



International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

IJAMSCR | Volume 9 | Issue 4 | Oct - Dec - 2021
www.ijamscr.com

ISSN:2347-6567

Research article

Medical research

High Performance Liquid Chromatographic Analysis of *Emblca officinalis* For Its Gallic Acid Content Obtained from Various Geographical Sources

S.Manimaran^{1*}, S.Kokila Vani¹, P.Jeevan Prasath¹, C.Manojkumar¹, C.Singaravelan¹,
L.Monisha¹, G.Arunachalam¹, R. Joseph Sahaya Raja² and R.Prasath²

¹Department of Pharmacognosy, P.G.P.College of Pharmaceutical Science and Research Institute, Namakkal,
Tamilnadu, India.

²Synthiya Research Labs Pvt. Ltd., Pondicherry.

Author for Correspondence: Prof.Dr.S.Manimaran

ABSTRACT

Standardization of herbal medicine is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive quantitative and qualitative values that carry on assurance of quality, safety, efficacy and reproducibility. The objective of present study is to carry out the standardization of herbal raw material *Emblca officinalis* locally called as Amla for its gallic acid content to check the content uniformity obtained from various soils by High Performance Liquid Chromatographic Technique (HPLC). 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. Based on the current research we strongly recommend to implement the Good Agricultural and Collection Practices (GACP) during medicinal plants cultivation to ensure the quality of herbal raw materials and also increasing the yield of the crop. The quality of raw materials will increase the trade of medicinal plants due to uniform content of phytoconstituents which is responsible for the therapeutic activity.

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

INTRODUCTION

In 1992, the WHO Regional Office for the Western Pacific invited a group of experts to develop criteria and general principles to guide research work on evaluating

herbal medicines. These WHO guidelines present general considerations of potentially hazardous contaminants and residues in herbal medicines and include guiding principles of assessing the quality of herbal medicines. It also recommended analytical methods for qualitative

and quantitative determination of such contaminants and residues and these guidelines intended to provide general technical guidance to member states in assessing quality relating to safety of herbal medicines and products classified as medicines, with regards to major and common contaminants and residues¹. There is limited scientific evidence for the safety and efficacy of plants used in 21st century. It is also called as phytomedicine or phytotherapy². Paraherbalism describe alternative and pseudoscientific practices of using unrefined plant or animal extracts as unproven medicines or health promoting agent³. Indeed, up to 80% of the rural population in Africa use herbal based traditional medicines for most of their healthcare. Rural South Africa also has a strong culture of traditional medicines that is base on the herbal medicines. In China and India, Herbal medicines accounts for about 50% of the total healthcare product consumption. Also in the USA, about 40% of the adult population has used herbal medicine.

The sales output of herbal medicine in Canada, Australia and Europe especially in Germany and France rapidly increasing⁴. Herbs such as black pepper, cinnamon, myrrh, aloe, sandal wood, red clove, bayberry and safflower are used to herbal wounds and sores⁵. Some herbs are used to neutralize the acid produced by the stomach, the gastric acid needed for proper digestion is retained by such herbs. Certain medicinal herbs have disinfectant property, which destroys diseases causing germs. They also inhibit the growth of pathogenic microbes that causes communicable diseases⁶⁻⁷. Monographs on herbs are available from a number of sources, including the European scientific cooperative on phytotherapy 1999, German commission E and the World Health Organization. Other resources that provide detailed information about herbal products in current use include the natural medicines comprehensive data base⁸.

Standardization of herbal medicine is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry on assurance of quality, safety, efficacy and reproducibility⁹. Methods of standardization should take into consideration that all the aspects of quality control of herbal drugs, namely correct identity of the sample, organoleptic evaluation and pharmacognostic evaluation, volatile matter,

quantitative evaluation, photochemical evaluation and biological activity etc¹⁰.

The aim of the present study is to determine the content variation of *Embllica officinalis* for its gallic acid content due to different soil and soil fertility. For the present study we have selected gallic acid as analytical marker to carry out the HPLC analysis. The Amla fruits were collected from different areas of various districts and subjected to HPLC standardization.

MATERIALS AND METHODS

Sample collection

The fresh raw materials of *Embllica 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 100meshes. The powdered raw materials were named I, II, III, IV and V 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 of volumetric flask and added 10ml of HPLC grade water and sonicated for 5minutes, make up the volume up to 25ml with HPLC grade water. Mixed well and filtered the solution through 0.45µ nylon filters 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 Size 4.6 μ
 Detector : Prominence Diode Array
 Wavelength : 270nm
 Flow rate : 1.3ml/min
 Injection volume : 20 μ l

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 constituents and used as an analytical marker for this study. The results are tabulated in Table. 2-3 and Fig. 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	I	7.998
		II	8.216
		III	8.233
		IV	7.721
		V	7.987

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

Sample No	Samples From Various Sources	Content of Gallic Acid (in %)
I	Salem (Yercaud adivaram)	0.58
II	Tirupathur (Pananthoppu)	0.43
III	Krishnagiri (Ittikal agaram)	0.29
IV	Coimbatore (Anamalai)	0.23
V	Thiruvarur (Thiruvanchyam)	0.22

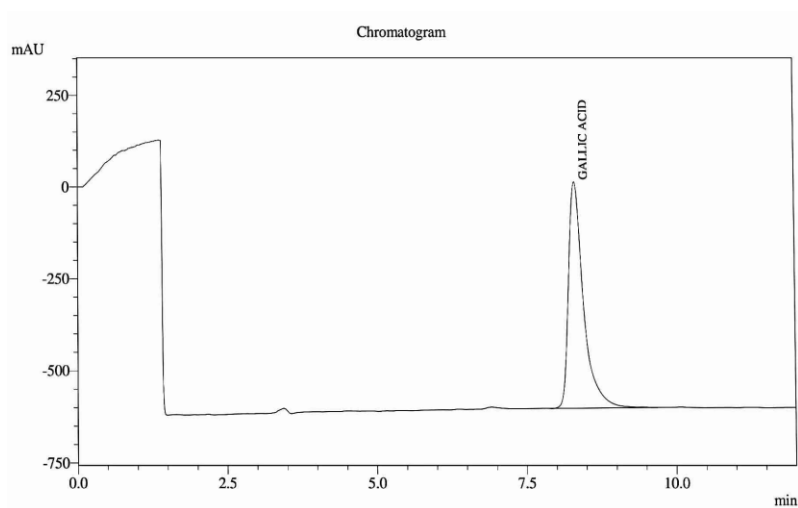


Fig 1:The HPLC Chromatogram of Standard Gallic Acid.

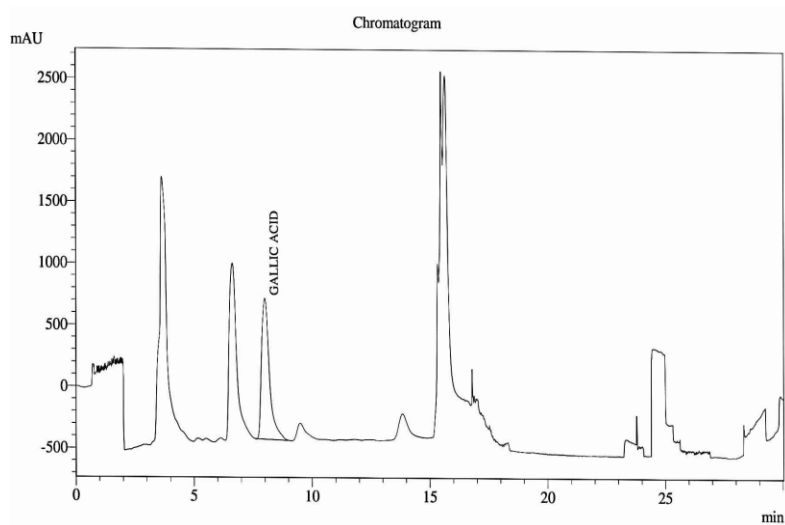


Fig 2:The HPLC Chromatogram of sample I, a raw material of *Emblca officinalis* containing Gallic Acid.

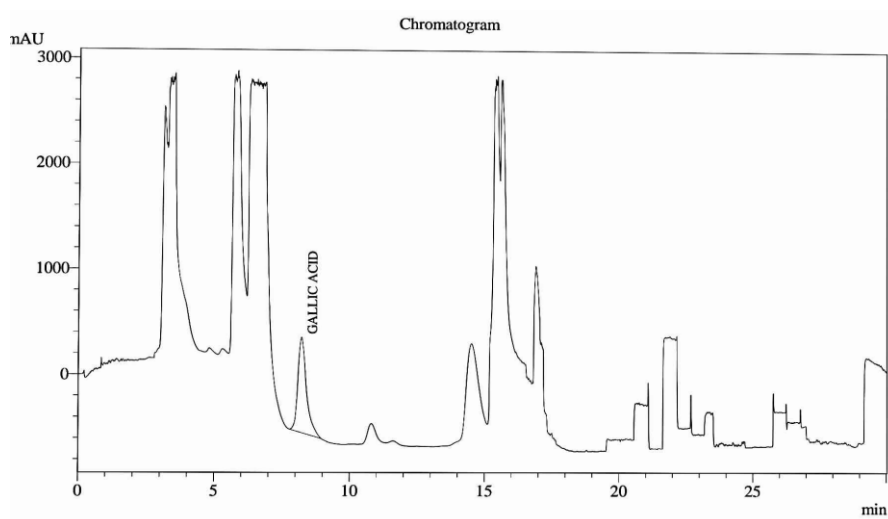


Fig 3:The HPLC Chromatogram of sample II, a raw material of *Emblca officinalis* containing Gallic Acid.

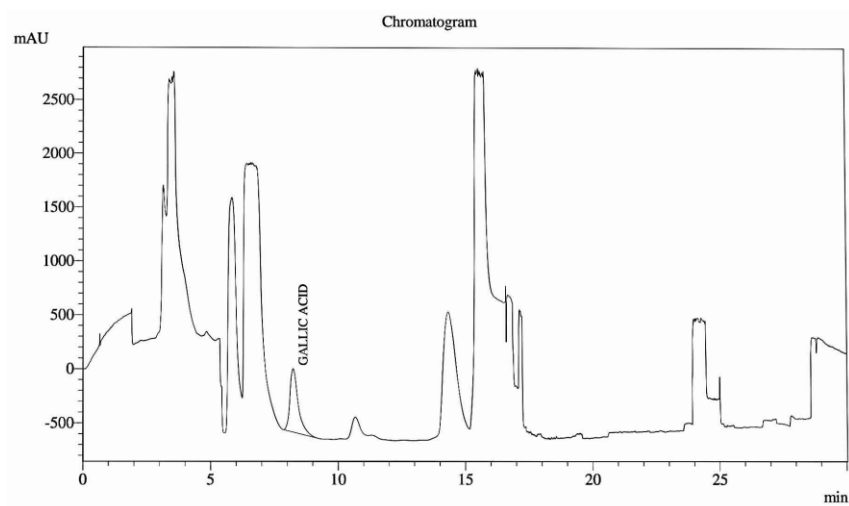


Fig 4: The HPLC Chromatogram of sample III, a raw material of *Emblica officinalis* containing Gallic Acid.

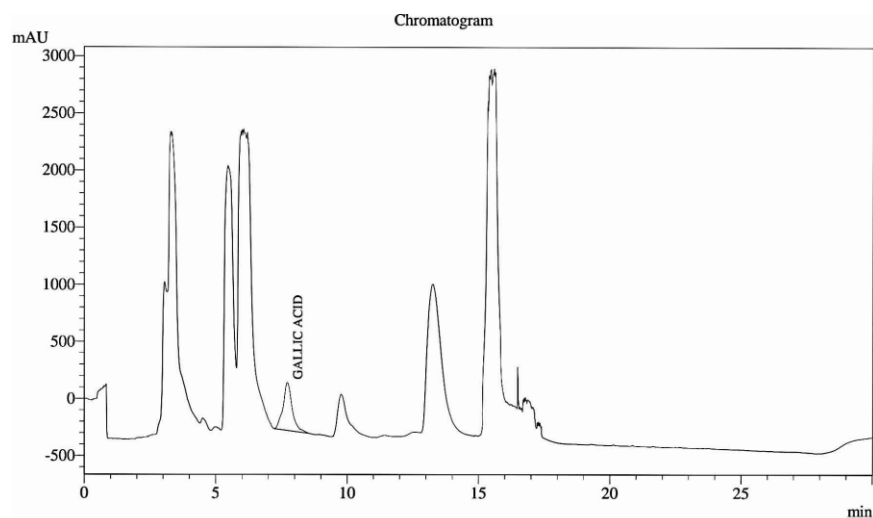


Fig 5: The HPLC Chromatogram of sample IV, a raw material of *Emblica officinalis* containing Gallic Acid.

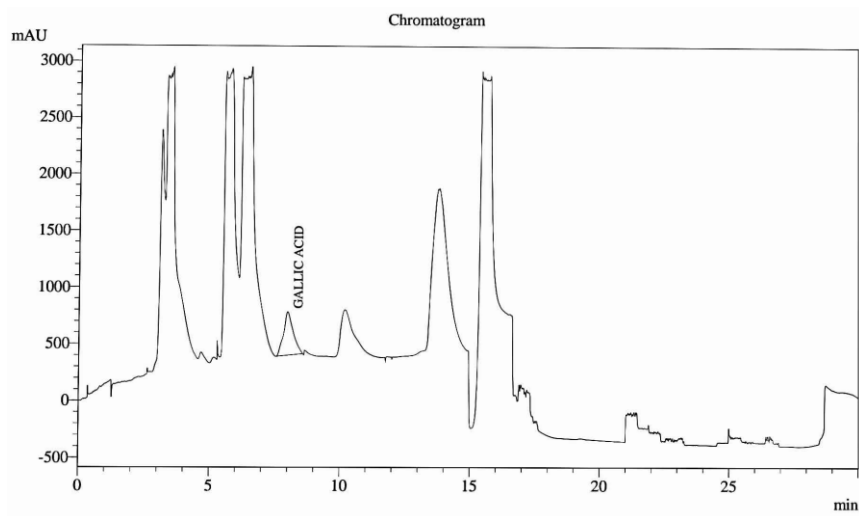


Fig 6: The HPLC Chromatogram of sample V, a raw material of *Emblica officinalis* containing Gallic Acid.

The natural herbal raw materials of Amla 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 7.998, 8.216, 8.233, 7.721 and 7.987 for samples I, II, III, IV and V respectively and confirmed the presence of gallic acid in all the collected samples. The content of gallic acid was estimated by comparing the peak area of standard and the same present in the samples. The amount gallic acid was found to be 0.58%w/w, 0.43%w/w, 0.29%w/w, 0.23%w/w and 0.22%w/w for samples collected from Salem, Tirupathur, Krishnagiri, Coimbatore and Thiruvavarur Districts respectively.

From the results it was clearly reveals that the content of gallic acid is high in samples collected from SALEM with 0.58% followed by TIRUPATHUR with 0.43%, medium in sample collected from KRISHNAGIRI with 0.29% and low in samples collected from COIMBATORE with 0.23% followed by THIRUVARUR with 0.22%.

SUMMARY AND CONCLUSION

WHO guidelines on Good Agricultural and Collection Practice (GACP) for medicinal plants, which addresses quality issues in the production of herbal raw material. It covers recommendations that range from the selection of appropriate seed material and cultivation sites to the avoidance of contaminations in post harvesting handling, training and working conditions of personnel and general rules for handling and

REFERENCE

1. WHO (1996), Annex11 *Guidelines for the assessment of herbal medicines*, (WHO Technical Report Serious No. 863)
2. Lack CW, et al., (2016), Critical thinking science and pseudoscience: Why we can't trust our Brains, P 212-214.
3. Tyler V.E, et al., (2000), Herbal medicine from the past to the future, *Public Health Nutrition*, Vol 3; P 447- 452.
4. Capasso R, et al., (2000), *Phytotherapy and quality of herbal medicines*, *Fitoterapia*, P 58-65.
5. Rahman A, et al., (2001), Bioassay Techniques for drug development, *Harwood*, Academic Publishers, Canada, P 9-11, 14-25, 65-67.
6. Cragg GM, et al., (2001), Natural product drug discovery in the next millennium, *Pharm Biol*, Vol 39; (suppl), P 8-17.
7. Spinella M, et al., (2001), The Psychopharmacognosy of herbal medicine, *MIT Press*, England, P 1-2.
8. Jellin J.M, et al., (2002), Natural Medicines comprehensive database (Pharmacists Letter Prescriber Letter), Stockton CA, Therapeutic Research faculty, P 1385-1560.

construction of tools and facilities. GAPC will affect the quality of herbal raw materials based on their cultivation procedures. Present study clearly reveals that the content of gallic acid was vary from soil to soil and shows a lot of variations. The content of gallic acid was high in sample collected from Salem District (0.58%w/w), followed by Tirupathur District (0.43%w/w), medium in samples collected from Krishnagiri District (0.29%w/w), low in samples collected from Coimbatore District (0.23%w/w), followed by Thiruvavarur District (0.22%w/w). The results reveal that the content of gallic acid is not uniform in all the collected samples. 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. We strongly recommend the implementation of GACP for medicinal plants to ensure the quality assurance of herbal medicine and also increasing yield of the crop. Also suggest that to give the proper cultivation and collection training to farmers which is enable maintain GAPC and this will lead to produce good quality herbal raw materials as well as herbal products.

ACKNOWLEDGEMENT

We gratefully thank M/S Synthiya Research Labs Pvt. Ltd., Pondicherry for their support and help to carry out the HPLC Analysis.

9. Kunle, et al., (2012), Standardization of Herbal Medicines, *International Journal of Bio diversity and conservation*, Vol 4(3); P 110-112.
10. Nikam Pravin H, et al., (2012), Future Trend in Standardization of Herbal Drugs, *Journal of Applied Pharmaceutical Science*, Vol 02 (06); P 38-44.

How to cite this article: S.Manimaran, S.Kokila Vani, P.Jeevan Prasath, C.Manojkumar, C.Singaravelan, L.Monisha, G.Arunachalam, R. Joseph Sahaya Raja and R.Prasath. High Performance Liquid Chromatographic Analysis of *Embllica officinalis* For Its Gallic Acid Content Obtained from Various Geographical Sources Int J of Allied Med Sci and Clin Res 2021; 9(4): 681-687.

Source of Support: Nil. **Conflict of Interest:** None declared.