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QUANTITATIVE ANALYSIS OF COUMARIN IN THE ROOTS OF *Chlorophytum borivilianum* BY HPLC

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ABSTRACT

Chlorophytum borivilianum Santapau & Fernandes (Liliaceae) is avery popular herbaceous medicinal plant. The roots of *C.borivilianum* contain various phytochemical used to treat various disease in our Indian medicinal system. Coumarin occurs naturally in many plants, this compound exhibit various pharmacological properties. The aim of this study is to analyse and compare the concentration of coumarin in plant roots of *C.borivilianum* collected from five experimental site of district Bareilly. The result of analyses show that the highest concentration of coumarin was found in the roots collected from Invertis University i.e.1938 µg/gm followed by Aonla, Fatehganjpurvi, Baheri & Meerganj i.e. 1624, 796, 595, 560 µg/gm.

Keywords: ***Chlorophytum borivilianum*** **Santapau, Fernandes, Coumarin, HPLC**

INTRODUCTION

Coumarins (1,2-benzopyrones) are ubiquitously found in higher plants where theyoriginate from the phenylpropanoid pathway. They contribute essentially to thepersistence of plants being involved in processes such as defence against phytopathogens, response to abiotic stresses, regulation of oxidative stress and probably hormonal regulation **[1]**.

The therapeutic use of medicinal plants is part of the history of humanity. Frequently the population of underdeveloped countries still depends strongly on medicinal plants due to its therapeutic properties and economic reasons **[2]**.

Chlorophytum borivilianum Santapau & Fernandes (Liliaceae) is an important medicinal plant commonly known as safedmusli, used in many Ayurvedic vital tonics and aphrodisiac formulations **[3]**. The saponins are considered to be a potent medicinal compound, found in tubers and impart medicinal value **[4]** but it also contain many other phytochemicals which also contain many therapeutic properties, one of them is coumarin.

Coumarin and its derivatives also show a wide range of bioactivities such asanticoagulant, oestrogenic, dermal photosensitizing, vasodilator, molluscicidal, anthelmintic, sedative, hypnotic, analgesic, hypothermic, antimicrobial, antiinflammatory, antifungal and antiulcer **[5]**.

Coumarin also use as component of natural flavourings agent, which is widely used in foods and pastries. The toxicity of coumarin has raised some concerns and food safety authorities have set a maximum limit of 2 mg/kg for foods and beverages in general **[6]**.

This paper describes quantitative determination of coumarins by HPLC in the roots of *Cholorophytum borivilianum.*

METHOD & MATERIAL

Collection and identification of plant

The experimental plant material *C. Borivilanum* was collected from five different area of Bareilly: Meerganj, Baheri, Aonla, Fatehganj Purvi and Invertis University. Further plant material was identified and voucher specimens were submitted in 'Herbarium' Department of Botany, Botanical survey of India, Allahabad. The plant material was dried under shade at room temperature for about 15 days. The dried plant samples were powdered by pastle & mortar and sieved to give particle size 40- 100 mm. The powder was stored in polythene bags at room temperature before extraction.

Chemicals

Toluene, acetone and chloroform used for thin layer chromatography (TLC analysis), extraction and isolation were of analytical grade. Acetic acid and acetonitrile used for HPLC analysis were of HPLC grade. All solvents& Standard were purchased from Sigma - Aldrich.

Extraction of Coumarin from roots of *C.borivilianum*

About 0.2 gm of tuber powder was taken in test tube and macerated with 4 ml methanol: ethanol (50:50) for 48 hr then sonicated for 15 min. and again leave it for 48 hr. with occasional shaking. After that centrifuge it and collect the supernatant.

TLC Analysis

The whole isolation procedure was monitored by using thin-layer chromatography (TLC) analysis and was performed on the TLC precoated silica gel plates. The TLC chromatograms were developed using a solvent system composed of Toluene, acetone and chloroform (9:11:1). The pure single compound on the TLC plate was spotted with the standard and the loaded plates were placed in the TLC jar which contained the solvent system. After the completion of the run the plates were taken out and kept at room temperature to get dried for 10 minutes. Chromatograms were observed under UV-lamp on 2 standard wavelengths, 254 nm and 366 nm.

HPLC Analysis

Quantitative analysis of *C. borivilianum* for analysis of coumarin was done by using Shimadzu SPD 20A (UV detector). LC solution software was used for data acquisition and computation and reserve phase HPLC with C18 Column was used for Chemical analysis. All the solvents used in this study were purchased from Sigma-Aldrich. The mobile phase was prepared by mixing acetic acid (0.5%) and acetonitrile. The mixture was filtered through a 0.2 µm pore size filter using vacuum pump and sonicated for 30 minutes. The flow rate and injection volume were 1 ml/min and 20 µL, respectively. The detection wavelength was 280 nm.

The stock solution of standard was prepared in methanol at a concentration of 10 mg/ml. Samples used for calibration curve was prepared by a series of dilutions from the stock solution with methanol at a final volume of 1 ml in the concentration range of 20, 40, 60, 80,100 µg/ml. Calibration curve of coumarin standard with linear relationship between the peak area at the Yaxis and the concentration of standard coumarin at the X axis **(Figure 1)** and the chromatogram of

coumarin standard shown in **Figure 2**, having retention time of 2.27 min.

RESULT & DISCUSSION

In this work, TLC utilized for the qualitative chemical analysis of coumarin in the extraction. Detection of developed spots is carrying out according to the principles of visualisation of chromatograms. Chromatograms are observed under UV-lamp on 2 standard wavelengths, 254 nm and 366 nm. Fluorescence of separated spots in comparing to fluorescence of standards could be considered reliable information in identification of analysed coumarin derivatives. This substance gives intensive fluorescent spots of blue-white as standards and got corresponding spots from the extracts of all area.

Qualitative analysis of coumarin was carried out on the basis of the standard curve obtained from the authentic marker [y = 7902.1x - 153061(R^2 = 0.9632)]. The standard curve was found to be linear in the range of 40 – 100 µg/ml and with the help of linear equation we can calculate the concentration of sample. Among all the five areas highest concentration of coumarin was found in the roots collected from Invertis University i.e.1938 µg/gm followed by Aonla, Fatehganjpurvi, Baheri & Meerganj i.e. 1624, 796, 595, 560 µg/gm.A chromatogram of coumarin from Invertis University sample (1 mg/ml) is shown in **Figure 3**.

As saponin is the potent medical compound in *C.borivilianum*, no one has quantify coumarin in *C.borivilianum* but quantitatively determined in other plants. Solaiman and Al-Zehouri, 2017 determine the concentration of coumarin in the extract of cinnamon bark by HPLC and found average concentration of coumarin in cinnamon bark extract is 916.71 mg/Kg **[6]**. Lucilia *et al*., 2015 analyse the concentration of coumarin in hydro-ethanolic extracts of oven dried and lyophilized leaves of *M. laevigata* is 775 µg/ml and 1131 µg/ml **[7]**.

Vaľko *et al*., 2018 quantitative determination of coumarins in different extracts (methanol, butanol, water) of leaves of *Philadelphus coronaries L., Philadelphus magdalenae Rehd., Philadelphus pekinensis Rupr., Philadelphus schrenkii Rupr., Philadelphus subcanus Koehne, Philadelphus tenuifolius Rupr. et Maxim. and Philadelphus zeyheri Schrad*. The content of coumarins was determined as umbelliferone using spectrophotometry. The highest content of coumarins was recorded in methanol extract (0.94%) of species *Philadelphus schrenkii* Rupr., and butanol (2.23%) and water extract (2.47%) of *Philadelphus subcanus* Koehne.

The results indicate higher content of coumarins in butanol and water extract compared to methanol extract **[8]**.

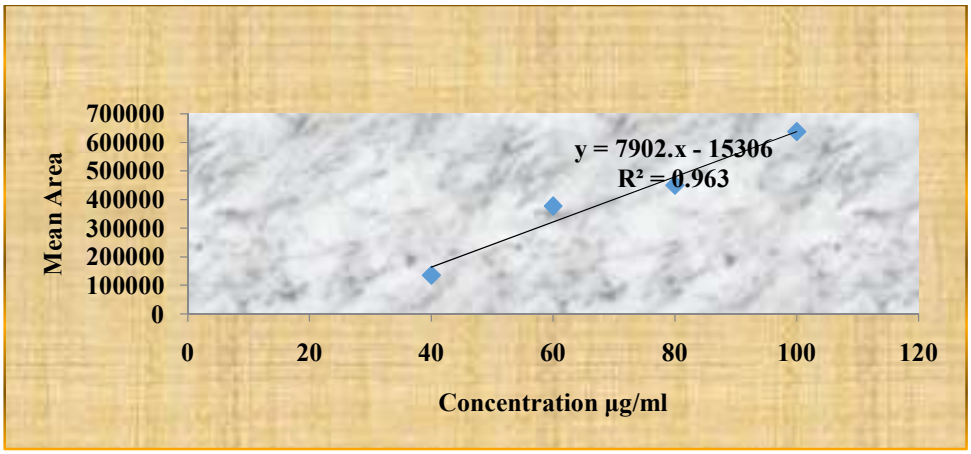

Figure 1: Calibration curve of mean area against concentration of coumarin standards

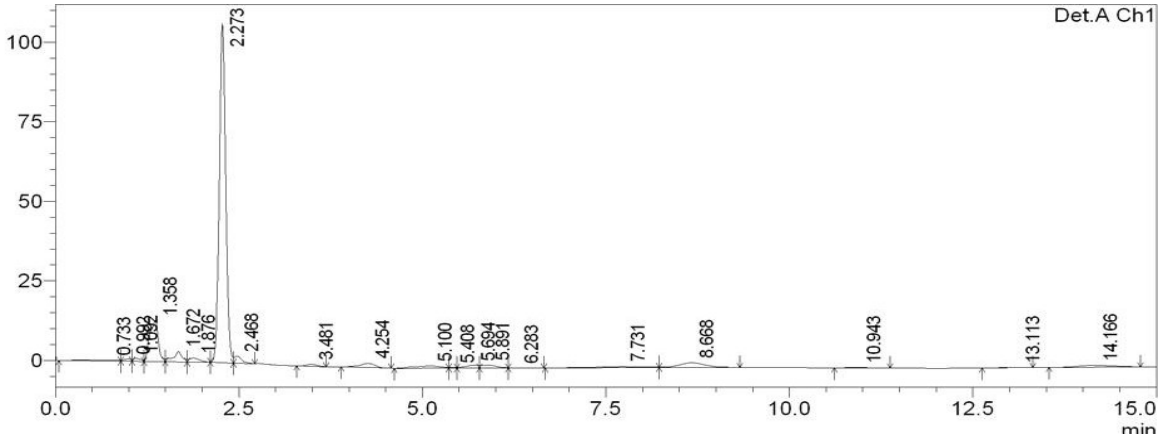

Figure 2: Chromatogram of standard (Coumarin) with concentration 60 µg/ml

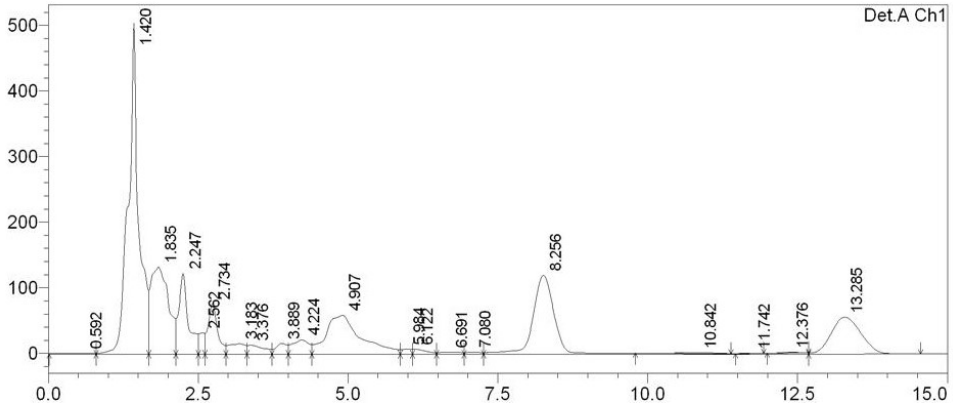

Figure 3: Chromatogram of Invertis University sample (1mg/mL)

CONCLUSION

This study deals with quantitative determination of coumarins in the root extract of *C.borivilianum* plant. The concontratio of coumarin was determined using HPLC. Among all the five areas highest concentration of coumarin was found in the roots collected from Invertis University and least concentration was found in the roots collected from Meerganj. From the result it could be conclude that variations in the concentrations of compound in all area maybe due to the change in location so the edaphic factors will also be changes and due to cross pollination genetic makeup of the plants was change. That is why different results were found **[9, 10]**.

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