



**HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC
PROCEDURE FOR THE QUANTITATIVE ESTIMATION OF TANNIC
ACID IN ROOTS OF *Chlorophytum borivilianum***

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ABSTRACT

Chlorophytum borivilianum Santapau & Fernandes (family: Liliaceae) is a small perennial herb with a condensed disc-like stem. The root of *C. borivilianum* is widely used for the treatment of various human ailments. Tannic acid occurs naturally in many plants, this compound exhibit various pharmacological properties. The aim of this study is to analyse and compare the concentration tannic acid in plant roots of *C. borivilianum* collected from five experimental site of district Bareilly. The result of analyses show that the maximum tannic acid content is 2804 µg/g in the roots of *C. borivilianum* which collected from the Aonla, followed by the samples of other locations Invertis University Campus, Fatehganj Purvi, Meerganj and Baheri as 2405, 2161, 1631 and 1168 µg/g, respectively. From the results it could be conclude that the difference in the concentration might be due to the change in location and genetic variation.

Keywords: *C. borivilianum*, estimation, HPLC, quantitative, roots, tannic acid

INTRODUCTION

Since ancient times, plants are widely used for the treatment of various ailments in Ayurveda, Siddha and Unani system of medicine. According to the World Health Organization, about 80% of the population of developing countries depends on traditional medicine to meet their primary healthcare needs; this may be due to availability and affordability of the plants [1] [2]. In recent years, the increasing awareness towards the use of traditional medicine in healthcare paved the way to explore the huge potential of plants knowledge for treating various diseases [3].

Chlorophytum borivilianum Santapau & Fernandes is a medicinal plant belonging to the family Liliaceae. It is commonly known as 'safed musli' and is widely used in Indian systems of medicine by indigenous practitioners. This plant is considered as a 'white gold' or 'Divya Aushad'. It is a small perennial herb with a condensed disc-like stem from which a whorl of leaves arise [4, 5]. The roots of *C. borivilianum* has been traditionally used to treat various kinds of human diseases, including arthritis, diabetes, high blood pressure, chronic leucorrhoea, delayed menopause and erectile dysfunction [6, 7]. It was found that the oral administration of aqueous extract from the dried roots of *C. borivilianum* in healthy male rats could increase the sperm count [8]. In addition,

oral administration of this plant extract in hyperglycaemic male rats may provide protection against sexual dysfunction [9].

C. borivilianum is in high demand both at the national and international drug market due to its phytochemical constituents. The roots of this plant contain the diverse groups of phytochemicals, such as alkaloids, vitamins, minerals, proteins, carbohydrates, steroids and amino acids [4].

Polyphenols are a chemically diverse group of secondary metabolites that occur naturally in many plants. Tannic acid is a water-soluble polyphenol that is present in various plants. This compound possesses various pharmacological properties, such as antibacterial, antienzymatic, astringent, antioxidant, anticarcinogenic and antimutagenic [10, 11].

Therefore, the aim of present study is to develop a high-performance liquid chromatography (HPLC) method for the quantitative determination of tannic acid in the roots of *C. borivilianum*.

MATERIALS AND METHODS

Materials

A total of five accessions of *C. borivilianum* were collected from different location of Bareilly district, Uttar Pradesh, India. The accession was authenticated by Central Region Herbarium of the Botanical

survey of India, Allahabad. The young roots of all the accessions were collected

for chemical fingerprinting analysis of Tannic acid.

Table 1: Details of *C. borivilianum* accessions used in the HPLC fingerprinting analysis

S. No.	Accession name	Collection Site	Latitude of site	Longitude of site
1.	Chr-1	Baheri	28.7780°N	79.4957°E
2.	Chr-2	Meerganj	28.5421°N	79.2085°E
3.	Chr-3	Fatehganj Purvi	28.0797°N	79.6320°E
4.	Chr-4	Aonla	28.2699°N	79.1605°E
5.	Chr-5	Invertis University	28.2919°N	79.4931°E

Chemical diversity analysis

Chromatographic condition for chemical fingerprinting

Chemical fingerprinting of *C. borivillianum* for analysis of Tannic acid was done by using Agilent technologies 1120 compact LC (UV detector). EZ chrome Elite software was used for data acquisition and computation and reserve phase HPLC with Eclipse Plus 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 methanol and water in the ratio of 50:50 v/v with pH 4.5. The mixture was filtered through a 0.2 µm pore size filter using vacuum pump and sonicated for 30 minutes.

Quantification of Tannic acid

The quantification of Tannic acid was done by using a method previously used by Durgawale *et al.* (2016) [12]. The roots of *C. borivilianum* were dried in shade at room temperature for 15 days. The dried samples were grind in mortar and pestle, and sieve it. The solution was filtered through Whatman filter paper and sonicated for 30 min and concentrated on water bath at 50

°C. After this step, the extracted samples were stored at 20°C till further use. All the samples prepared for HPLC analysis were filtered through a 0.45 µm syringe membrane filter before use. Quantification of Tannic acid was done at 270 nm wavelength with flow rate 1 ml/min and 10 min run time of sample. Quantitative analysis of tannic acid was carried out on the basis of standard curve obtained from the authentic marker ($y = 65211x - 166048$, $R^2 = 0.9838$). The curve was found to be linear in the range of 20–100 µg/mL. The concentration of tannic acid in the samples was calculated with the linear equation. Calibration curve of mean area against different concentrations of tannic acid standard is shown in **Figure 1** and the chromatogram of Tannic acid standard shown in **Figure 2**, having retention time of 1.8 min.

RESULTS & DISCUSSION

In the studied samples, the highest concentration of tannic acid was 2804 µg/g in aqueous extract of *C. borivilianum* roots that collected from Aonla (Chr-4), followed by Invertis University (Chr-5), Fatehganj Purvi (Chr-3), Meerganj (Chr-2) and Baheri

(Chr-1) as 2405, 2161, 1631 and 1168 $\mu\text{g/g}$, respectively. A chromatogram of tannic acid from Aonla sample (1 mg/mL) is shown in **Figure 3**. 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 [13, 14].

From the above result it could be conclude that the environment of the Bareilly is suitable to cultivate the *C. borivilianum*. As this plant contains various phytochemicals having many medicinal properties so it could also be cultivated on the commercial level in Bareilly specially in the area of Aonla because the concentration of tannic acid found in high concentration compare to the other locations. The edaphic factor of Aonla (Chr-4) found more suitable for the cultivation of plant. There is huge demand of phytochemicals in pharmaceutical industries for the production of new drugs to treat various diseases.

C. borivilianum is medicinal plant so it contains many of the phytochemicals having various medicinal properties. Tannic acid one of the phenolic compound found in the plant having various medicinal properties i.e. anticarcinogenic, anti-microbial, anti-inflammatory or anti-AIDS activities [15] also used in many of the

industries i.e. dyestuff industry, food industry to clarification of fruit, beer and wine [16].

Durgawale *et al.* 2016 quantify the tannic acid in *Pueraria tuberosa* and found 50 $\mu\text{g/mL}$ of aqueous extract contained 13.98 $\mu\text{g/mL}$ of tannic acid [12]. Niranjana *et al.*, 2009 estimated total phenolic compound in *C. borivilianum* root were 4.9 mg/g GAE [17]. It was found that the fruits of *Terminalia chebula* contains 55% of condensed tannins [18]. Hussein *et al.*, 2017 reported that 853 $\mu\text{g/gm}$ of tannic acid occurs in the fruits of *Rubus idaeus* by reverse phase-HPLC analysis [19]. Boligon *et al.* 2012 reported the tannin content in the stem bark of *Scutia buxifolia* by HPLC/DAD method that ranges 176.70 ± 0.24 to 66.67 ± 0.17 mg/g [20]. The highest concentration (844.59 ± 10.38 mg/g) of tannic acid was found in ethanolic extract of *Peltophorum pterocarpum* flowers, whereas the lowest concentration (9.54 ± 6.98 mg/g) in ethyl-acetate extract of leaves [21]. Gupta and Garg, 2014 estimated the concentration of tannic acid using HPLC method in raw material of *Aegle marmelos*, *Embllica officinalis*, *Terminalia belerica*, *Terminalia chebula* and *Trichyspermum ammi* as 4.79 ± 0.98 , 6.17 ± 0.37 , 8.67 ± 0.03 , 13.96 ± 0.745 and $0.668 \pm 1.002\%$ w/w, respectively; and also in three identical batch of Bhuvnesvara vati BV-I,

BV-II and BV-III as 2.62 ± 0.75 , 2.59 ± 0.36 , 2.63 ± 0.24 % w/w, respectively [22].

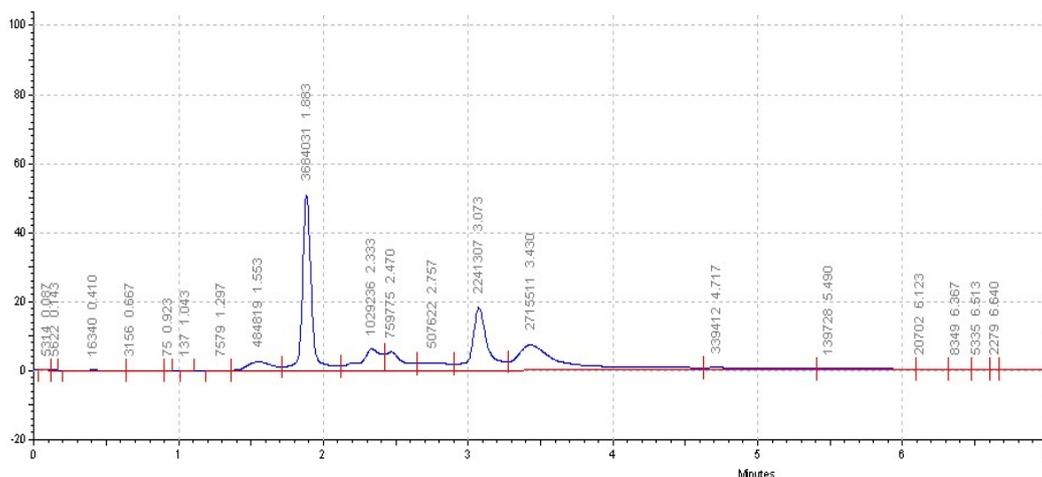


Figure 1: Chromatogram of tannic acid standard (60µg/mL)

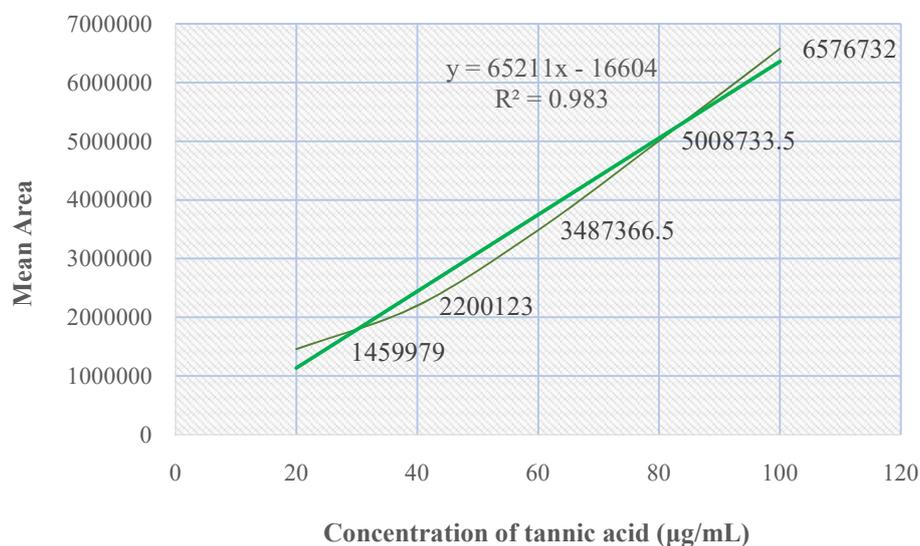


Figure 2: Standard curve of mean area against different concentration of tannic acid

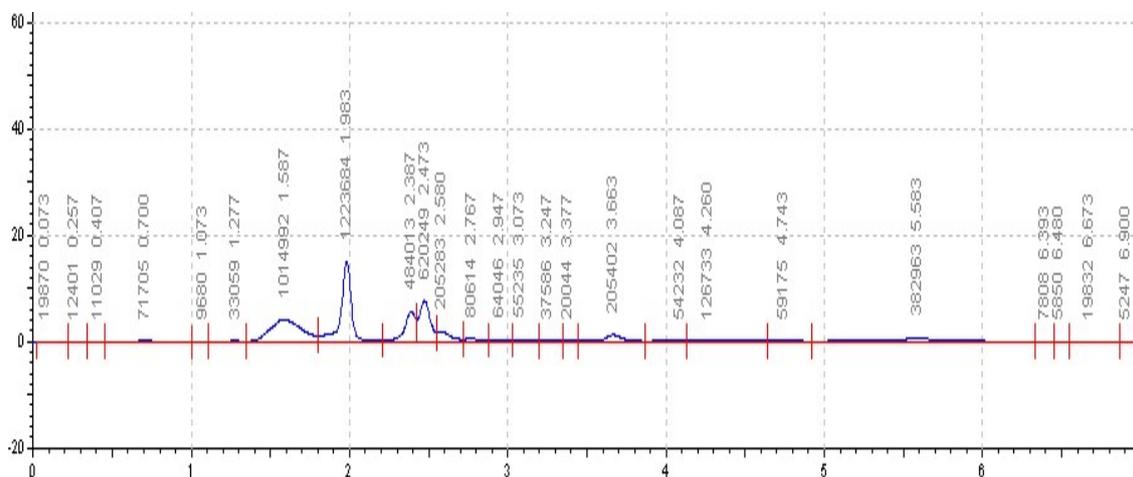


Figure 3: Chromatogram of tannic acid from Aonla sample (1 mg/mL)

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