

Estimation of Ursolic acid in *Bauhinia racemosa* Lam. by HPTLC method

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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.

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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].

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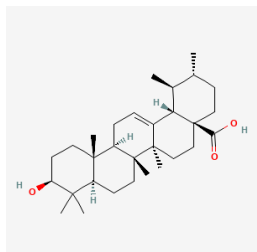


Figure 1. Structure of Ursolic acid

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 occurs in India, Sri Lanka, and China. Many applications of plant parts in folk medicine have been described, including flowers as a diuretic. 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 phytopharmacological 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 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.

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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.

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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

The leaf powder was extracted using Soxhlet apparatus using hydroalcoholic solvent (60:40). 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

To make stock solutions of Ursolic acid, dissolve 1 mg in 1 mL of methanol (final concentration $\mu\text{g/mL}$). A standard graph was prepared using a series of dilutions ranging from 100 to 500 $\mu\text{g/mL}$.

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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.

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3. HPTLC method development

3.1 Preparation of stock

Stock solution - Suspend 10 mg of Ursolic Acid 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.

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3.3 Optimization of chromatographic conditions

Merck cat. No. 5548 HPTLC silica gel 60 F254 was used as a stationary phase. 10 μ l of sample and 1 μ l 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. The plate was sprayed with freshly prepared Vanillin Sulphuric Acid Reagent. Heat the plate at 110°C for 5-10 minutes and detection was done at 540 nm wavelength.

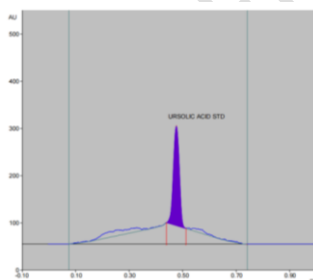


Figure 1a. Typical Chromatogram of standard ursolic acid

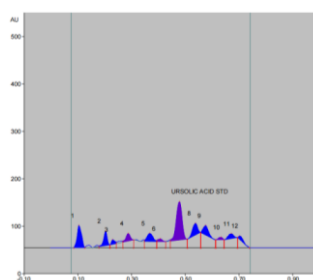


Figure 1b. Chromatogram of ursolic acid in *B. racemosa*

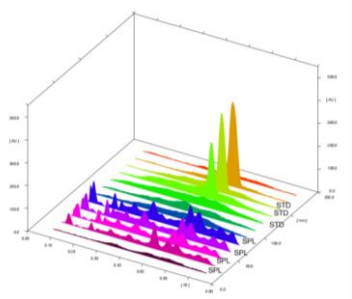


Figure 2. 3D spectra of Ursolic acid + *B.racemosa*

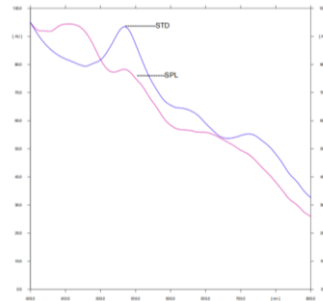


Figure 3. Spectral comparison of Standard Ursolic acid and *B.racemosa*

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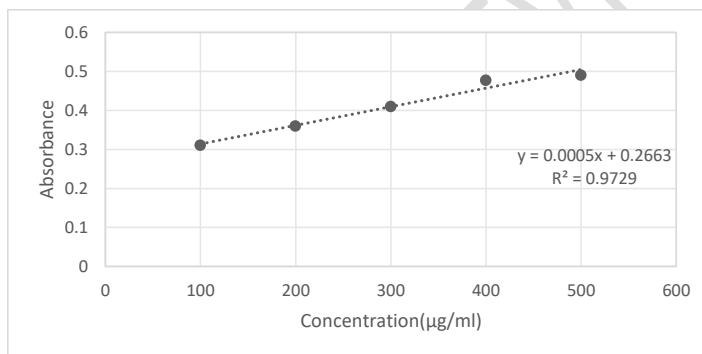


Figure 4. Graphical representation of the calibration curve of standard Ursolic acid

4. Results and Discussion

In the current study, the quantification of the contents of Ursolic acid and the HPTLC method was developed in *B. racemosa*. The chromatograms (Figure 1a,1b,2,3) were obtained upon scanning at 540 nm. The content of ursolic acid in the present plant sample was found to be 0.09 ng/µg. The calibration plots (Figure 4) 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.

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,

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which may then be utilized to assess not only the presence or absence of certain markers, but also the proportion of all detected analytes [17]. HPTLC uses much less solvents, making it more cost-effective and environmentally friendly. Furthermore, it is a rapid method of analysis that allows for the simultaneous processing of a high number of data while minimizing memory effects. Moreover, it enables the identification of all molecules, including those that are substantially maintained at baseline [18].

A novel HPTLC approach has been developed for detecting and quantifying ursolic acid in apple peel extract [19]. 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 [20]. 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* [21] and *Wattakaka volubilis* [22]. An HPTLC densitometric approach was devised to quantify ursolic acid in *U. dioica* leaves [23]. In *Alstonia scholaris*, a quantitative method was developed and quantified for monitoring ursolic acid [24].

Moreover, UA is gaining popularity because of its therapeutic benefits, which include anti-inflammatory, antioxidant, anti-apoptotic, and anti-carcinogenic effects [25]. 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.

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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, antioxidant, anti-wrinkle, anticancer, anti-hepatotoxic, antipyretic, and antidiabetic), and the fair content of that compound in the leaves was observed. We could presuppose that this plant may be appreciated in domains of pharmacology, pharmacognosy, cosmetology, and oncology.

Abbreviations

UA: Ursolic acid

HPTLC: High-performance thin layer chromatography

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.

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