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Quantification of Ursolic Acid from Different Geographical Locations in Order Myrtales

Research Article

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Abstract

Order Myrtales represents homogenous complexes of different chemical composition. Enormous bioactive compounds are believed to be significantly present in the different plant families which are proved to be potential for pharmaceutical uses. Once such notable active compound identified in the current research is Ursolic acid from the members of order Myrtales. Ursolic acid a pentacyclic terpenoid contributes to a wide range of pharmaceutical properties. In the current study 16 sample of healthy plants were collected from the different geographical regions of Maharashtra i.e., Mumbai (18.9781154°N 72.8367457°E) and Lonavala (18.7557°N,73.4091°E) in different seasons. The identification and quantification of Ursolic acid was carried out using spectrophotometric analysis.

Keywords: Myrtales; UrsolicAcid; Spectrophotometer; Bioactive Molecule; Maharashtra

Introduction

Identification of bioactive molecules in plants has laid down the foundation of modern therapeutic agents for the treatment of different health aliments in humans. Secondary metabolites play significant role in the physiological and biological functions in [1] A quality information of these significant chemicals can lead to the betterunderstanding of its therapeutic utility. With an advent of modernization, role of primarymetabolites has been better understood in development of plants, right from cell division,growth, respiration, and reproduction with various interlinked processes such as the Kerbscycle or citric acid cycle, glycolysis, photosynthesis, and associated pathways [2]"Secondary metabolites "or" Natural products" are produce dinascarce amount, and serve as main contributor of odours, tastes, and colours in plant, they are also identified as a key components of active defence mechanisms against various pest and plant pathogen.[3]The secondary metabolites are broadly classified into five main groups viz. Alkaloids, Flavonoids, Glycosides, Polyphenols and Terpenoids.

Terpenoids are one of the largest classes of natural products constitute incredible chemicaldiversity. Ursolic acid (3β-hydroxy-urs-12-ene-28-oic acid, UA,) a pentacyclic terpenoidcontributes to a wide range of pharmaceutical properties [4] It's a secondary metabolite present in a wide variety of plants in the form of a free acid and a glycone of triterpenesaponins chemically also known as merotine. The other synonyms of Ursolic acid are Urson, Prunol, Micromerol and Malol. [5] The natural source to Ursolic acid is stem bark, leaves, or fruit peel. The percentage composition of Ursolic acid might differ in different species, due to varying presence of enzymes responsible for synthesis.

The records of Ursolic acid from the natural sources are documented in the peel of Apple (*Malus domestica)*, Marjoram (*Origanummajorana*) leaves, Oregano (*Origanumvulgare*) leaves, Rosemary (*Rosmarinus officinalis*) leaves, Sage (*Salvia officinalis*) leaves, Thyme (*Thymus vulgaris*) leaves, lavender (*Lavandula angustifolia*) leaves and flowers, Eucalyptus (*Eucalyptus*) leaves and bark, black elder (*Sambucus nigra*) leaves and bark, hawthorn (*Crataegus* spp.) [4,6] Besides the above-mentioned plant species many other plants might carry potential.

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Quantity of UA. It consists of several pharmacological properties and modulates numerous signaling pathways counteract on chronic diseases. It also exhibits thermogenic anti-carcinogenic, anti-diabetic, antioxidant, anti-obesity, anti inflammatory, neuroprotective, hepatoprotective, and cardioprotective effects. The active mechanism of UA may involve nuclear factor- kappa B (NF-kB) and apoptoticsignaling in cancer cells, Marker expression in cardiac damage, insulin signaling in adipose tissue, inflammation, and the level of antioxidants in the brain, metabolic signaling and oxidants level in the liver, and atrophy signaling and metabolic signaling in skeletal muscles [1].

Finding a novel compounds (secondary metabolites or phytochemicals) from the plants isan achievement towards eliminating various human diseases [7] These Phyto-chemical analyses are invaluable tools for taxonomic differentiation within species, or for evaluating environmental factors affecting the presence of metabolites or variation in the biosynthesis of these metabolites [8,7] These metabolites in the form of biomarkers are widely used in medicine, biology, ecology,taxonomy and environmental chemistry due to its ability to cure certain diseases such as cancer, coronary heart disease [9].

The current study focuses on Ursolic acid (UA), a pentacyclic terpenoids with wide rangeof pharmaceutical importance [4] The presence of UA is widely documented in higher plants namely *Oscimum sanctum* L.,*Vaccinum myrtillus* L., *Harpagophytum procumbens* DC., *Sambucus nigra* L., *Mentha piperita* L., *Lavandula augustifolia* Mill, *Origanum vulgare* L., *Thymus vulgaris* L. *Crataegus laevigata (Poir)* DC, *Prunus laurocerasus* L., *Arctostaphylos uvaursi., Coffea arabica., Eucalyptus spp., Malusdomestica, Melissa officinalis Nerium oleander, Plantago major, Rosmarinus officinalis* [5]. The selection of marker compounds is based on certain factors, including value in identification of botanical source, significance in therapeutic or health relevance, availability of analytical methods and standards, ease of analysis, use as an indicator of quality and stability, and many more(American herbal products association 2001). These markers are also known as a valuable tool for fundamental and appliedresearch [10].

Plants biological component s selectively absorbs certain wavelength of light while reflecting others [11]. Concentration of various compound scan be determined by spectrophotometric method, a classical method at the same time the simplest way of identifying major compounds in the mixture. Identification of compounds solelybased on absorbance however represents strong limitations. Overlapping of absorbance band of the compounds presents complicates the estimation of individual compound concentration. The current study on quantification of Ursolic acid was carried out using Spectrophotometric Analysis.

Materials and Methods

Preparation of Methanolic extracts

Methanolic solutions were prepared by weighing about 0.1g of dried leaves powder in 10ml of Methanol, refluxed on boiling water bath at 60^{0}c for 1 hour. The extract was filtered using Whatman filter paper 41. The fresh filtrate was used for further analysis.

Preparation of standards

Stock solutions of Ursolic acid was prepared by dissolving1mg in1mL of methanol (final concentration mg/ml). A standard graph was prepared, considering series of dilutionsrangingfrom 100 mcg –500 mcg.

Estimation of Ursolic acid[12]

A volume of 200 µl methanolic solution was transferred in a test tube and was evaporated to dryness in a boiling water bath. A volume of 0.3 ml of 5% vanillin glacial acetic acid and 1ml of perchloric acid solution were added to the tube. The sample solutions were heated at 60˚C for 45 min and cooled under the tap water. To this cooled solution, a volume5ml of glacial acetic acid was added. The reaction mixture was uniformly mixed using thevortex mixture. The Optical density of the samples was measured at 544 nm on JASCO model V-530spectrophotometer, one of the most versatile economical and reliable instruments for UV/Visible.

Results

The optimized UV – Spectrophotometric method was used for the estimation of Ursolicacid, 16 different plant samples were considered under study using standard equivalence. Stock solutions of Ursolic acid was prepared by dissolving 1 mg/1 mL of methanol. Astandard dilution series were prepared ranging from 100 mcg – 500 mcg. The graph wasplotted corresponding absorbance of Ursolic acid (Y-Axis) and concentration of Ursolic acid (X-axis). Thus, the graph

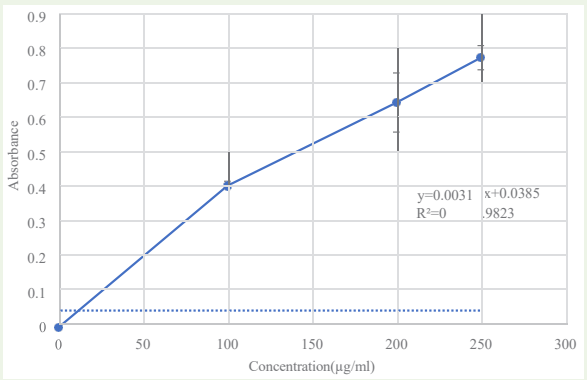

Figure 1: Calibration curve for Absorbance vs.Concentration of Ursolic acid

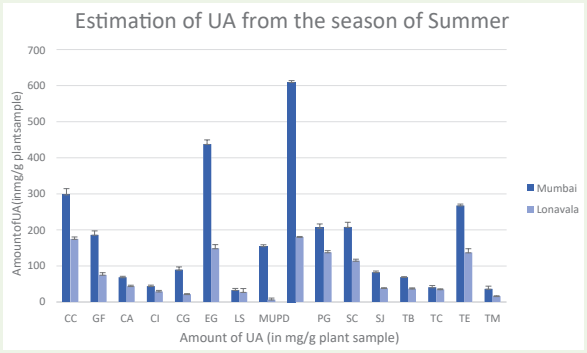

Figure 2: Estimation of UA from the season of Summer, in Mumbai and Lonavala

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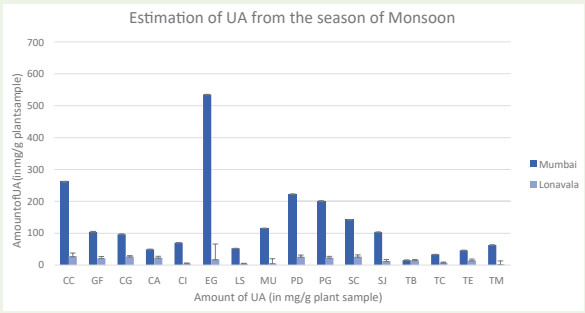

Figure 3: Estimation of UA from the season of Monsoon, in Mumbai and Lonavala

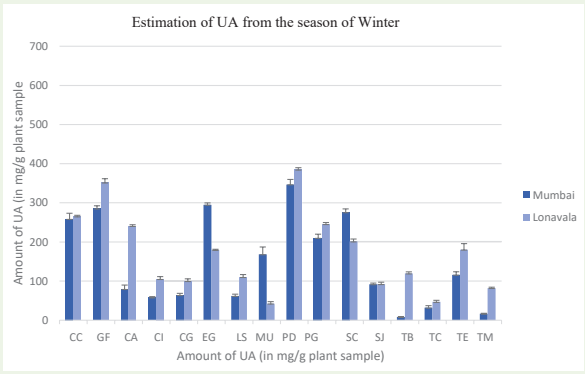

Figure 4: Estimation of UA from the season of Winter,in Mumbai and Lonavala

represents line ar response between 100 µg/ml concentrations to 250 µg/ml with a linear regression of 0.9823.

Where, CC-*Callistemon citrinus*, GF-*Getonia floribunda*, CI-*Combretum indicum*, CG-*Couroupitaguanensis*, EG-*Eucalyptusglobulus*, LS-*Lagerstroemiaspeciosa*, MU-*Memecylonumbellatum*, PD-*Pimentadioica*, PG-*Psidiumguajava*, SC-*Syzygiumcumini,* SJ-*Syzygiumjambos*, TB-*Terminaliabellirica*, TC-*Terminaliacatappa*, TE-*Terminaliacrenulata,* TM-*Terminalia mantaly.*

Conclusion

In the level of secondary metabolites observed in the study, this might be due the influence of environmental, geographical, seasonal or interaction with another organism etc.The content and composition of effective components,i.e.,the secondary metabolites in medicinal plants are vary with changes in the growth seasons, growth years and environment, increases or decreases under developmental process or stress conditions. (Ranaetal., 2020) [13]. Ursolic acid (UA) is a promising triterpenoid compound present in several parts of plants. The compound shows abroad range of pharmaceutical properties and therapeutic effects and exhibit thermogenic effects anti-carcinogenic, anti-diabetic, antioxidant, antiobesity, anti-inflammatory, neuroprotective, hepatoprotective, and cardioprotective [1].

The percentage composition of Ursolic acid might differ in different species, due to the presence of enzymes responsible for synthesis. The present study summarizes the presence of Ursolic acid from the plant sample collected in winter (Mumbai) and in summer (Lonavala) are adequate.

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