization (ICH) guidelines we have applied different concentrations of lupeol as standard on HPTLC plates for the quantification of lupeol from the petroleum ether extract in bark of *Crateava religiosa*. The retention factor of lupeol was found to be 0.43*f*. The developed and validated HPTLC method was employed for lupeol in petroleum ether extract from bark of *Crateava religiosa* for standardization of the content of the marker. The results obtained in the validation assays are promising, with an average error of less than 0.5%. The results obtained in validation assays indicate the accuracy and reliability of the developed HPTLC method for the quantification of lupeol in petroleum ether extract from bark of *Crateava religiosa.*

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INTRODUCTION

The global drug industry continues to grow at an alarming rate with the recent US market value exceeding $1 trillion annually in 2017 [1]. The pharmaceutical industry can be divided into three different segments - small molecules and biologics, which are often genetically engineered or synthetically modified; chemical drugs such as steroids; High-performance thin layer chromatography is a technique used for the separation and purification of mixtures [2]. It uses a liquid solvent (usually water) to determine the purity of a mixture. The

technique is used in analysis and pharmaceutical research, forensics, forensic toxicology, pharmacognosy, drug discovery and development. It can be used to separate biomolecules such as proteins from other organic compounds like carbohydrates or lipids [3]. HPTLC was developed at the beginning of WWI. In the early 1900s, it was found that patients were dying from impure medicaments. The discovery of HPTLC allowed companies to test each batch with greater accuracy and efficiency than before, even after all manufacturing steps had been completed [1].

HPTLC is a method for purification of aqueous extract. It is an acronym for high-pressure, temperature, and time. High-pressure refers to the use of pressure above 100 MPa (1,000 psi), temperature refers to the use of temperatures as high as 160°C (320°F), and time refers to the extraction process lasting up to 24 hours. HPTLC is used in materials science, biochemistry, and pharmaceutical industries [4]. Here, we will discuss about the use of HPTLC and also see some of its benefits and limitations. HPTLC is an affordable solution to produce quality content at scale. HPTLC is a method used to analyze the ingredients of a medication. It is widely used in quality control and compliance for pharmaceutical companies that are required by law to perform HPTLC analysis on finished drug products [5]. It is important in analysis because it gives the assurance that medications will be safe and effective, especially since incorrect dosage or toxic substances can lead to serious health issues like kidney failure or death if administered in small doses over a long period of time.

Phytochemical evaluation is the process of identifying and quantifying the bioactive constituents in plant materials. The process of evaluating phytochemicals is complex, time-consuming and highly dependent on the expertise of a trained chemist [6]. Phytochemical evaluation is an analytical method used to determine the presence of natural chemicals in plant materials, such as leaves, fruits, and seeds. It is a qualitative analysis. Phytochemical evaluation is a qualitative analysis that determines the presence of natural chemicals in plant materials. It can be done with different methods such as extraction, chromatography and spectroscopy. The secondary metabolites are the small molecules that are not a part of the primary metabolism [7]. They are produced by a living organism in response to its environment and can be used for communication with other organisms. The secondary metabolites can be found in plants, animals, fungi, bacteria and even archaea. The phytochemical evaluation is important to know about these metabolites because it helps in identifying the compounds that might have therapeutic value or may cause toxicity in humans and animals. Secondary metabolite screening is done with the help of various techniques such as chromatography, HPLC-UV, GC-MS and NMR spectroscopy [8].

Secondary metabolites are chemicals that are produced by plants and animals as a defense mechanism. They are also involved in plant growth, defense, and reproduction. Phytochemical evaluation is the process of screening for secondary metabolites in plants. It is done by extracting a sample of the plant material, usually with organic solvents like methanol or hexane [9]. The extract is then applied to a solid phase chromatography column where it can be separated into fractions based on their polarity. The fractions are then analyzed using various spectroscopic techniques such as ultraviolet-visible spectroscopy, infrared spectroscopy, nuclear magnetic resonance spectroscopy and mass spectrometry. Secondary metabolites have different effects on humans depending on their chemical structures and target receptors [10]. HPTLC is a method of phytochemical screening that uses high-performance liquid chromatography. It is a very fast and sensitive technique for the analysis of plant materials. HPTLC analysis can be used to identify the presence of active compounds in plant extracts, raw materials, and even finished products. The HPTLC system can be used to test for the presence of active compounds in plant extracts, raw materials, and even finished products. This method is effective for identifying qualitative information about certain substances such as their type, quantity, concentration, and purity. A phytochemical screening using HPTLC has many benefits such as its high sensitivity and specificity which makes it an efficient tool to detect the presence or absence of certain substances in plant extract samples [11].

The *Crataeva religiosa* is a species of tree that grows in the forests of the Mediterranean region. It has an interesting taste and texture which can be described as sweet, pungent, bitter, and astringent in nature. The leaves and the bark of this tree are used for medicinal purposes. The leaves and the bark of the *Crataeva religiosa* are used for medicinal purposes. The leaves are used for treating fevers and pain, while the bark is used to treat diarrhea, dysentery, and other gastrointestinal diseases [12]. *Crataeva religiosa* is a medicinal herb that is used for various urinary disorders. In this article, we will explore the benefits of *Crataeva religiosa* and how it can help with urinary disorders. It is a medicinal herb that has been used for various urinary disorders for centuries. It has been found to be effective in

treating conditions such as bladder spasms, urine retention and other similar conditions. It is also used as an antispasmodic, anti-inflammatory and diuretic agent [13]. The plant can also be found in many traditional medicines around the world. However, it has only recently been studied extensively by scientists in recent years to understand its potential uses and benefits. *Crataeva religiosa* is a litholytic herb that is used to cure people of benign prostate hyperplasia. It is also used as a treatment for diabetes, and it can be found in the form of an extract in many dietary supplements. The most common use of *crataeva religiosa* is as a treatment for benign prostate hyperplasia [14]. This condition occurs when there are too many cells in the prostate, leading to inflammation and pain. This herb can be found in dietary supplements, but it's most commonly found as an extract from the plant itself. *Crataeva religiosa* is an herb that has been used in traditional medicine to treat a variety of ailments. It is also used in alternative medicine and folkloric practices. *Crataeva religiosa*, also known as cratty, is a perennial herb with woody stems and leaves that grows up to three feet tall. The plant has been used for centuries for various purposes including as a remedy for snakebites and rheumatism. The present study was designed to develop a new simple, precise, rapid and selective HPTLC method for *Crataeva religiosa*. The purpose was to determine whether the plant contains Lupeol that might be used as medicine [15].

MATERIALS AND METHODS

Chemicals and Reagents

Lupeol (purity 99%) was procured from Sigma-Aldrich, New Delhi. All the chemicals, including solvents such as n-hexane, ethyl acetate, chloroform, methanol and petroleum ether were of analytical grade and were procured from E. Merck, India.

Collection of Plant Material

Crataeva religiosa Hook & Frost. (Capparidaceae), was procured from the surroundings of Tirupathi and used for the present study. Bark of *Crataeva religiosa* Hook & Frost. (Capparidaceae) 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 *Crataeva religiosa* Hook and Frost

Bark of *Crataeva religiosa* Hook & Frost. is 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 petroleum ether (60-80^{0}C), in a soxhlet apparatus [16]. Extract was filtered, the solvent was evaporated and accurate weight of the extract was taken [Figure 1].

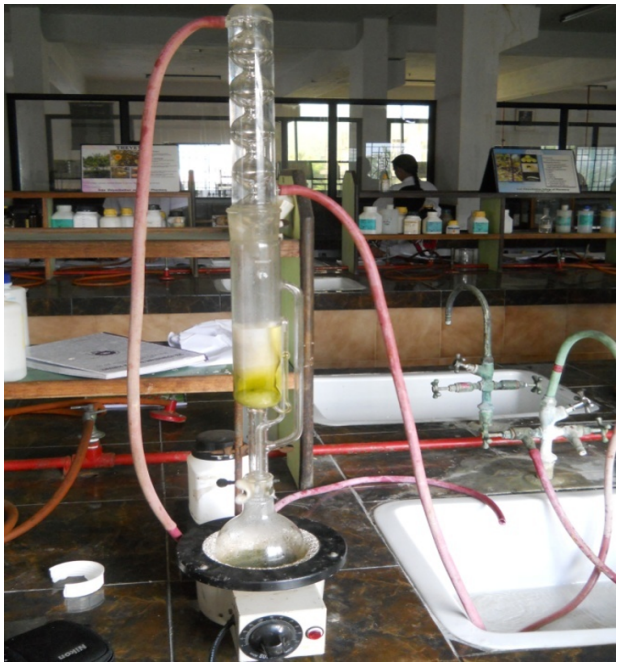

Figure 1: Extraction of *Crataeva religiosa* Hook and Frost

HPTLC Working Principle

HPTLC is a novel technology that uses a combination of HPLC and LC-MS to identify compounds in a sample. The technology can be used to identify the presence or absence of an analyte in a sample, or the identity of certain compounds. HPTLC is an analytical technique that has been used since the 1970s. It works by separating and analyzing molecules from a mixture using HPLC (high performance liquid chromatography) and mass spectrometry (MS). HPTLC is a water-based solution that is used to remove organic contaminants from water. This includes bacteria, viruses, and other microorganisms [15].

The working principle of HPTLC is that it uses oxidizing agents to break down organic contaminants into smaller molecules. The oxidizing agent in the process is hydrogen peroxide or ozone gas. The reaction creates a hydroxyl radical which breaks-down all types of organic contaminates into their respective compounds. This process can be used for both drinking and industrial applications, but it is mainly used for drinking water purification due to its low cost and the speed at which it can provide results [17].

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).

Sample and Standard Preparation for HPTLC Fingerprinting

Preparation of *Crataeva religiosa* Extract

Sample was prepared by taking 250mg & 380mg of extract in 10 mL of chloroform: Methanol (50:50).

Preparation of Lupeol Solution

6.14mg of lupeol was weighed and dissolved in chloroform: methanol (50:50) [18].

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 Whatmann filter paper lining.

Stationary Phase

TLC aluminium sheet precoated with silica gel 60 F_{254} (3x10 cm) was used as stationary phase, obtained from MERK [19].

RESULTS AND DISCUSSION

HPTLC is a process that involves washing, drying, and grinding of a soil sample in order to extract organic compounds in the soil. HPTLC is important for many agricultural purposes. It can be used for the analysis of soils and plants as well as their nutrients. Traditionally HPTLC was done with a laboratory instrument called an oven-drying apparatus. However, this instrument was expensive and took up valuable laboratory space. The process has been automated through the use of digital cameras and computers at some labs now which makes it faster, less expensive, more accurate, and more efficient than traditional HPTLC techniques. HPTLC is an acronym for "High-Performance Thin-Layer Chromatography." It is a technique of chromatography involving precipitation. HPTLC separates the analytes from each other by exploiting differences in their solubility in nonpolar organic solvents.

An HPTLC instrument consists of three parts; the solvent, the solvent chamber and the chromatographic column. The solvent chamber is where all

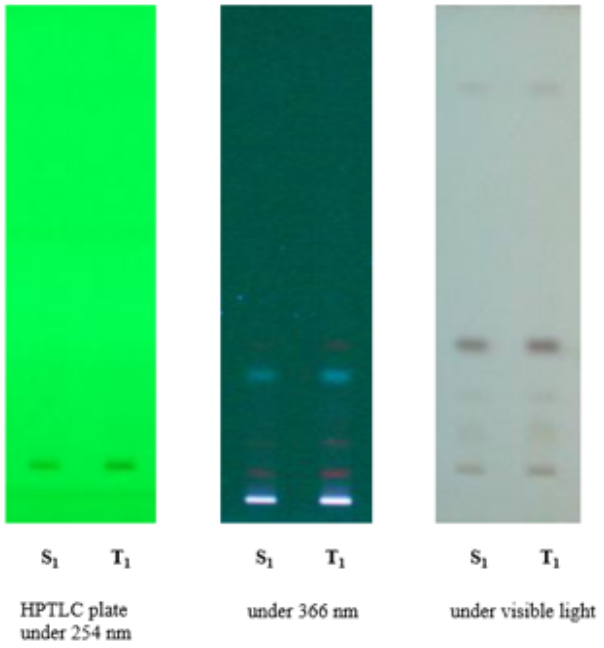

Figure 2: HPTLC report of *Crataeva religiosa* Hook and Frost, UV Detection of HPTLC plates at 254, 366 and visible light, S: Standard & T: Sample

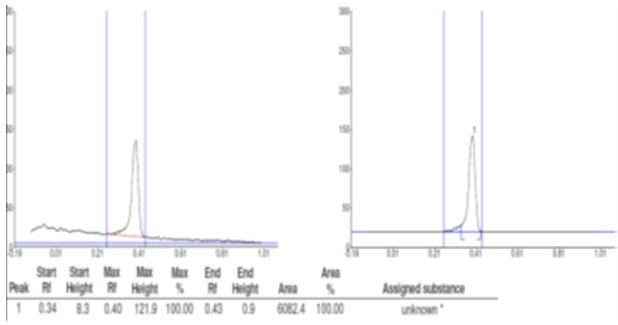

Figure 3: HPTLC Chromatogram of Lupeol

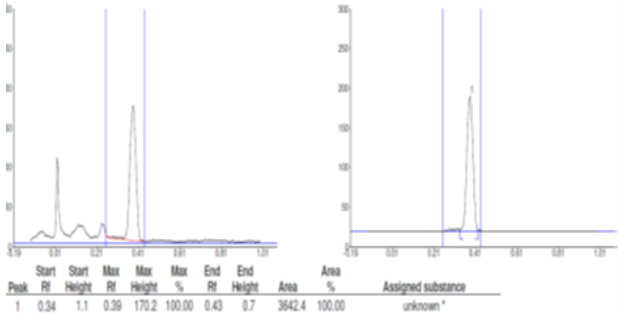

Figure 4: HPTLC Chromatogram of Petroleum Ether Extract of *Crataeva religiosa* Hook and Frost

adulteration tests and cleanup occur. All supplies required for HPTLC are stored in this area while they are being processed.

The chromatographic column includes a stationary phase (a mat) and a mobile phase (a liquid). The stationary phase determines the separation while the mobile phase provides energy to drive it by transporting analytes to it via capillary interaction between them [20].

HPTLC guidelines are important in analyzing herbal drugs. In the context of Ayurveda, they contain all the information one needs to accurately analyze a plant or herb. HPTLC stands for High Performance Thin Layer Chromatography, which can be seen as a method of separating different types of molecules and this process determines the identity of plants and herbs [Figure 2]. One must remember that HPTLC requires appropriate reagents to work correctly. HPTLC studies of the petroleum ether extract of bark of *Crateava religiosa* are carried out to standardize the presence of lupeol.

HPTLC Fingerprinting of *Crataeva religiosa* Extract

In precoated aluminium TLC plate 6μl and 9μl of *Crataeva religiosa* Hook & Frost extract and standard lupeol were applied as 6mm band using Linomat V applicator with Hamilton syringe. Applied plate was developed in a twin trough chamber containing n-Hexane: Ethyl acetate (90:10) as mobile phase. The plate was developed for a migration distance of 75mm. Developed plate was then derivatised with Libermann Buchard's reagent by dipping in it and dried at 110^0C in a hot air oven for 10 min. It was then scanned under all the three wavelengths and photo documented using Camag Reprostar. The Rf value and peak area of the extracts and standard were interpreted by using the software. The extract of *Crataeva religiosa* Hook & Frost was subjected to HPTLC analysis by using specific solvent systems like Hexane: Ethyl acetate (60:32) and detected under UV at different wavelengths. The HPTLC images indicate that all sample constituents were clearly separated without any tailing and diffuseness [Figure 3 and Figure 4]. The extract of *Crataeva religiosa* Hook & Frost has shown four different well-defined peaks indicating the occurrence of at least four different components. Standard lupeol was detected at Rf 0.43 and the same spot was revealed by extract of *Crataeva religiosa* Hook & Frost. The standard and sample spots were clear under visible light after derivatization. This confirms the presence of lupeol in the extract.

CONCLUSION

In conclusion, the HPTLC method has been developed with a few modifications and it can be used for the quantitative determination of lupeol from the petroleum ether extract of bark of *crateava religiosa*. The modified HPTLC method is more sensitive than the original one. It also has a wider range of detection limit. The new HPTLC method is based on the use of a solvent mixture of methanol, chloroform and water. This method is more specific in terms of the reaction time and temperature compared to the previous methods. The new HPTLC method also uses a shorter wavelength UV light source compared to the previous methods that used a longer wavelength UV light source. The new HPTLC method is one step closer to being able to determine lupeol in plant extracts with high accuracy and precision. HPTLC has many advantages over other types of HPLC systems. It is a simpler, more accurate and selective HPLC system that delivers the same quality results. HPTLC is a non-destructive, easy-to-use, and affordable process for separating chemical components in mixtures. The system is simple in design because it doesn't have any moving parts like pumps or valves. Lupeol is a natural compound that has been found to have anti-inflammatory and analgesic properties. The average recovery values of lupeol were found to be about 99.80%, which showed the reliability and suitability of method. The main component of this product is lupeol, which has been found to have a number of benefits when it comes to treating various ailments.

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Conflict of Interest

The authors declare that no conflict of interest associated with this work.

Contribution of Authors

Authors declare that the work done 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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