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Research Article

**ANALYSIS OF CALCIFEROL AND ELEMENTS IN AQUEOUS
EXTRACT OF *MUNTIGIA CALABURA* FRUIT USING
LC-MS/MS AND ICPMS TECHNIQUES****K.B. Leneeygreen^{1*}, K. Rasika¹, K. Rajathi²**¹ Department of Biochemistry, Dr. N.G.P. Arts and Science College, Coimbatore-641 048,
Tamil Nadu, India.² Professor, Department of Biochemistry, Dr. N.G.P. Arts and Science College, Coimbatore-641
048, Tamil Nadu, India.**Abstract:**

Muntigia calabura fruit have a lot of medicinal properties like antimicrobial, antioxidant, anti-inflammatory, and anticarcinogenic activities. It is used to reduce headaches, chronic pain, arthritis, blood pressure, prevent infection and boost immunity. This study planned to find out the amount of Calciferol, Cu, Zn and Selenium present in *Muntigia calabura* fruit. Aqueous fruit extract were prepared and analyzed by above mention tests using LCMS/MS and ICPMS analytical chemistry techniques. These both techniques are very advanced and assist to find out the accurate quantitative results. The sample preparation, analyzer method and calculations are carried out with reference to AOAC protocol. In this fruit extract contains 0.91 μ g/100g of Calciferol, 2.22mg/kg of Copper, 0.1mg/kg quantity of Selenium and Zinc metals were observed.

Key words: Copper, Zinc, Selenium, LCMS/MS, ICPMS, Calciferol, *Muntigia calabura*

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INTRODUCTION:

Muntingia calabura (Jamaica Cherry) is a plant of the Elaeocarpaceae family encountered in almost all tropical area due to its high adaptability. It is still used in Peru, Mexico, Colombia, Vietnam and the Philippines countries [1]. It is a fast-growing tree of slender proportions, grow 25 to 40 ft in height, with spreading, nearly horizontal branches [2]. The *M. calabura* leaf extract have high level of phytochemical, especially flavonoids, polyphenols. The plant fruit extract contains antioxidant activity, antinociceptive, antiproliferative, anti-inflammatory, and anticarcinogenic [1]. This plant is usually known for its antiseptic and antispasmodic properties besides being a proven hypotensive drug. *M. calabura* flowers has been used as anti-septic, anti-spasmodic, antidyspeptic, diaphoretic, tranquilizer, tonic and for the treatment of headache [3].

Liquid Chromatography/Mass Spectrometry (LC-MS) is fast becoming the preferred implement of liquid chromatographers. It is a wonderful analytical technique that combines the resolving power of liquid chromatography with the detection specificity of mass spectrometry. Liquid chromatography separates the sample components and then inserts them to the mass spectrometer. The MS creates and find charged ions. The LC-MS data may be used to provide information about the molecular weight, structure, identity and quantity of particular sample components [4]. The inductively coupled plasma (ICP), which has been introduced for a light source of atomic emission spectrometry (AES) of solutions, was first used as an ion source of mass spectrometry (MS) in 1980 by Houk and his co-workers. After the pioneering work by Gray, who used capillary arc plasma as an ion source [5]. Today, ICP MS is routinely deployed in diverse fields such as geochemistry, environmental and life sciences, industries (food, chemical, semiconductor, nuclear), forensic science and archaeology. After introduction of the first commercially available instrument in 1983, the technique has continuously improved. Several manufacturers produce reliable and robust instruments with very low detection limits (ppt) and high spectral resolution (10 000) for multi element isotope detection [6]. In the medical field, about 80%

of the drugs tested are derived from natural sources or are modified semi synthetically. The ultimate goal for any discovery is to offer appropriate and efficient antimicrobial drugs for the betterment of the patient [7]. In this present investigation is carried out for analyzing Calciferol and some elements in *Muntingia calabura* (Jamaica Cherry) fruit aqueous extract using LCMS/MS and ICPMS techniques.

***Muntingia calabura* systemic Classification**

Kingdom: *Plantae*

Order: *Malvales*

Family: *Muntingiaceae*

Genus: *Muntingia L.*

Species: *M. Calabura*

SAMPLE COLLECTION AND FRUIT EXTRACT PREPARATION

The fruit *Muntingia calabura* were collected from Krishnaraja Pura, Bengaluru. The fruit of *Muntingia calabura* is thoroughly washed in distilled water, fresh fruit aqueous extract is used following work.

MATERIAL AND METHODS:

Micro centrifuge tube, Pasteur pipet, Centrifuge, Water bath, Microwave digester, Nitric acid, Formic acid-LCMS grade, Methanol-LCMS grade, Acetonitrile-LCMS grade, Kinetix C18 column.

LCMS/MS and ICPMS advanced technology was used to detect the vitamin and elements. The Chromatographic and Mass spectrometer instrument settings are followed 2016.05B and 2016.05D protocol. Table-1 and Table-2 are clearly explain the instrument settings.

Table-1: Chromatographic instrument settings

Instrument Parameter	Value
Mobile Phase A	Aqueous with 0.1 formic acid
Mobile Phase B	Methanol 100%
Column	Kinetex C18
Oven Temperature	40°C
Chillier Temperature	15°C
Injection Volume	3µl
Initial Flow rate	0.6mL/min

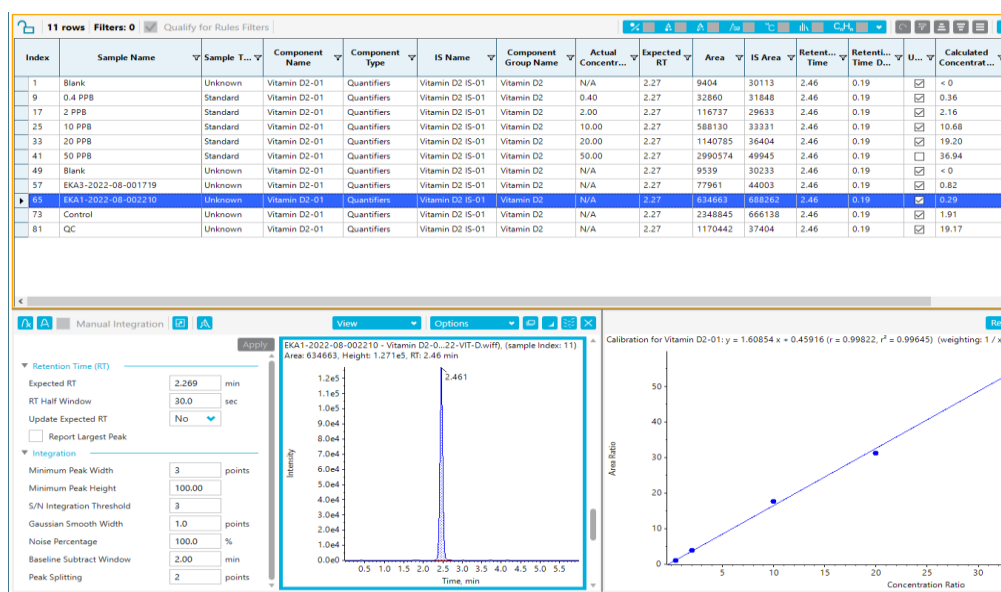
Table-2: Mass spectrometer instrument settings

Instrument parameter	Value
Ionization mode	ESI
Curtain gas	30 psi
Nebulizer gas	40psi
Heater gas	40psi
Collision gas	N2
Source temperature	300°C
Ion spray voltage	5500V

RESULT AND DISCUSSION:

The (AOAC 2016.5) official protocol is used for LCMS samples preparation and analysis [8]. Vitamin D is sensitive to light and it was Carry out all steps under UV shielded lighting. Vitamins are generally extracted from the saponified mixture with an organic solvent or a mixture of organic solvents. Liquid-liquid extraction method used for vitamin sample preparation, which usually requires multiple steps of extractions. The extracted mixture of vitamin-D concentrated into a vial is then dissolved in

acetonitrile to further eliminate other organic components insoluble in this solvent before LCMS/MS analysis. The prepared samples were loaded to LCMS/MS analyzer, C18 column used for sample separation and ESI ion source was used for sample ionization. After that MS was detected the quantity of vitamin-D in fruit extract using m/z ratio. The chromatogram peak was obtained 2.49 RT. 0.91µg/100g of vitamin-D was observed in *Muntigia calabura* fruit.

Figure-1: Chromatogram peak of vitamin-D

Sample is taken in microwave digester tube then added nitric acid and kept it for 30 mins pre digestion. After 1 hour of micro digestion it is cooled at room temperature. This sample diluted with double distilled water. The prepared samples was loaded to ICPMS analyzer, mainly 3 elements copper, zinc and selenium are analyzed. 2.22mg/kg amount of copper and 0.1mg/kg of Selenium and Zinc elements was observed in this fruit extract.

CONCLUSION:

This fruit have excellent amount of phytochemicals and other antibiological properties. Its contains good vitamin-D and copper properties. Basically vitamin-D helps to boost immunity and reduce the risk of autoimmune diseases. Copper also one of the essential nutrients of the body, it maintains healthy bone, blood vessels and immune function.

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DECLARATION OF INTEREST

The authors report no conflicts of interest.

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