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Geographical Variation Studies of *Emblica officinalis* Using Gallic Acid as Analytical Marker by HPLC Technique

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ABSTRACT

Standardized extracts are high quality extracts containing constituents levels of specified compounds and they are subjected to rigorous quality control during all phases of the growing, harvesting and manufacturing process. The active principles are unknown marker substances should be established for analytical purpose and standardization. The standardization of herbal raw material has become very essential as there is increase in the demand and frequent usage of the herbal raw materials. The herbal raw material containing *Emblica officinalis* fruits were collected from the various geographical sources and standardized for their gallic acid content by High Performance Liquid Chromatographic Technique (HPLC) is our objective of present study. The collected fresh fruits were cut in to small pieces, dried under shade and made to fine powder. The powdered raw materials were subjected to HPLC analysis to estimate the gallic acid content. The percentage of gallic acid was estimated by comparing the peak area of standard and the same present in the samples. The results reveal that there are lots of variations between the samples and the percentage of gallic acid is not uniform in all the collected samples. To get the good quality herbal products in the market the Good Agricultural and Collection Practices (GACP) to be maintained throughout the cultivation of medicinal plants to ensure the quality and uniform content of phytoconstituents which is responsible for the therapeutic activity.

Keywords: HPLC Analysis, *Emblica officinalis*, Raw material, Gallic acid.

INTRODUCTION

The World Health Organization (WHO) estimates that 80 percent of some Asian and African countries presently use herbal medicine for some aspects of primary healthcare. WHO has also issued guidelines for the assessment of herbal medicines (1996).These guidelines defined for the basic criteria for the evaluation of quality, safety and efficacy of herbal

medicine with the goal of assisting national regulatory authorities, scientific organizations and manufactures in assessing documentation submissions and dossiers in respect such products[1]. Herbal medicine is the study of Pharmacognosy and the use of medicinal plant, which are the basis of traditional medicines. The herbal medicine commonly includes plants, fungi and bee products, as well as minerals, shells and certain animal parts[2-3]. Many herbs are used as blood purifiers to alter or changing a long standing condition by eliminating metabolic toxins. The most of the traditional medicines are derived from medicinal plants, minerals and organic matter etc[4-5].

Standardized extracts are high-quality extracts containing constituents levels of specified compounds and they are subjected to rigorous quality control during all phases of the growing, harvesting and manufacturing process. Some manufactures are used the term standardization incorrectly to refer uniform manufacturing practices. The presence word "Standardized" on a supplement label does not necessarily indicate product quality. The active principles are unknown then the marker substances should be established for analytical purposes as well as to carry out the standardization[6].

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. For our present study we have selected gallic 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 gallic acid content by HPLC Technique to check the content variation of gallic acid.

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 in to 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

Prepared 25mg/ml concentration of gallic acid in HPLC grade water and used as standard solution.

Sample preparation

Accurately weighed quantity of raw material equivalent to 1g/ml of sample transferred into a 25ml volumetric flask and added 10ml of HPLC grade water and sonicated for 5minutes, make up the volume to 25ml with HPLC grade water. Mixed well and filtered the solutions through 0.45µ nylon filter paper and used as sample solution.

Chromatographic conditions

Solvent A - Dissolved 0.0272gm of anhydrous potassium dihydrogen orthophosphate [KH2PO4] in 1800ml of HPLC grade water and added 0.5ml of orthophosphoric acid. Add water to the above to make up the volume upto 2000ml.The above solution was filtered through 0.45µm membrane and degasses it in a sonicator for 5 minutes.

Solvent B - Acetonitrile solution

Table 1: Gradient Solution

TIME(min)	BUFFER CONCENTRATION (SOLVENT A)	ACETONITRILE CONCENTRATION (SOLVENT B)
00.01	95	05
10.00	95	05
12.00	20	80
23.00	20	80
24.00	95	05
30.00	95	05

Column : Agilent Zorbax SB C-18 Sizex4.6µ
Detector : Prominence Diode Array
Wavelength : 270nm
Flow rate : 1.3ml/min
Injection volume : 20µl

RESULTS AND DISCUSSION

The HPLC analyses of raw material of *Emblica officinalis* obtained from various geographical sources were subjected to HPLC analysis to estimate their gallic acid content. Gallic acid is one of the chemical constituent of the amla fruit and used as analytical marker for this study. The results are tabulated in Table No.2 & 3 and Fig No.1-6.

Table 2: Results of HPLC Analysis With Respect to Retention Time.

Name of the Marker	Standard Retention Time	Sample No Allotted	Retention Time of Samples
Gallic Acid	8.314	A	7.777
		B	8.135
		C	8.002
		D	7.809
		E	7.884

Table 3: Results of HPLC Analysis With Respect to Percentage of Gallic Acid

Sample No	Samples From Various Sources	Content of Gallic Acid (in %)
A	Trichy (Mukkombu)	0.44%
B	Dharmapuri (Solakottai)	0.36%
C	Namakkal (Unangalpatti)	0.29%
D	Erode (Parapalayam)	0.24%
E	Karur (Vangal)	0.19%

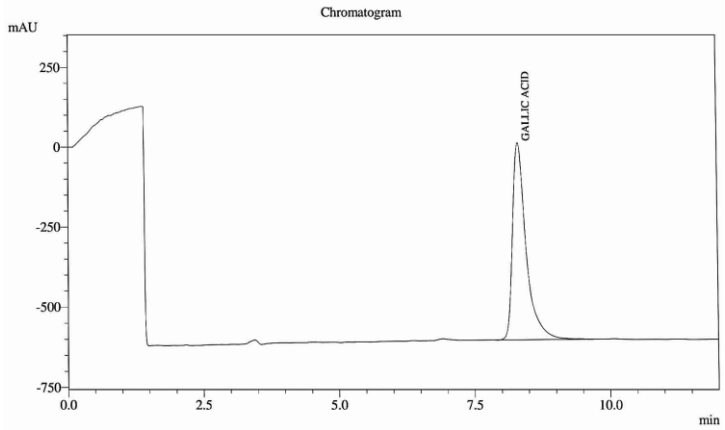

Fig 1: The HPLC Chromatogram of Standard Gallic Acid.

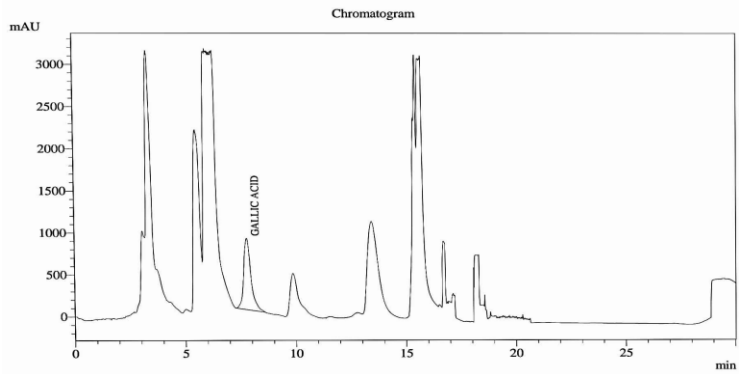

Fig 2: The HPLC Chromatogram of sample A, a raw material of *Emblica officinalis* containing Gallic Acid.

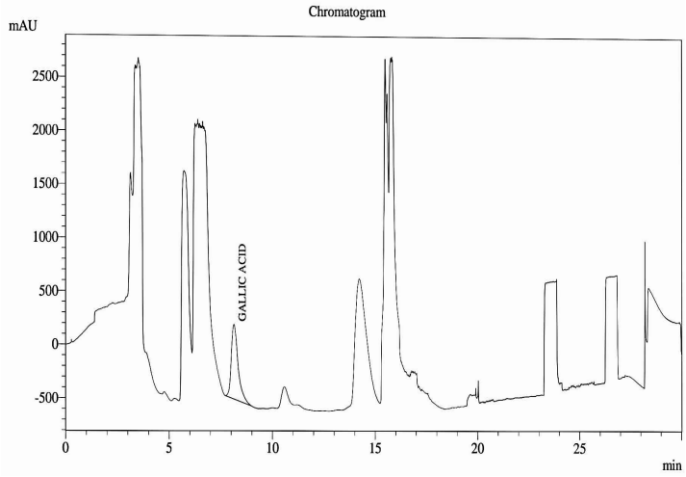

Fig 3: The HPLC Chromatogram of sample B, a raw material of *Emblica officinalis* containing Gallic Acid.

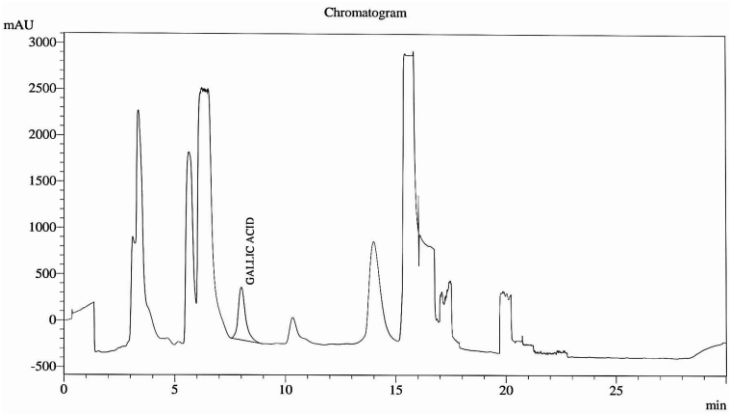

Fig 4: The HPLC Chromatogram of sample C, a raw material of *Emblica officinalis* containing Gallic Acid.

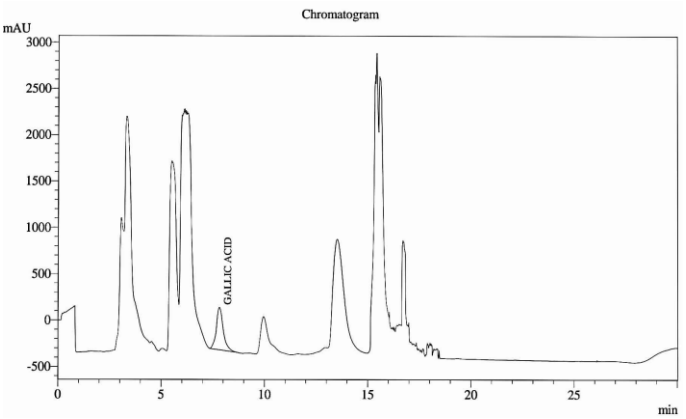

Fig 5: The HPLC Chromatogram of sample D, a raw material of *Emblica officinalis* containing Gallic Acid.

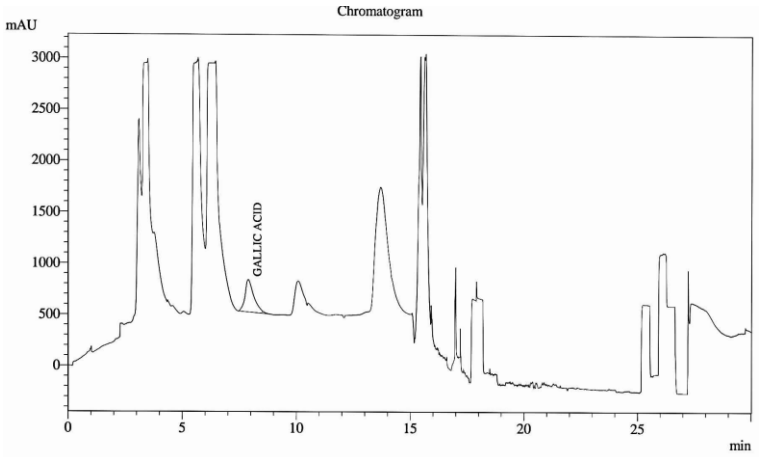

Fig 6: The HPLC Chromatogram of sample E, raw material of *Emblica officinalis* containing Gallic Acid.

The herbal raw materials of Amla (*Emblica officinalis*) collected from various places were taken for HPLC analysis. The retention time of the standard gallic acid was found to be 8.314 and the retention time of gallic acid present in the various collected raw materials were found to be 7.777, 8.135, 8.002, 7.809 and 7.884 for samples A, B, C, D and E respectively and confirmed the presence of gallic acid in all the samples. The content of gallic acid was estimated by comparing the peak area of standard and the same present in the samples. The amount of gallic acid was found to be 0.44%w/w, 0.36%w/w, 0.29%w/w, 0.24%w/w and 0.19%w/w from the area collected Mukkombu (Trichy), Solakottai (Dharmapuri), Unangalpatti (Namakkal), Parapalayam (Erode) and Vangal (Karur) respectively.

From the results it was clearly reveals that the content of gallic acid is high in samples collected from MUKKOMBU (TRICHY) with 0.44% followed by SOLAKOTTAI (DHARMAPURI)) with 0.36% and medium in samples collected from UNANGALPATTI (NAMAKKAL) with 0.29% followed by PARAPALAYAM (ERODE) with 0.24%, and low in sample collected from VANGAL (KARUR) with 0.19%.

SUMMARY AND CONCLUSION

Medicinal plants are considered as rich resources of ingredients which can be used in drug development either Pharmacopoeial, non-pharmacopoeial or synthetic drugs. Apart from that, these plants play a critical role in the development of human cultures around the whole world.

Moreover, some plants are considered as important source of nutrition and as a result of that they are recommended for their therapeutic values. Some of these plants include ginger, green tea, walnuts, aloe, pepper and turmeric etc. Some plants and their derivatives are considered as important source for active ingredients which are used in various ailments.

Good Agricultural and Collection Practices (GACP) for medicinal plants is only the first step in quality assurance, on which the safety and efficacy of herbal medicinal products directly depend upon, and will also play an important role in the protection of natural resources of medicinal plants for sustainable use. Under the overall context of quality assurance and quality control of herbal medicines, WHO developed the Guidelines on Good Agricultural and Collection Practices (GACP) for medicinal plants, providing general technical guidance on obtaining medicinal plant materials of good quality for the sustainable production of herbal products.

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 five different geographical sources and analyzed for their gallic acid content by HPLC technique as gallic acid is one of their chemical constituents and used as analytical marker.

The results reveal that the content of gallic acid is vary from soil to soil and shows a lot of variations. The content of gallic acid is high in sample collected from Trichy District with 0.44%w/w followed by samples collected from Dharmapuri District with

0.36%w/w and medium in sample collected from Namakkal District with 0.29%w/w followed by samples collected from Erode District with 0.24%w/w and low in sample collected from Karur District with 0.19%w/w. The results clearly reveal that the content of gallic acid is not uniform in all the collected samples and shows lots of variation. It is concluded that, to get the good quality herbal products in the market, we need to get quality raw materials and to get the good quality raw materials everyone those who are cultivating the medicinal plants have to follow the Good Agricultural and Collection Practices (GACP) to ensure the quality and uniform content of phytoconstituents which is responsible for the therapeutic activity.

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