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Research Article

Content Variation Studies of *Emblica officinalis* for its Ascorbic acid Collected from Various Geographical Sources by HPLC Technique

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ABSTRACT

The fruit of *Emblica officinalis* Gaertn (Euphorbiaceae), also knownas amla in Ayurveda are considered to be a rich source of ascorbic acid. However the antioxidant activities exhibited by *Emblica officinalis* extract are superior to those of ascorbic acid itself. The objective of present study is to carry out the standardization of herbal raw material *Emblica officinalis* for its ascorbic acid content to check the content uniformity obtained from various soils by HPLC technique. The collected fresh fruits were cut into small pieces, dried under shade and made into a fine powder. The powdered raw materials were subjected to HPLC analysis to estimate the ascorbic acid content. The percentage of ascorbic acid was estimated by comparing the peak area of standard and the same present in the collected samples. The results reveal that there are variations between the samples and the percentage of ascorbic acid is not uniform in all collected samples. Based on the current research that the content of ascorbic acid is not uniform in all the collected samples and it is concluded that the variation may be due the soil & soil fertility and climatic conditions.

Keywords: HPLC Analysis, *Emblica officinalis*, Raw material, Ascorbic acid

INTRODUCTION

The World Health Organization defines herbal medicine as a practice which includes herbs, herbal materials, herbal preparations and finished herbal products that contains as active ingredients parts of plants or other plant materials or combinations. These herbs are derived from plant parts such as leaves, stems, flowers, roots and seeds(1). Globaly 25 percent of the drugs prescribed are derived from Plants. Out of the 252 drugs in the World Health Organizations essential Medicine list, 11 percent are exclusively of plant origin(2). The use of plants as medicine dates 60,000 years ago according to ancient Babylon reports. There are various herbal medicinal system practices and philosophy of each is influenced by the region within which it is first evolved. In China, they have their own system known as the Traditional Chinese Medicine which has been used throughout history. Herbal drugs contain active ingredients, plant parts or plant materials in the processed or crude state with certain excipients, i.e., dilutions, solvents or preservatives. These active ingredients protect plants from damage and disease and contribute to the plants aroma, flavor and color. Scientifically, they are known as phytochemicals which include several classes such as saponins, flavonoids, glycosides, tannins, alkaloids and terpenoids(3).

The pharmacological treatment of disease began long ago with the use of herbs, one of the most important advantages of these supplements is that they come of various natural sources. As these supplements come from various foods, the body has a better chance of balancing them out in the system. The body in turn absorbs all the essential nutrients and has no side effects like chemical medicines[4]. Standardization of herbal formulations is essential in order to assess of quality drugs based on the concentration of their active principles, physical, chemical, physic-chemical standardization and invitro-invivo parameters[5].

Standardization is a system that ensures a predefined amount of quantity, quality and therapeutic effect of ingredients in each dose. Herbal product cannot be considered scientifically valid, the drug tested has not been authenticated and characterized in order to ensure reproducibility of manufacturing the product. Moreover, many dangerous and lethal side effects have been recently reported, including direct toxic effect, allergic reaction, effect from contaminants and interaction with herbal drugs. The development of authentic analytical method which can reliably profile the phytochemical composition including quantitative analysis of bio active compound and other major constituents is a major challenge to scientists[6]. Botanical characters, sensory evaluation, foreign organic matter, microscopic, histological, biochemical assessment, quantitative measurements, physical and chemical identity, fingerprints chromatography, ash values, moisture content, volatile oil and alkaloids tests, microbial contamination and radioactive contamination are followed[7]. The aim of the present study is to determine the content variation of herbal raw material of *Emblica officinalis* collected from various geographical sources to check soil and soil fertility and other climatic conditions. For our present study we have selected ascorbic acid as analytical marker present in the amla for the HPLC analysis. The amla fruits were collected from different areas of various Districts, standardized for their ascorbic acid content by HPLC Technique.

MATERIALS AND METHODS

Sample collection

The fresh raw materials of *Emblica officinalis* were collected from different geographical area of various districts. The collected fresh fruits were cut into small pieces, dried under shade and made to fine powder after passing through 100 meshes. The powdered raw materials were named A,B,C,D, and E based on the area of collection.

Standard preparation

Accurately weighed about 10mg of Ascorbic acid working standard and transfer into 250ml of clean and dried volumetric flask, added 30ml of methanol (Diluent-1). Then sonicated for 5minutes to dissolve the content and then cool to room temperature and make upto volume with buffer (Diluent-2) and mixed well.

Sample preparation

Accurately weighed 1gm of raw material and transfer into a 100ml of clean and dried volumetric flask, added 30ml of methanol (Diluent-1). Then sonicated for 10minutes to dissolve the content and then cool to room temperature and make upto volume with buffer (Diluent-2) and mixed well. Filtered the solution through 0.45µm nylon membrane filter. Further pipette out 1ml of solution and transfer into 100ml of clean and dried volumetric flask and make upto volume with buffer (Diluent-2) and mixed well.

Chromatographic conditions

Mobile phase (solvent)

Buffer preparation – Dissolved 900mg of 1-Hexane sulphonic acid (C_6 H_{14} O_3 S) in 990ml of HPLC grade water and added 10ml of acetic acid. The above solution was filtered through 0.45µm membrane and degasses it in a sonicated for 10 minutes.

Taken 700ml of buffer solution and mixed with 200ml of methanol (CH_3OH). Added HPLC grade water to the above to make up the volume upto 1000ml. The above solution was filtered through 0.45µm membrane filter.

Column: Inerstil C-18, size: 250mm × 4.6mm × 5µm.
Detectors : UV-detector
Wave length : 280nm
Flow rate : 1ml / min
Injection volume : 20µl
Pump mode : Isocratic mode
Diluents : 1. Methanol
2. Buffer

RESULTS AND DISCUSSION

The raw material of *Emblica officinalis* collected from various geographical sources were subjected to HPLC analysis to estimate their Ascorbic acid content. Ascorbic acid is one of the important active chemical constituent and used as analytical marker for this study. The results are tabulated in Table No.1 & 2 and Fig.No.1-6.

Table 1: Results of HPLC Analysis with Respect to Retention Time

Name of theMarker	Standard Retention Time	Sample NoAllotted	Retention Time ofSamples
ASCORBIC ACID	5.656	A	5.665
		B	5.658
		C	5.666
		D	5.662
		E	5.682

Table.2: Results of HPLC Analysis With Respect to Percentage of Ascorbic Acid

Sample No	Samples From Various Sources	Content of AscorbicAcid %w/w
A	Salem (Yercaud adivaram)	3.81
B	Namakkal (Unangalpatti)	2.97
C	Thiruvarur (Thiruvanchyam)	3.98
D	Erode (Parapalayam)	3.01
E	Coimbatore (Anamalai)	3.92

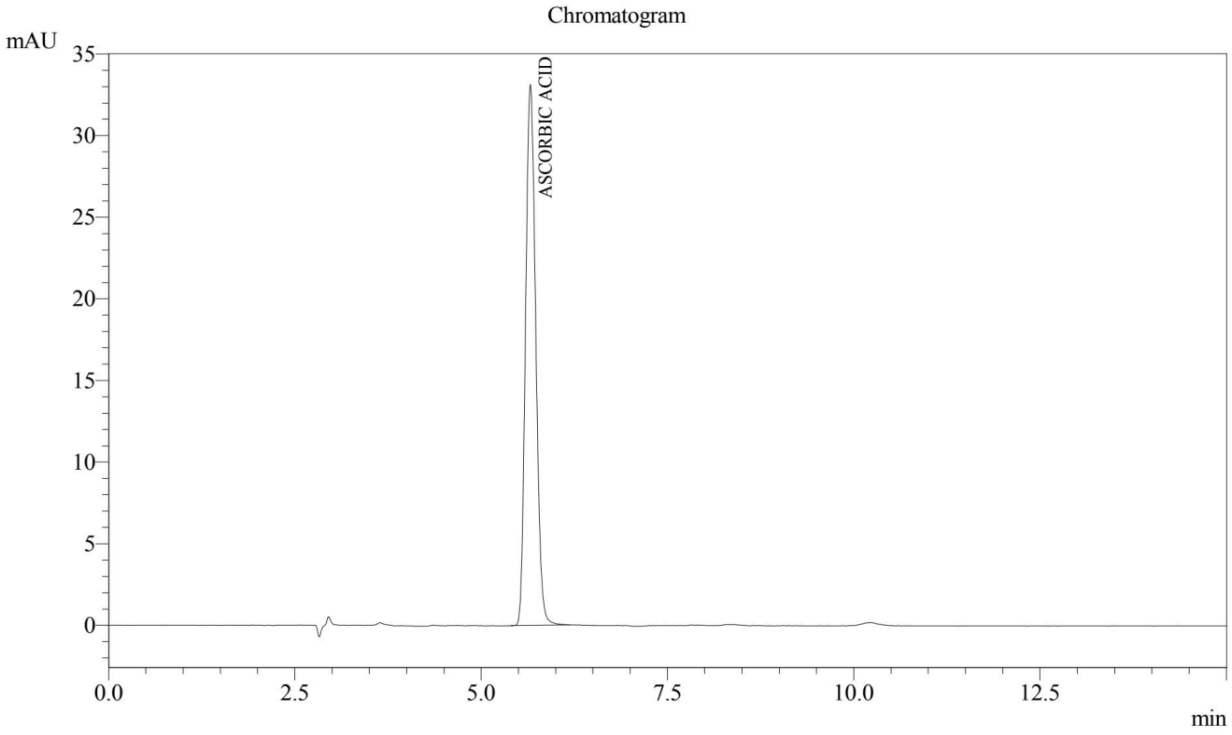

Fig 1: The HPLC Chromatogram of Standard Ascorbic Acid.

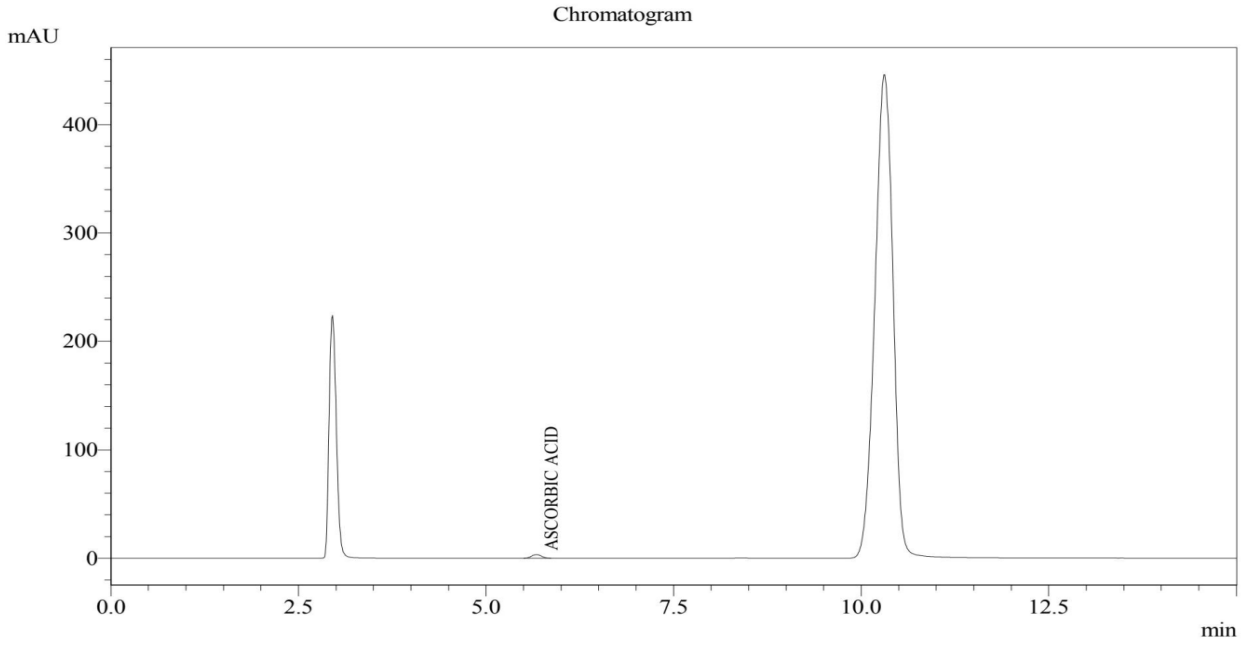

Fig 2: The HPLC Chromatogram of Sample A, a raw material of *Emblica officinalis* Containing Ascorbic Acid.

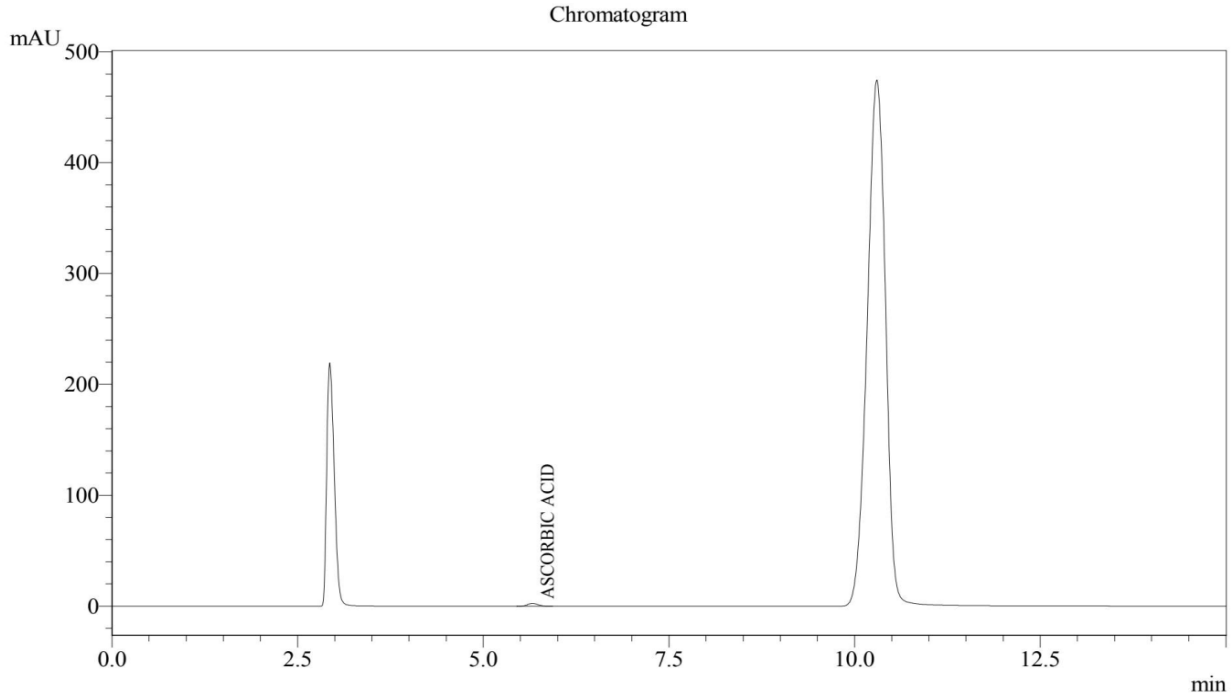

Fig 3: The HPLC Chromatogram of Sample B, a raw material of *Emblica officinalis* Containing Ascorbic Acid.

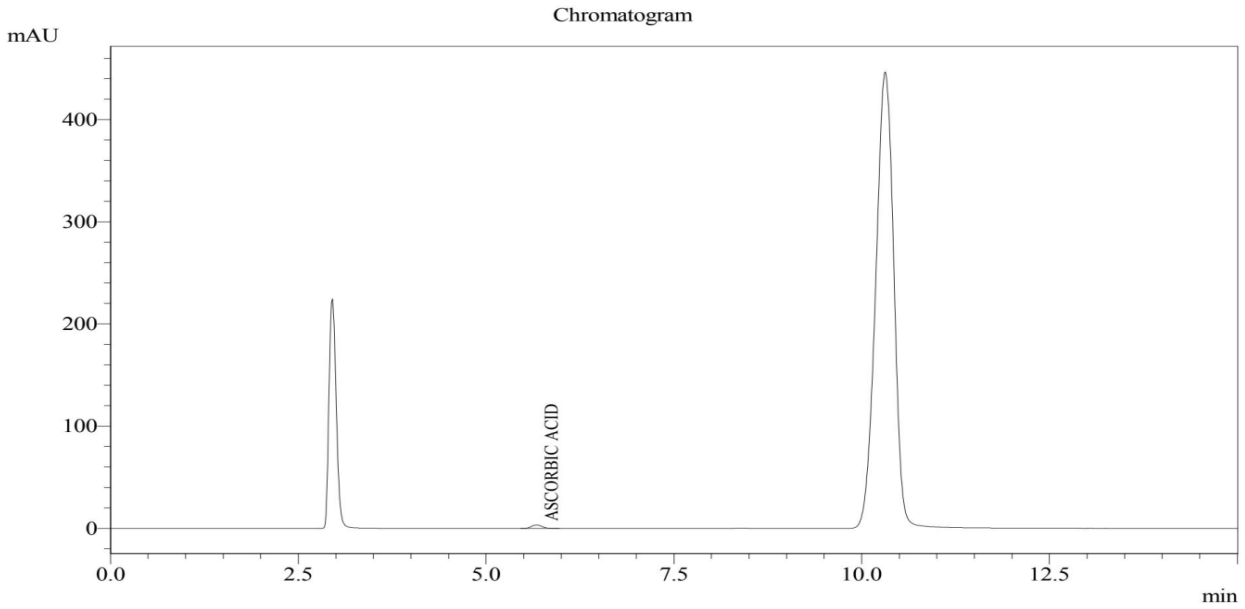

Fig 4: The HPLC Chromatogram of Sample C, a raw material of *Emblica officinalis* Containing Ascorbic Acid.

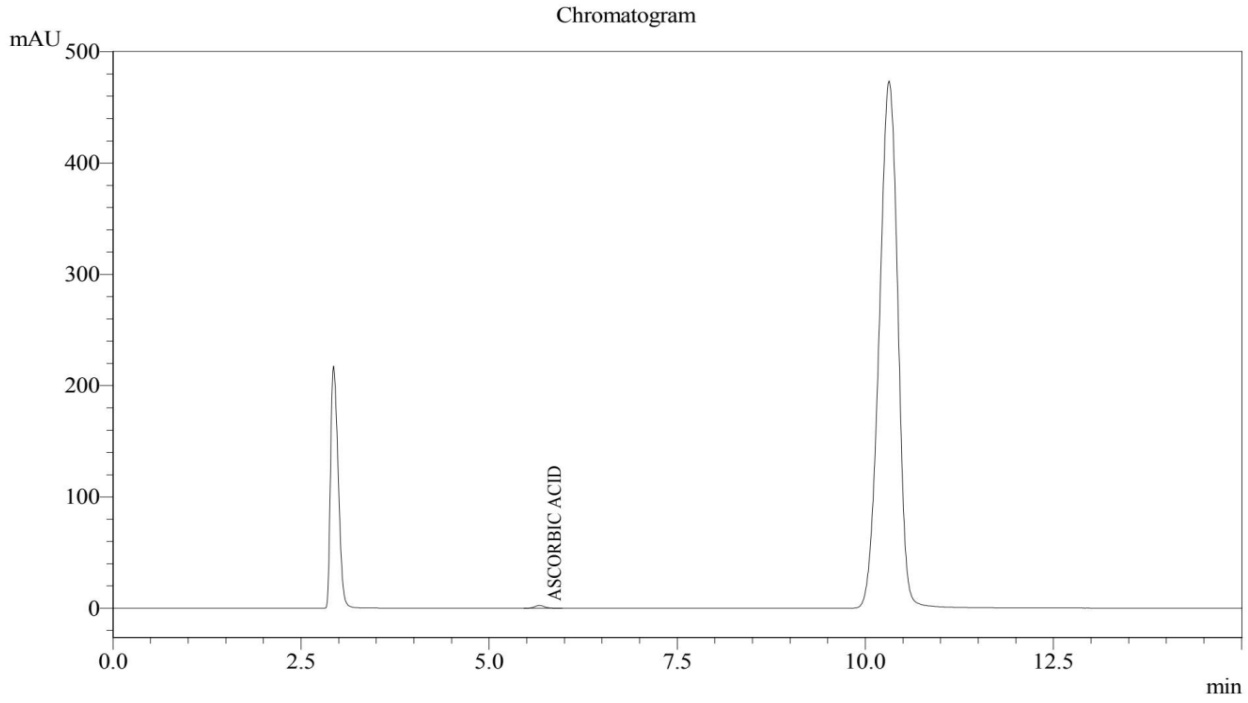

Fig 5: The HPLC Chromatogram of Sample D, a raw material of *Emblica officinalis* Containing Ascorbic Acid.

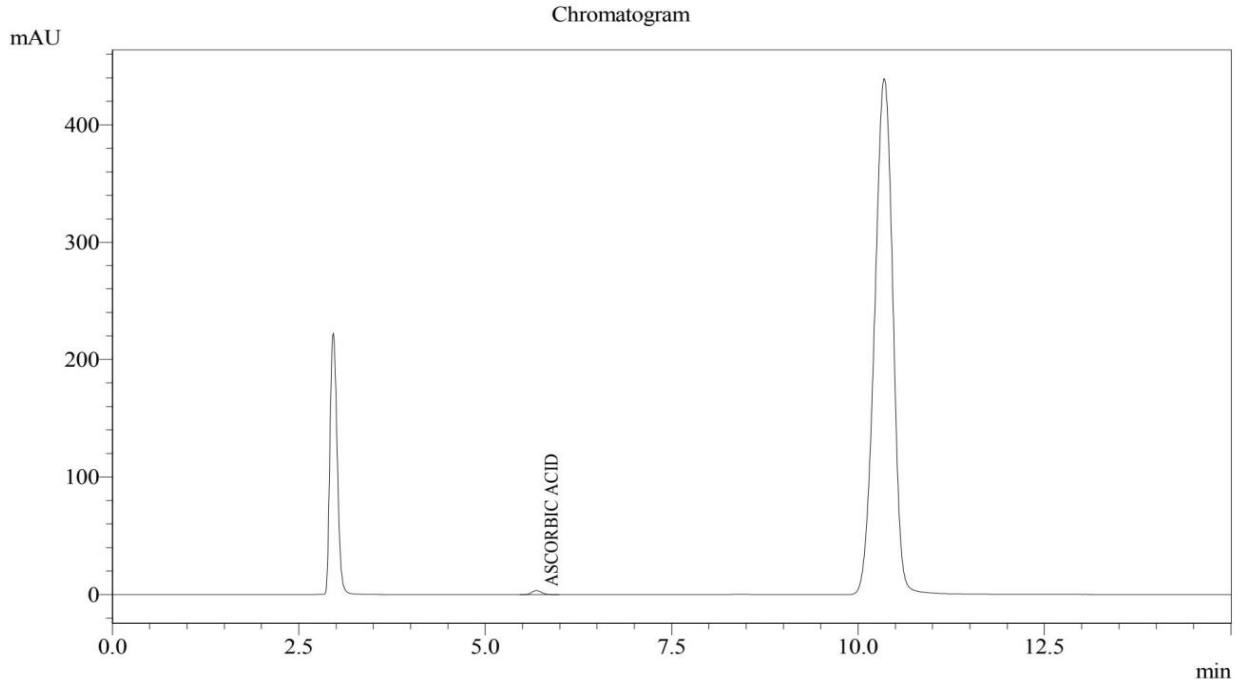

Fig 6: The HPLC Chromatogram of Sample E, a raw material of *Emblica officinalis* Containing Ascorbic Acid.

The herbal raw materials of *Emblica officinalis* collected from various places taken for HPLC analysis. The retention time of the standard ascorbic acid was found to be 5.565 and the retention time of ascorbic acid present in the various collected raw materials were found to be 5.665, 5.658, 5.666, 5.662 and 5.682 for samples A, B, C, D and E respectively and confirmed the presence of ascorbic acid in all the collected samples. The content of ascorbic acid was estimated by comparing the peak area of standard and the same present in the samples. The amount of ascorbic acid was found to be 3.81% w/ w, 2.97% w/ w, 3.98% w/ w, 3.01% w/ w, and 3.92% w/ w for samples collected from Salem, Namakkal, Thiruvarur, Erode and Coimbatore Districts respectively.

From the results it was clearly reveals that the content of ascorbic is high in samples collected from Thiruvarur with 3.98% w/w followed by Coimbatore with 3.98% w/ w and medium in samples collected from SALEM with 3.81% w/w and low in samples collected from Erode with 3.01% w/ w and Namakkal with 2.97% w/w.

SUMMARY AND CONCLUSION

Soil is frequently referred to as the "fertile substrate", not all soils are suitable for growing crops. Ideal soils for agriculture are balanced in contributions from mineral components (sand: 0.05–2 mm, silt: 0.002–0.05 mm, clay: <0.002 mm), Soil Organic Matter (SOM), air, and water. The balanced contributions of these components allow for water retention and drainage, oxygen in the root zone, nutrients to facilitate crop growth; and they provide physical support for plants. The distribution of these soil components in a particular soil is influenced by the five factors of soil formation: parent material, time, climate, organisms, and topography. Each one of these factors plays a direct and overlapping role in influencing the suitability of a soil for agriculture (Jenny, 1941)[8].

Often referred to as the master variable of soil, pH controls a wide range of physical, chemical, and biological processes and properties that affect soil fertility and plant growth. Soil pH, which reflects the acidity level in soil, significantly influences the availability of plant nutrients, microbial activity, and even the stability of soil aggregates. At low pH, essential plant macronutrients (i.e., N, P, K, Ca, Mg, and S) are less bioavailable than at higher pH values near 7, and certain micronutrients (i.e., Fe, Mn, Zn) tend to become more soluble and potentially toxic to plants at low pH values (5–6) (Brady and Weil, 2008)[9]. Aluminum toxicity is also a common problem for crop growth at low pH (<5.5). Typically, soil pH values from 6 to 7.5 are optimal for plant growth; however, there are certain plants species that can tolerate — or even prefer — more acidic or basic conditions. Maintaining a narrow range in soil pH is beneficial to crop growth. SOM and clay minerals help to buffer soils to maintain a pH range optimal for plant growth. In instances where the pH is outside a desirable range, the soil pH can be altered through amendments such as lime to raise the pH. Ammonium sulfate, iron sulfate, or elemental sulfur can be added to soil to lower pH (Havlin, *et al.*, 2005)[10].

Based on the above facts that we have selected the research work to check the geographical variation studies on *Emblica officinalis* a medicinal plant having very good antioxidant properties and therapeutically valued chemical

constituents. We have collected the raw materials from ten different geographical sources and analyzed for their ascorbic acid content by HPLC technique as ascorbic acid is one of their important chemical constituents and used as analytical marker.

The results reveal that the content of ascorbic acid is vary from soil to soil and shows a lot of variations. The content of ascorbic is high in samples collected from Thiruvarur with 3.98% w/w followed by Coimbatore with 3.98% w/w and medium in samples collected from Salem with 3.81% w/w and low in samples collected from Erode with 3.01% w/ w and low in samples collected from Namakkal with 2.97% w / w. The results clearly reveal that the content of ascorbic acid is not uniform in all the collected samples and it is concluded that the variation may be due the soil & soil fertility and climatic conditions.

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