RESEARCH ARTICLE

Estimation of Ursolic acid in *Bauhinia racemosa* Lam. by High-performance thin layer chromatography method

Abstract

Plants have been utilized since ancient times to cure and manage a variety of diseases. Their capacity to heal or prevent ailments is emanates from the presence of various phytochemicals such as alkaloids, flavonoids, glycosides, terpenoids, steroids, tannins, phenols, etc. Pentacyclic triterpenoids have attracted the interest of researchers due to their diverse pharmacological activities.One of the Pentacyclic triterpenoids is Ursolic acid (UA) with significant biological effects, including anti-inflammatory, anticancer, antidiabetic, antioxidant, and antibacterial properties. *Bauhinia racemosa* Lam. is used in folk medicines for the treatment of various disorders. In the present work, a subtle HPTLC method has been developed for determination of Ursolic acid in *Bauhinia racemosa* Lam. The demonstrated method is simple, rapid, accurate and can be used for routine qualitative testing.

Keywords: Secondary metabolite, Pentacyclic triterpenoids, Ursolic acid, *Bauhinia racemosa* Lam, HPTLC

1. Introduction

Medicinal plants and their secondary metabolites are being utilized more often as supplemental medicine to cure diseases. A range of potent phytoconstituents may be found in the therapeutic plants [1]. The importance of plant secondary metabolites in industry, agriculture, and medicine has shed light on several studies concerning their biological activity, synthesis, and biosynthesis. It has been estimated that these active natural commodities are the source of at least 40% of medications [2]. Terpenes and their derivatives are an important class of natural compounds. Recently, pentacyclic triterpenoids have gained popularity as a class of terpenes. Ursolic acid (UA) (Figure 1) belongs to the C30 class of isoprenoid compounds. It is a pentacyclic triterpenoid with a molecular weight of 456.68 g/mol and a melting point of 283–285 °C [3].

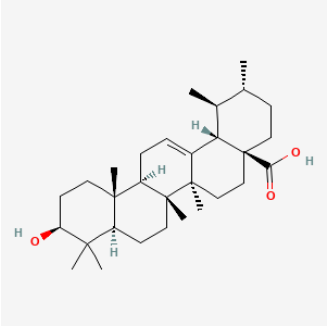

Figure 1. Structure of Ursolic acid

(Source: PubChem CID:64945)

Different pharmacological activities of ursolic acid are established, including anti-inflammatory [4], hepatoprotective [5], hair growth stimulant, and anti-tumor activity [6]. Pharmacological studies have shown that UA can interfere with a variety of signaling pathways involved in the survival and development of cancer and treat inflammatory or oxidative stress-related disorders, including cancer, cardiovascular disease and neurodegeneration [7].

Bauhinia racemosa Lam belongs to the Fabaceae family. It is found in India, Sri Lanka, and China. Many applications of plant parts in folk medicine have been described, including flowers as a diuretic [8]. The treatment for dysentery includes the use of flowers, buds, and dried leaves. The root bark is utilized in inflammation of the liver [8,9]. The seeds are tonic and aphrodisiac. Leaves are anti-diabetic in nature [10]. General phyto-pharmacological screening of the plant has showed that the ethanol extract of *B. racemosa* leaves has analgesic, antipyretic, and antiplasmodic properties [11] as well as antimicrobial activity and antihistaminic effect [10,12]. The plant's fresh flower buds demonstrated antiulcer action [13], as well as hypotensive and hypothermic activity [14,15].

Chromatographic technology is essential for both qualitative and quantitative research. HPTLC is an advanced method used to estimate biomarker levels. Synthetic medications increase the chance of developing illnesses such as cancer, diabetes, and neurological disorders, endangering global health. Natural herb-based treatments are urgently needed to address this issue. Indigenous remedies can mitigate the negative

effects of manufactured pharmaceuticals, providing a viable solution to the global health issue. The aim of the study is to develop a simple and rapid method for ursolic acid estimation since it has many pharmacological properties and wide application in therapeutics.

2. Material and Methods

2.1 Collection and Authentication of plant material

B.racemosa Lam. healthy leaves were obtained from Rani Baug in Mumbai, India. The species was identified and verified at St. Xavier's College Blatter Herbarium (Accession no. PD-431) Mumbai. Leaves were dried in the shade, then chopped into small pieces and roughly powdered.

2.2 Preparation of extract

3g of leaf powder was extracted in 300 ml of hydroalcoholic solvent (60:40) using Soxhlet apparatus. The extraction method comprised 12 siphon cycles lasting 8 hours, producing a dark green solvent that separated phytoconstituents from the plant. The extract was collected, and the solvent was removed using simple evaporation at ambient temperature. The concentrated extract was used for further analysis.

2.3 Preparation of standard stock solution for estimation of ursolic acid by UV Vis Spectrophotometer

To make stock solutions of Ursolic acid (Sigma-Aldrich), dissolve 1 mg in 1 mL of methanol (final concentration µg/mL). A standard graph was prepared using a series of dilutions ranging from 100 to 500 µg/ml.

2.4 Estimation of Ursolic acid [16]

A 200 µl methanolic solution was placed into a test tube and evaporated in a boiling water bath. The tube was filled with 0.3 ml of 5% vanillin glacial acetic acid and 1 ml of perchloric acid solution. The sample solutions were heat at 60˚C for 45 minutes and cooled under tap water. To the cooled solution, 5 ml of glacial acetic acid was added. The reaction mixture was stirred evenly using a vortex. The OD was measured at 544 nm on UV Vis Spectrophotometer (Labindia Analytical).

3. HPTLC method development

3.1 Preparation of stock

Stock solution - Suspend 10 mg of Ursolic Acid (Sigma-Aldrich) in 10 ml of methanol in a volumetric flask. Shake and sonicate the solution for 5 to 10 minutes. Further dilute the solution to 10 ml of methanol to prepare working solution.

3.2 Sample preparation

Accurately weighed 0.2 gm of sample in a 5 ml volumetric flask. Add methanol, shake and sonicate the solution for 5 to 10 minutes. Dilute the solution to 5 ml with methanol.

3.3 Optimization of chromatographic conditions

Initially, a variety of solvent combinations were used to achieve the best possible separation of the components. The solvents were selected depending on the polarity and type of the phytoconstituents. However, the best resolution was obtained using the optimized solvent system of Toluene: Ethyl acetate : Formic Acid (5: 1.5: 0.5 v/v/v). Merck cat. No. 5548 HPTLC silica gel 60 F254 was used as a stationary phase. 10µl of sample and 1µl of standard were loaded with a band width of 8mm.

3.4 Chromatography Determination and Derivatization

The pre-derivatized plates were developed with a mixture of Toluene: Ethyl acetate : Formic Acid (5: 1.5: 0.5) (v/v/v) as a mobile phase. Before development, the chamber was saturated with mobile phase vapour for 20 minutes. After development, the plates were dried with a dryer.The plate was sprayed with freshly prepared Vanillin Sulphuric Acid Reagent. Heat the plate at 110°C for 5-10 minutes. To confirm the identification of ursolic acid, the UV spectra of the samples and the standard were compared to ensure they had the same retardation factor (R_f). The plates were examined at wavelength of 540 nm.

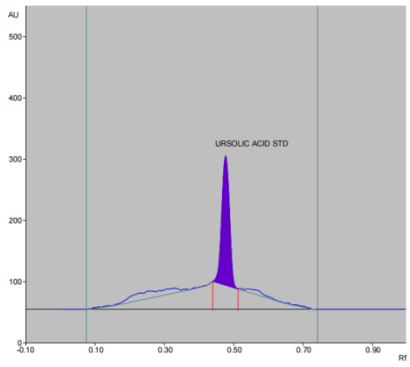

Figure 2a. Typical Chromatogram of standard ursolic acid

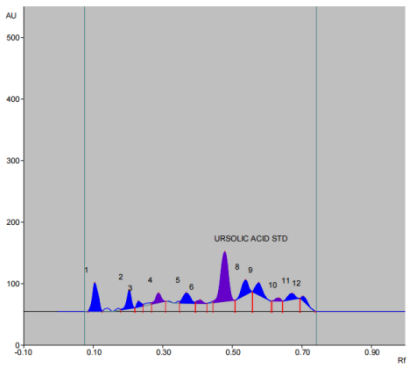

Figure 2b.Chromatogram of ursolic acid in *B.racemosa*

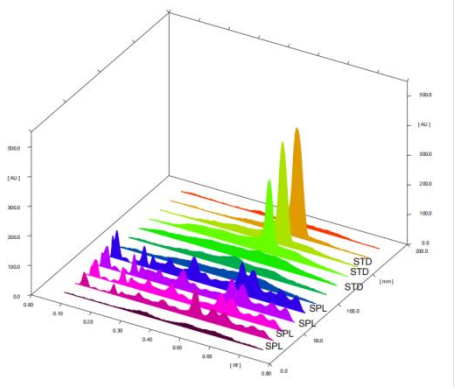

Figure 3. 3D spectra of Ursolic acid + *B.racemosa*

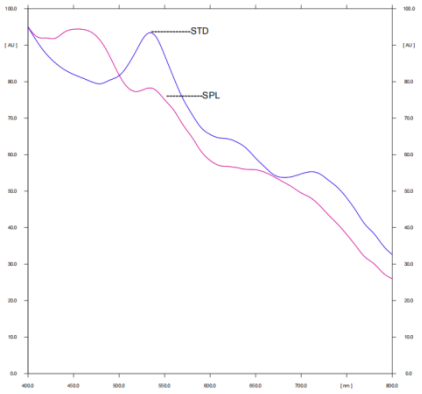

Figure 4. Spectral comparison of Standard Ursolic acid and *B.racemosa*

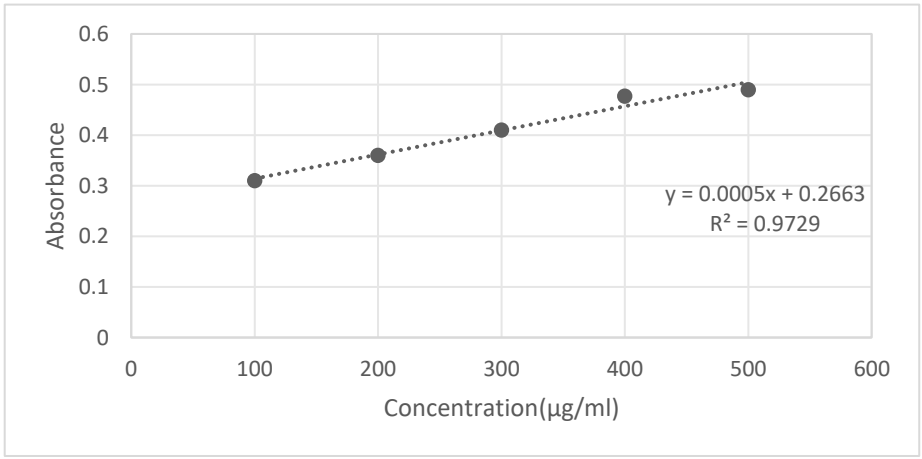

Figure 5. Graphical representation of the calibration curve of standard Ursolic acid

4. Results and Discussion

In the present study, the quantification of the contents of Ursolic acid and the HPTLC method was developed in *B. racemosa*. The proper solvent system for estimation is Toluene: Ethyl acetate : Formic Acid in the ratio 5: 1.5: 0.5. The chromatograms (Figure 2a,2b,3, & 4) were obtained upon scanning at 540 nm with Vanillin Sulphuric Acid Reagent.The content of ursolic acid in the present plant sample was found to be 0.09 ng/µg. The calibration plots (Figure 5) were linear in the range of 100-500 (µg/ml), and the correlation coefficient (r) was 0.9729 which indicates good linear dependence of peak area on concentration.

HPTLC's progress is recorded in a series of publications that demonstrate its present state-of-the-art [17]. Key features include the use of fine particle layers for efficient separations, sorbents with diverse properties for selectivity, semi-automated sample application and detection, accurate in situ chromatogram recording and quantification, biochemical detection to supplement spectroscopic methods, and the development of interfaces for mass spectrometric identification. These innovations have considerably increased the efficiency and accuracy of HPTLC [18].

UV spectroscopy is a low-cost method that can be used in laboratories without high-tech analytical instruments, particularly in developing countries where expensive instruments and skilled personnel are limited, as compared to complex, expensive, and time-consuming HPTLC methods [19]. However, HPTLC offers more resolution and the possibility of analyzing various analytes simultaneously as compared to UV-Vis spectrophotometry, which is commonly limited to single analyte quantification. In addition, HPTLC gives a visual representation of separation of the analyte and this makes possible qualitative and quantitative analysis in complex mixtures.

Chemical fingerprinting has been shown to be a thorough and realistic way of assessing the quality and identifying the species of traditional medications. It employs chromatographic methods to create distinct patterns of identification for herbal drugs, which may then be utilized to assess not only the presence or absence of certain markers, but also the proportion of all detected analytes [20-21].

A novel HPTLC approach has been developed for detecting and quantifying ursolic acid in apple peel extract [22]. UA has been discovered in the cuticular wax of numerous edible fruits, including cherry, loquat, pear, peach, quince, apple, and bilberry. The cuticular wax of five Rosaceae fruits contained high levels of this triterpene [23]. Several HPTLC investigations have been published for the quantitative measurement of ursolic acid in numerous plant extracts, such as the aerial portions of *Nepeta deflersiana* [24] and *Wattakaka volubilis* [25]. An HPTLC densitometric approach was devised to quantify ursolic acid in *U. dioica* leaves [26]. In *Alstonia scholaris,* a quantitative method was developed and quantified for monitoring ursolic acid [27].

Moreover, UA is gaining popularity because of its therapeutic benefits, which include anti-inflammatory, antioxidant, anti-apoptotic, and anti-carcinogenic effects [28]. This study attempted to establish a method which was found to be simple, accurate, specific and robust for the analysis of ursolic acid in crude drug samples.

5. Conclusion

In summary, leaves of *B. racemosa* Lam. can be considered as a good source of Ursolic acid. Since the diverse biological activities of ursolic acid reported in folk medicine and recent studies (such as antibacterial, antifungal, antiviral, anti-inflammatory, anti-oxidant, anti-wrinkle, anticancer, anti-hepatotoxic, antipyretic, and antidiabetic), and the fair content of that compound in the leaves was observed. This study thus opens the door to a more in-depth exploration of this indigenous plant, revealing novel possibilities for harnessing their benefits, ranging from dietary supplements to pharmaceutical formulations, and ultimately contributing to both scientific and practical applications.

Abbreviations

UA: Ursolic acid

HPTLC: High-performance thin layer chromatography

R_f : Retardation factor

ng: Nanogram

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Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

Conflict of interest

Authors declare that there is no conflict of interest.

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