Quantitative estimation of ursolic acid by HPTLC in methanolic extract of leaves *Streblus asper* and *Melissa officinalis*

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Abstract

Chromatographic techniques (HPTLC) was used for qualitative and quantitative determination of ursolic acid in methanolic leaves extract of *Streblus asper* and *Melissa officinalis* from the Moraceae and Lamiaceae family. It is considered to contain ursolic acid, confirmed by TLC and by qualitative test. Thus it was quantified using HPTLC a sensitive method for development of marker compounds. The method was carried out on precoated TLC aluminum plates with silica gel F 254 as stationary phase using solvent system as toluene: ethyl acetate: glacial acetic acid (7:3:0.2) in both extract. with Rf value of 0.636. Quantitative analysis was carried out in the absorbance at 530 nm. A good linear relationship (r2 = 0.9939 respective to peak area and height, Respectively) was observed between the concentration ranges of 200ng/spot to 1000ng/spot Thus HPTLC method provides a faster and cost effective quality control for the routine analysis of ursolic acid in extracts containing *Streblus asper and Melissa officinalis.*

Introduction

The human race is mainly dependent upon the plants and herbs for food and medicine. As per reports a total of 7000 species of plants are available throughout the world which are edible. Plants can be a storehouse of energy and wellness in terms of health benefits because of various phytochemicals and micro as well as macronutrients in them. The traditional use of plants is playing an important role acting as ethnomedicines amongst various tribes and communities. For example, medicinal plants are used as functional meals for medical and replacement needs as well as daily foods in China, Japan, Korea, and other nations (1).

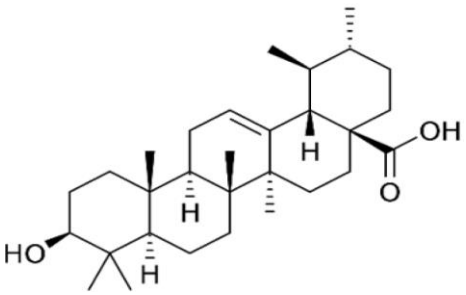
One such plant is the *Streblus asper* Lour from the family Moraceae. The plant is indigenous to India, Philippines, Malaysia, Thailand and Sri Lanka. The plant is believed to have antimicrobial, anti-inflammatory, antioxidant, anti-pyretic, anti-plaque, activities and also used in diarrhea and dysentery (2,3,4). The plant is vastly used in India mostly in the North Eastern states of the country. The local people use the plant as dental sticks to brush the teeth and it helps to get rid of dental problems and periodontal problems. In Assam the plant is called as ‘sora’ and people believe that the use of this as a dental stick can prevent all the ailments related to teeth and from ancient time it has been a reason for the long lasting teeth for the aged people in the state. The scientists have found that the plant is very rich in cardiac glycosides, triterpinoids as well as phytosterols (5). The tree has a number of uses. In Thailand it is one of the potential sources for paper making. Streblus

asper is a multipurpose plant that is primarily found in Vietnam. Its edible fruits are tasty, and it also produces leaves that can be used for cleaning and tea, medicinal seeds, animal feed, valuable timber, and ornamental potential.

Within the Lamiaceae family, Melissa officinalis L., or lemon balm, is one of the most well-known and traditional medicinal plants. Although it originated in the eastern Mediterranean region, it is now widely grown in both Europe and the US. The aromatic dried leaves that are traditionally utilized have antiviral and antispasmodic properties [6], and no negative effects have been reported to yet. The flavonoids (luteolin, kaempferol, and quercetin), phenolic acids (caffeic, chlorogenic, and rosmarinic acids), and volatile monoterpenes (citral and citronellal) that are present in alcoholic leaf extracts have been linked to their antiviral, antioxidant, antibacterial, anticholinesterase, and antiproliferative properties [7, 8]. More specifically, to better understand and treat infectious diseases and diabetes or obesity, respectively, the identification of antibacterial substances and α-glucosidase inhibitors of lemon balm leaf extract is required [9,10].

The pentacyclic triterpenoid ursolic acid (3b-hydroxy-urs-12-en-28-oic acid) found in Melissa officinalis and Streblus asperis is accountable for the plant's diverse pharmacological properties (Fig. 1). Ursolic acid is utilized as a starting material for the manufacture of powerful anti-tumor drugs [14], and it has antitumor, anti-inflammatory, and cardioprotective properties [11, 12, 13, 14, 15]. It also reduces muscular atrophy and stimulates muscle development activity in mice [13, 14].Various topical medicines in japan contain ursolic acid used for prevention of skin cancer [15]. The goal of the current study is to determine the best extraction technique and solvent to maximize the extraction process of ursolic acid from the leaves of Melissa officinalis and Steblus asper. This will be accomplished by quantitatively analyzing the ursolic acid through HPTLC in various

Ursolic acid



Material and Methods

Collection and authentication of plant material

Leaves of *Melissa officinalis* were collected in march 2023 from Mphmi Nursary, Delhi NCR and were authenticated by Dr. Sunita Garg Former Chief Scientist , Head ,RHMD, CSIR-NIScPR in Delhi.Leaves of *Streblus asper* were collected from Kadipur town, Sultanpur Local Area, They were collected in January 2023 and were authenticated by Dr. Sunita Garg Former Chief Scientist , Head ,RHMD, CSIR-NIScPR in Delhi.

Preparation of extracts

Samples of plants were cleaned with water, allowed to air dry for seven days at room temperature, and then oven dried at 40ºC to eliminate any remaining moisture. After being dried, the leaves were ground up and put away in an airtight container for later use. Secondary metabolites from plants exhibit a variety of biological functions. Methanol was employed in the extraction process because of its strong polarity, which allows it to extract a wide range of polar and relatively non-polar molecules such alkaloids, sterols, flavonoids, and carbohydrates. Methanol was used in a soxhlet device to extract the dried and coarsely ground material (10g) each. After the solvent extraction of the extracts to dryness, their consistent extractive values were noted [16].

Qualitative Phytochemical Analysis

The extracts were tested for the presence of bioactive compounds by using standard methods[17].

Flavonoids:

Extract mixed with few fragments of magnesium turnings. Concentrated HCl was added drop wise. Appearnce of pink scarlet colour after few minutes indicates the presence of flavonoids is indicated by the appearance of a pink scarlet color after a few minutes.

Tannins and Phenols: The sample was combined with 2 milliliters of a 2% FeCl3 solution. Phenols and tannins are indicated by a blue-green or black coloring.

Saponins: Combine extract and 5 milliliters of distilled water in a test tube and shake briskly. One way to determine whether saponins are present is to look for the creation of stable foam.

Alkaloids: Combine 2 milliliters of 1% HCl with crude extract and gently heat. Mayer's and Wagner's reagent was added to the mixture. Turbidity of the resulting precipitate is taken as evidence for the presence of alkaloids.

Phytochemical screening of methanolic leaves extract of *Melissa officinalis and Streblus asper*

Phytochemical	**Methanolic leaves extract of *Melissa officinalis***	**Methanolic leaves extract of *Streblus asper***
Steroids	+	-
Terpenoids	+	+
Flavonoids	+	
Phenols	-	+
Tannins	+	+
Anthraquinones	-	-
Carbohydrates	+	+
Glycosides	+	+
Alkaloids	+	+
Quinones	-	-
Saponins	+	+
Proteins	-	-
Amino acids	-	-

Procedure:

Standard ursolic acid solution:

A 100 mcg/ml solution of standard ursolic acid was made in methanol for preparation of the standard curve.

Procedure:

The five standard levels (4, 8, 10, 12 and 16 µl) of standard ursolic acid were used for calibration curve for which 4,8,10,12and 16 µl of standard solution was applied in duplicate on a TLC plate using a semi automatic Linomat V sample applicator. The chromatograph was developed for15 mints, dried at room temperature and the scanned at 530 nm; average peak areas of two standards were calculated. The calibration curve of the standard drug concentration (X-axis) over the average peak height / area (Y-axis) was prepared to get a regression equation by Win Cats software, which was used for the estimation of ursolic acid in *Melissa officinalis* and *Streblus asper.*

Chromatographic conditions:

Sample applicator (Linomat V) made by CAMAG (Muttenz, Switzerland) and a 100 µl syringe (Hamilton, Bonaduz) make up the HPTLC equipment. Aluminum HPTLC plates measuring 10 by 10 and 20 by 10 centimetres that had been precoated with silica gel F 254 (E. Merck, Darmstadt, Germany; provided by Anchrome Enterprises (I) Pvt. Ltd.) were used for the chromatographic separation. Version 1.4.8 of the VISIONCATS software and TLC scanner 4 were used to perform the absorbance mode of HPTLC densitometric scanning. Using a Camag Twin trough glass chamber, the solvent solution was created by mixing, toluene: ethyl acetate: glacial acetic acid (7:3:0.2) in both extract.

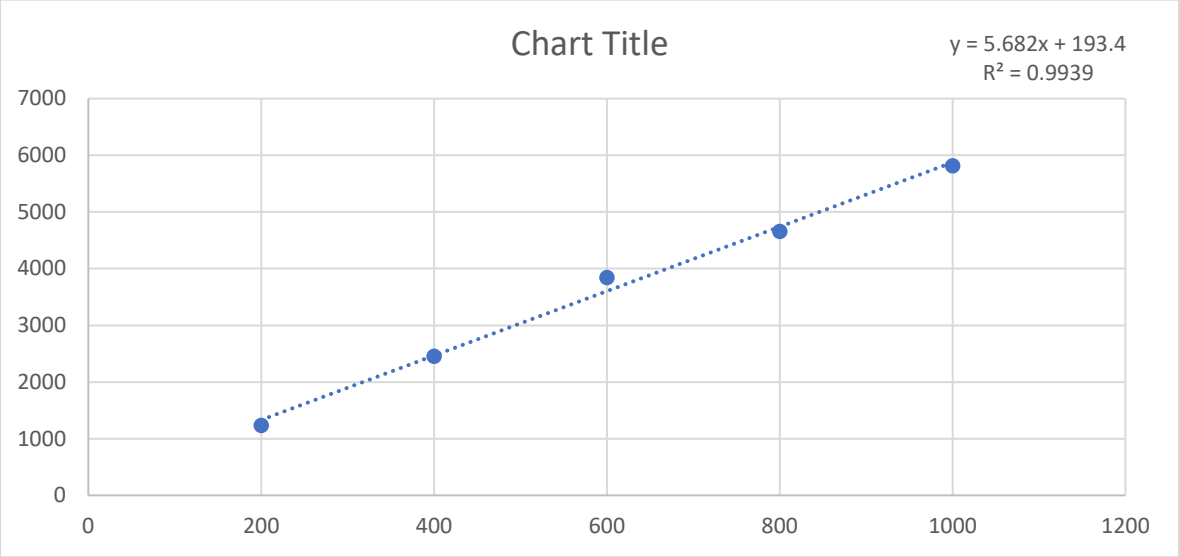
Estimation of ursolic acid in test sample:

The mean peak height / area of duplicate sample were calculated and the content of ursolic acid was quantified using the regression equation obtained from the standard curve.

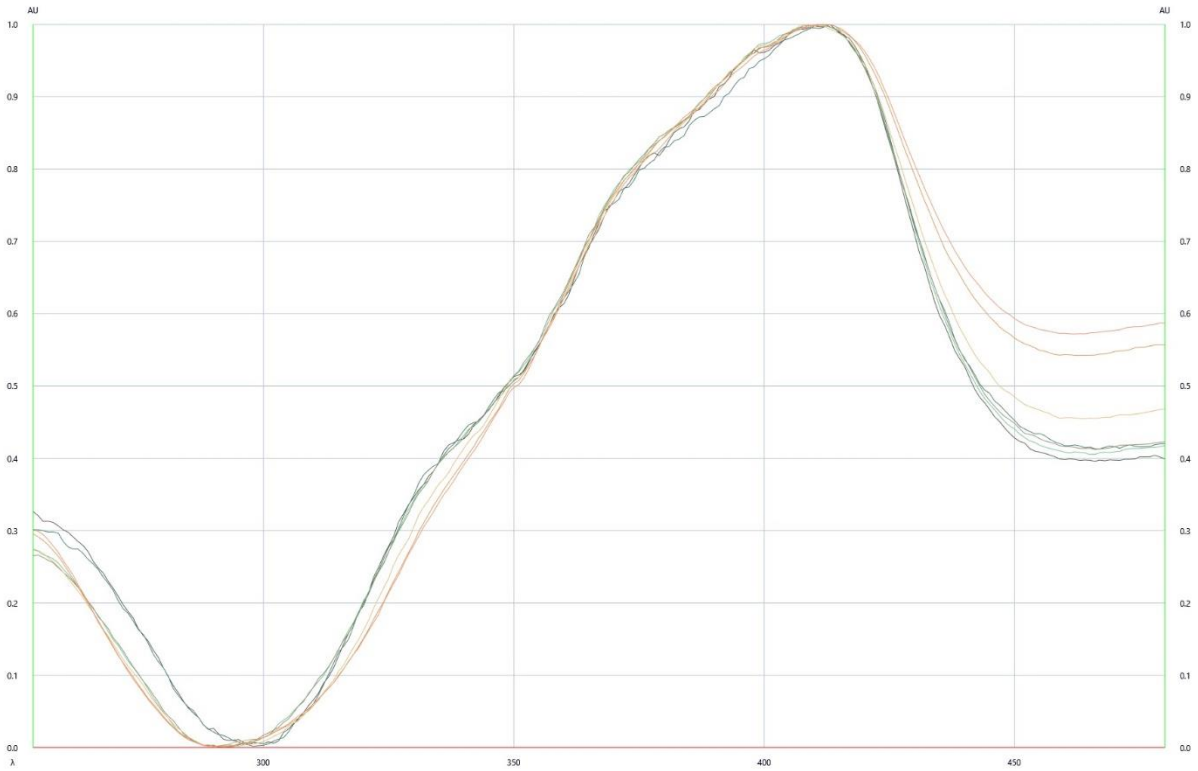
Result and Discussion

The standard ursolic acid has Rf value of 0.636 A good linear relationship (r2 = 0.9939 respective to peak area and height, respectively) was observed between the concentration ranges of 200ng/spot to 1000ng/spot. The regression equation was found to be Y= 5.682X +193.4 with respect to area, where Y is the peak area and X is concentration of ursolic acd. The highest content of ursolic acid was found to be 0.2 mg/100 mg in *Melissa officinalis* and 0.34 mg/100 mg in *Streblus asper* using the present HPTLC method.

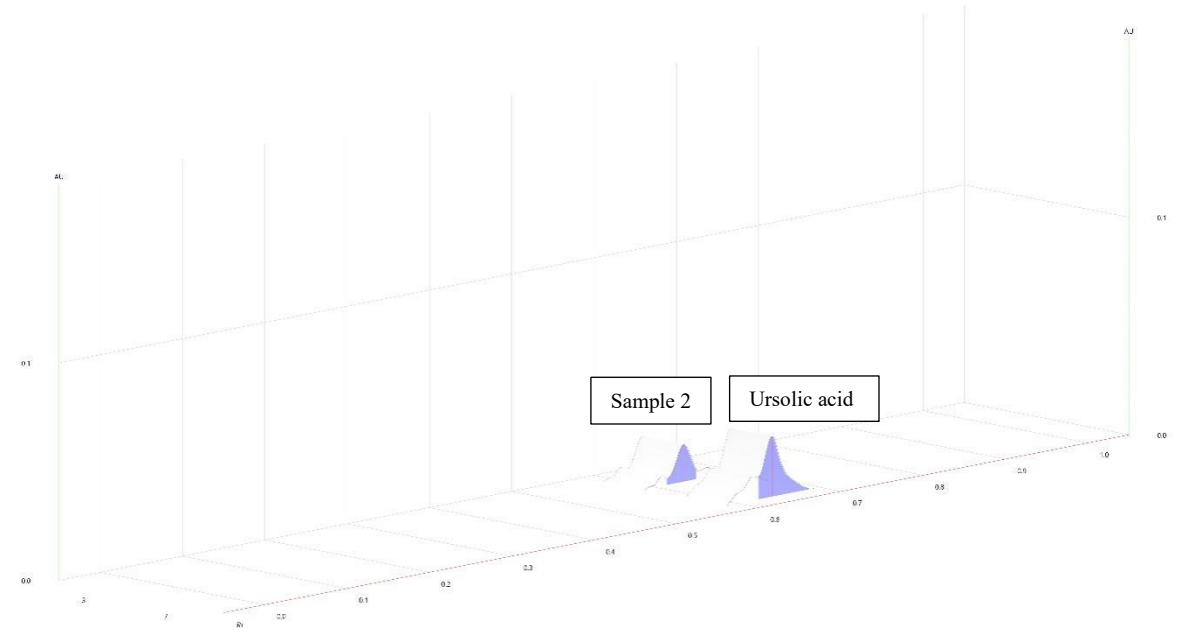
The UV spectrum of test sample is super imposable with that of standard ursolic acid indicating purity of peak. Simplicity, specificity and sensitivity of newly developed method makes it the apt choice for monitoring ursolic acid content for standardization of raw materials at the time of formulating a preparation as well as for the quality control of
finished product.



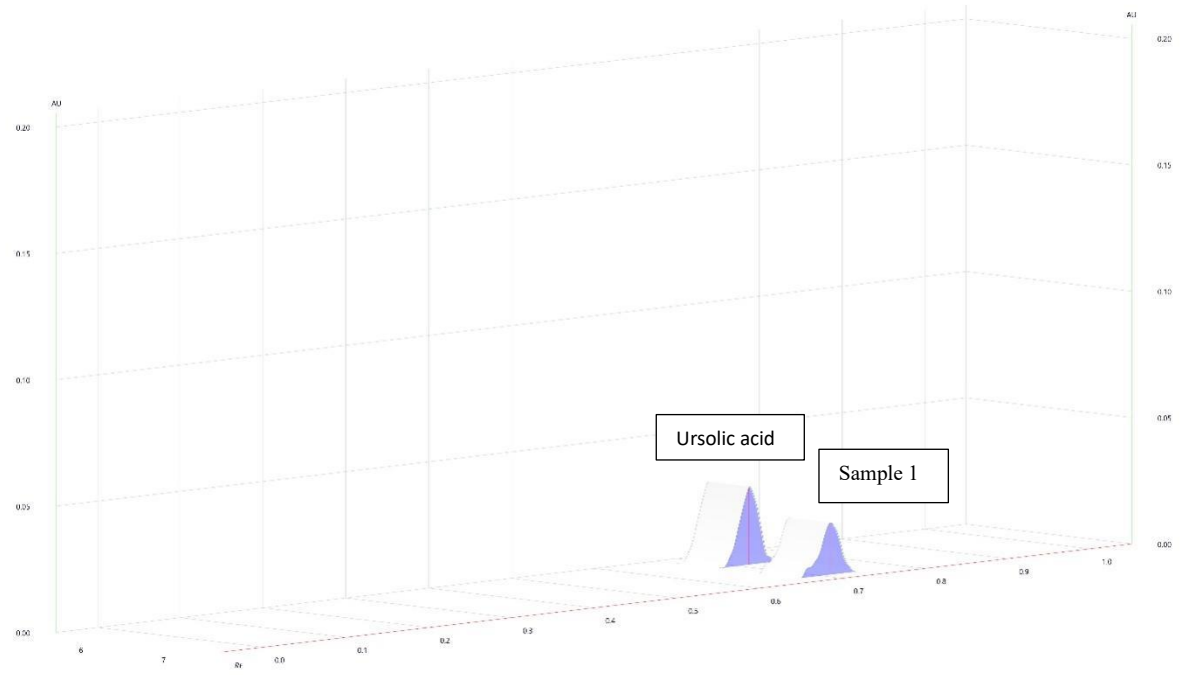
Calibration plot with respect to peak area by HPTLC at different concentration levels of standard ursolic acid.



Spectral scan of ursolic acid and sample



Chromatogram of Standard ursolic acid with Methanolic extract of leaves *Melissa officinalis*



Chromatogram of standard ursolic acid with Methanolic extract of leaves *Streblus asper*

Conclusion

Lack of standardization techniques fails to identify the drug from its originality which thereby exploits the usage of drug from itsTraditional System of Medicine. The plant *Melissa officinalis and Streblus asper* is used from the ancient time for its great medicinal values as a remedy in day today life but in this aspect adulterations are also done which leads

to its extinct. It may be stated that the approach given for the standardization of ursolic acid using the HPTLC fingerprint method should be followed for standardization of all Unani and Ayurvedic compound. The scientific, quality assessment parameter accepted by the World Health Organization (WHO) evolved in the present investigation will be helpful in checking the identity and quality and to detect adulterants/ substitutes.

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