Research Article

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Estimation of Ascorbic Acid from *Emblica Officinalis* collected from different Geographical Sources by HPLC Technique

S.Manimaran[1]*, M.Ranjith Raj[1], S.Zahir Hussain[1], R.Ranjithkumar[1], R.Aravindan R.Ajith[1], G.Arunachalam[1], R. Joseph Sahaya Raja[2] and R.Prasath[2]

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

[2]Synthiya Research Labs Pvt. Ltd., Pondicherry.

ABSTRACT

Standardization of herbal drug or extract means confirmation of its identity, determination of its quality and purity by carry out the proper approved procedures. In order to obtain quality oriented herbal products, care should be taken right from the proper identification of plants, season and area of collection and their extraction and purification process. The standardization of herbal raw materials has become very important as there is increase in demand. The herbal raw materials containing *Emblica officinalis* fruits were collected from various geographical sources and standardized for their ascorbic acid content by High Performance Liquid Chromatographic Technique (HPLC) is our objective of present study. The collected fresh fruits were cut into small pieces, dried under shade and made to 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 sample. The results reveal that there are some variations between the samples and the percentage of ascorbic acid is not uniform in all the collected samples. 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 (WHO) has defined traditional medicine as the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses[(1)]. According to the figures provided by WHO in the report on the World Medicines Situation, between 70% and 95% of citizens in many developing countries are traditional medicine for the management of health and as a primary source of health care. They turn to traditional medicine mainly because it is close in hand, easily affordable, readily available, cheap and consistent with indigenous cultures or either group, though its effectiveness needs proving[(2)]. Herbal products are often used as a first line therapy for conditions such as benign prostatic hyperplasia (BPH) in Germany, Italy, and elsewhere. German physicians receive medical school training in medicinal herbs and must pass a test on herbal medicine to become licensed. Approximately 80% of German physicians regularly prescribe herbs. A survey of 21,923 adults in the Northwest region of England patients reported taking herbal medicines. Twenty seven percent of outpatients in Spanish gastroenterology clinic had used herbs in the previous year[(3)].

The commercialization of the herbal medicine has become an important issue due to safety, quality and efficacy of medicinal plants. The herbal raw material is prone to a lot of variation due to several factors, the important ones being the identity of the

Author for Correspondence: Prof.Dr.S.Manimaran

plants and seasonal variation, the ecotype, genotypic and chemotypic variations, drying and storage conditions and the presence of xenobiotic[4]. Standardization as defined by American Herbal Product Association refers to the body of information and control necessary to product material of reasonable consistency. Methods of standardization should take into consideration of all aspects that contribute to the quality of herbal drugs, namely correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation, volatile matter, quantative evaluation, phytochemical evaluation, test for the presence of xenobiotics, microbial load testing, toxicity testing and biological activity. Of these, the phytochemical profile is of special significance since it has a direct bearing on the activity of herbal drugs. The fingerprint profiles serve as guideline to the phytochemical profile of the drug in ensuring the quality, while quantification of the marker compounds would serve as an additional parameter in assessing the quality of the sample[5]. Phytotherapeutic agents are normally marketed as standardized preparations in the form of liquid, solid or viscous preparations[6]. Standardization of herbal formulation requires implementation of Good Manufacturing Practices (WHO guideline in 1996). In addition, study of various parameters such as pharmacodynamics, pharmacokinetics, dosage, stability, self-life, toxicity evaluation, chemical profiling of herbal formulation is considered essential[7]. Standardiztion is an important aspect for maintaining and assessing the quality and safety of the Polyherbal formulation as these are combinations of more than one herb to attain the desire therapeutic effect[8]. Standardization minimizes batch to batch variation, assure safety, efficacy, quality and acceptability of the Polyherbal formulations[9].

The aim of the present study is to determine the content variation of *Emblica officinalis* for its ascorbic acid content due to different soil and soil fertility. For the present study we have selected ascorbic acid as analytical marker to carry out 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 *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) andmixed 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 filteredthrough 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
Wavelength	:	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 and 2 and Fig.1-6.

Author for Correspondence: Prof.Dr.S.Manimaran

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

Name of the Marker	Standard Retention Time	Sample NoAllotted	Retention Time ofSamples
ASCORBIC ACID	5.656	A	5.649
		B	5.666
		C	5.658
		D	5.665
		E	5.666

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

Sample No	Samples From Various Sourse	Content of Ascorbic Acid %w/w
A	Tirupathur (Pananthoppu)	3.97
B	Krishnagiri (Ittikal agaram)	3.81
C	Dharmapuri (solakottai)	3.61
D	Karur (Vangal)	3.00
E	Trichy (Mukkombu)	3.84

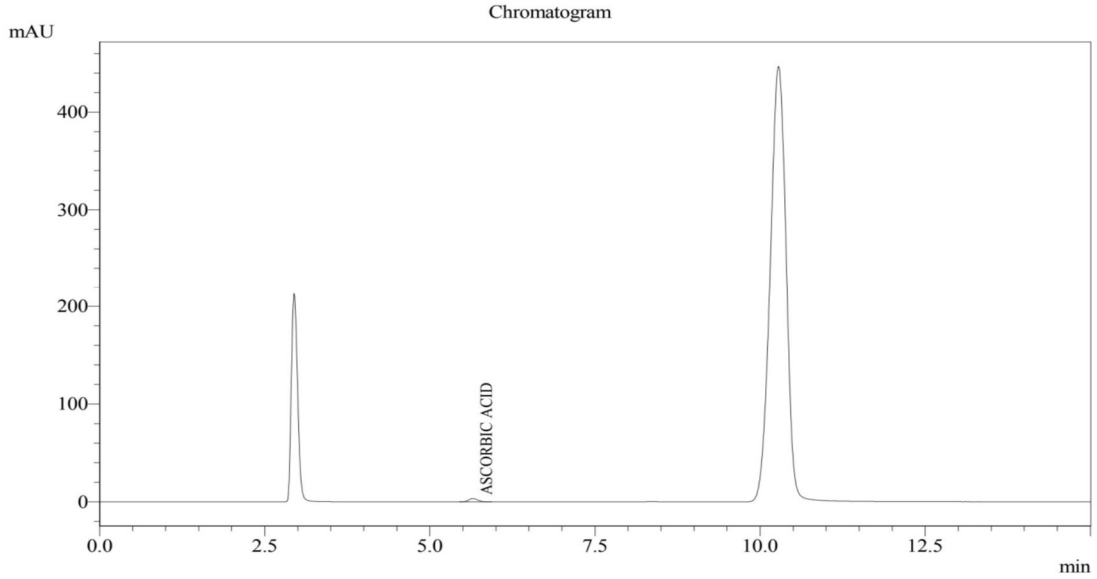

Fig 1: The HPLC Chromatography of Standard Ascorbic Acid.

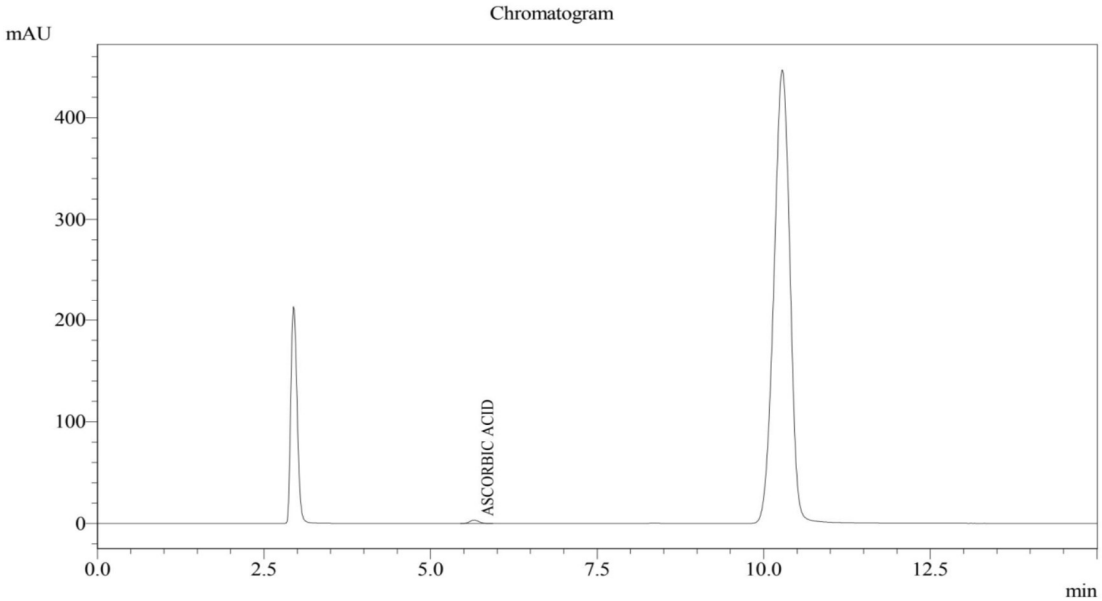

Fig 2: The HPLC Chromatogram of Sample A, a raw material of *Emblica officinalis* Containing Ascorbic Acid.

Author for Correspondence: Prof.Dr.S.Manimaran

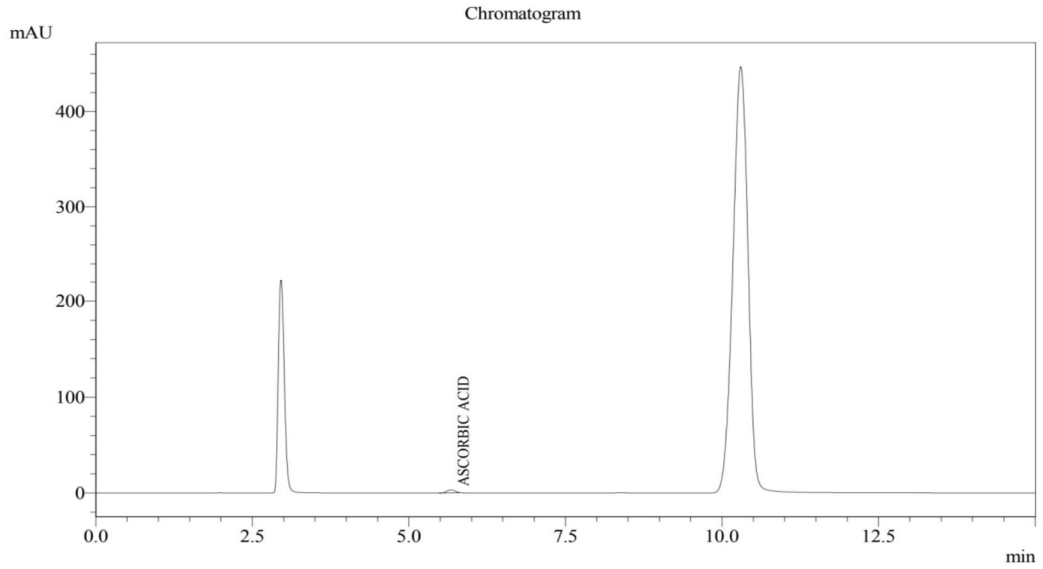

Fig 3: The HPLC Chromatogram of Sample B, a raw material of *Emblica officinalis* Containing Ascorbic Acid.

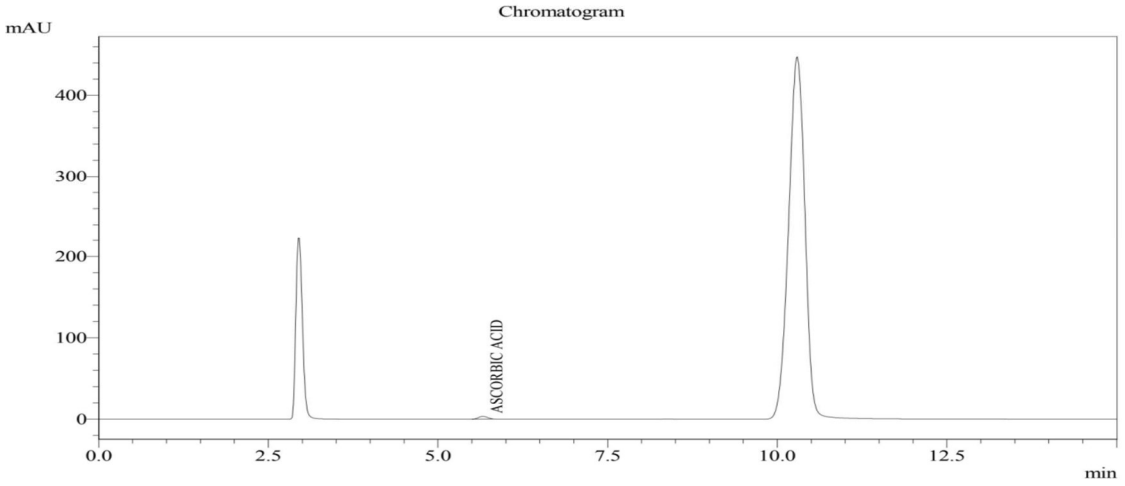

Fig 4: The HPLC Chromatogram of Sample C, a raw material of *Emblica officinalis* Containing Ascorbic Acid.

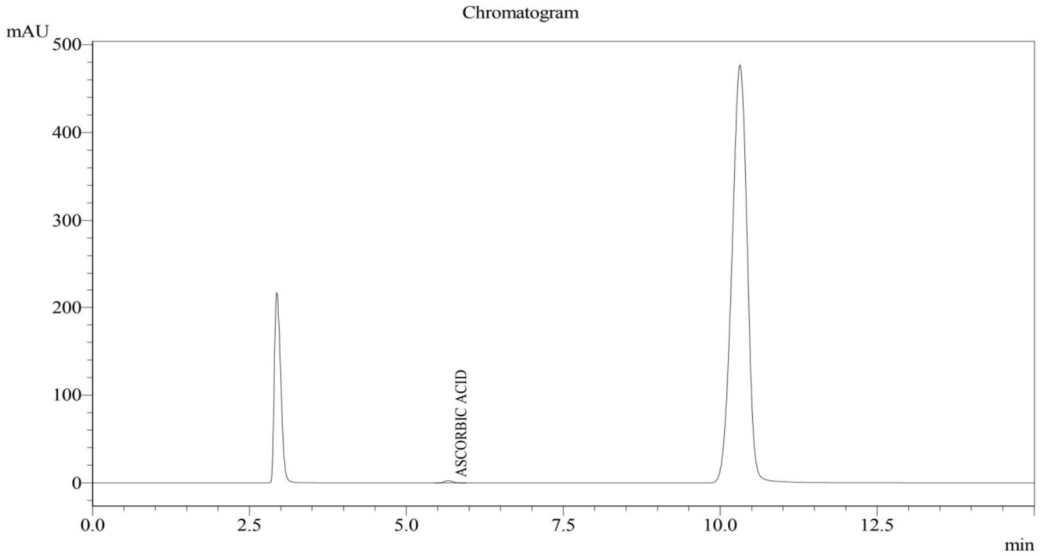

Fig 5: The HPLC Chromatogram of Sample D, a raw material of *Emblica officinalis* Containing Ascorbic Acid.

Author for Correspondence: Prof.Dr.S.Manimaran

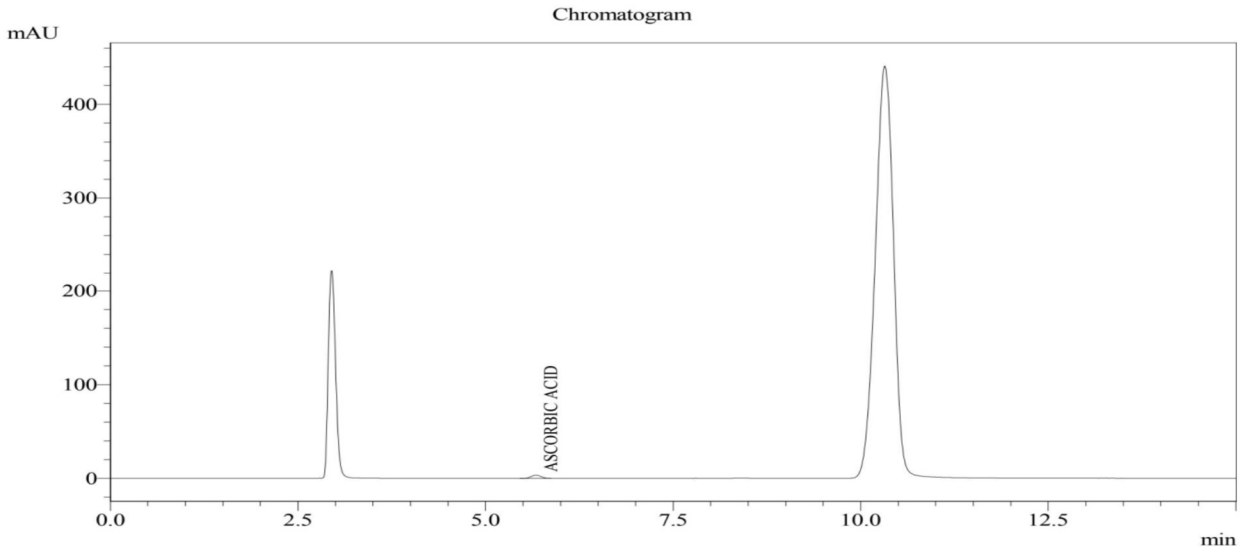

Fig 6: The HPLC Chromatogram of Sample E, a raw material of *Emblica officinalis* Containing Ascorbic Acid.

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 5.649, 5.666, 5.658, 5.665 and 5.666 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 3.97% w/w, 3.81% w/w, 3.61% w/w, 3.00 % w/w, and 3.84 % w/w for samples collected from Tirupathur, Krishnagiri, Dharmapuri, Karur and Trichy Districts respectively.

From the results it was clearly reveals that the content of ascorbic is high in samples collected from TIRUPATHUR with 3.97 % w/ w followed by TRICHY with 3.84% w/w and KRISHNAGIRI with 3.81% w/ w and medium in samples collected from DHARMAPURI with 3.61% w/w and low in samples collected from KARUR with 3.00 % w/w.

Plants grow better in high-quality soil because there are more nutrients in it. Soil quality is a measure of the level of nutrients in soil and its structure. They grow less well in lower-quality soils because there are not sufficient nutrients. Soil helps anchor plants and provides them essential elements of water and nutrients. The best soil for most plants to ensure optimum growth is a rich, sandy loam. However, there are many plants that are well adapted and can grow in particular types of soil. Soil is a major source of nutrients needed by plants for growth. The three main nutrients are Nitrogen (N), Phosphorus (P) and Potassium (K). Together they make up the trio known as NPK. Plants take up essential elements from the soil through their roots and from the air (mainly consisting of nitrogen and oxygen) through their leaves. Nutrient uptake in the soil is achieved by cation exchange, wherein root hairs pump hydrogen ions (H^+) into the soil through proton pumps (Ordonez.J.C, *et al.,* 2009)[10], (Wright.I.J, *et al.,* 2017)[11], (Atkin.O.K, *et al.,* 2015)[12].

There is ample evidence that large-scale variation of individual plant traits is related to environmental gradients. Early plant biogeographers suggested that climate and soils together shape plant form and function but could not propose a more precise theoretical framework describing these fundamental relationships. Over the last decades, examples have thus accumulated without an overall framework in which to place them. For instance, tree height depends on water availability, while leaf economics traits depend on soil properties, especially soil nutrient supply, as well as on climatic conditions reflected in precipitation. Leaf size, leaf dark respiration rate, specific leaf area (SLA), leaf N and P concentration, seed size and wood density, all show broad-scale correlations with climate and soil properties (Anser.G.P, *et al.,* 2016)[13], (Moles.A.T, *et al.,* 2007)[14], (Blume.H.B, *et al.,* 2016)[15].

CONCLUSION

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 variations. The content of ascorbic is high in samples collected from Tirupathur with 3.97 % w/ w and medium in samples collected from Trichy with 3.84% w/ w followed by Krishnagiri with 3.81% w/w and Dharmapuri with 3.61% w/w and low in samples collected from Karur with 3.00 % w/w. The results clearly reveal that the content of ascorbic acid is not uniform in all the collected samples and it is

Author for Correspondence: Prof.Dr.S.Manimaran

concluded that the variation may be due the soil & soil fertility and climatic conditions.

REFERENCES

1. WHO. Fact sheet. Geneva, Switzerland: Traditional Medicine; 2003. p. 18-21.
2. Sen S, Chakraborty R, De B. Challenges and Opportunities in the advancement of herbal medicine: India's position and role in a global context. J Herb Med. 2011;1(3-4):67-75. doi: 10.1016/j.hermed.2011.11.001.
3. Fletcher SW 2009. Overview of herbal medicine, *Journal of Homeopathy and*.
4. Dixit VK, et al. Recent approaches in herbal drug standardization. Integr Biol. 2008;2(3):195-203.
5. Waldesh, et al. Herbal medical products – scientific and regulatory basis for development quality assurance and marketing authorization, published by medpharm stuttaguart and CRC Press, Washington, DC; 2003. p. 37-42.
6. Schulz, et al. Rational phytochemistry, A physicians guide to herbal medicine. The Int J Aromatheraphy. 1996;11(4):229-30.
7. Mosihuzzaman M, Choudhary MI. Protocols on safety, efficacy, standardization, and documentation of herbal medicine (IUPAC Technical Report). Pure Appl Chem. 2008;80(10):2195-230. doi: 10.1351/pac200880102195.
8. Sharma, et al. A rapid and simple scheme for standardization of Polyherbal drugs. Int J Green Pharm. 2009;3:134-40.
9. Patil PS, et al. An advancement of analytical techniques in herbal research. J Adv Sci Res. 2010;1(1):8-14.
10. Ordoñez JC, van Bodegom PM, Witte JM, Wright IJ, Reich PB, Aerts R. A Global study of relationships between leaf traits, climate and soil measures of nutrient fertility. Glob Ecol Biogeogr. 2009;18(2):137-49. doi: 10.1111/j.1466-8238.2008.00441.x.
11. Wright IJ, et al. Global Climatic drivers of leaf size, leaf evolution. Plant Ecol. 2017;5:917-21.
12. Atkin OK, Bloomfield KJ, Reich PB, Tjoelker MG, Asner GP, Bonal D, Bönisch G, Bradford MG, Cernusak LA, Cosio EG, Creek D, Crous KY *et al* Global variability in leaf respiration in relation to climate, plant functional types and leaf traits. New Phytol. 2015;206(2):614-36. doi: 10.1111/nph.13253, PMID 25581061.
13. Asner GP, Knapp DE, Anderson CB, Martin RE, Vaughn N. Large scale climatic and geophysical controls on the leaf economic spectrum. Proc Natl Acad Sci U S A. 2016;113(28):E4043-51. doi: 10.1073/pnas.1604863113, PMID 27354534.
14. Moles AT, Ackerly DD, Tweddle JC, Dickie JB, Smith R, Leishman MR, Mayfield MM, Pitman A, Wood JT, Westoby M. Global patterns in seed size. Glob Ecol Biogeogr. 2007;16(1):109-16. doi: 10.1111/j.1466-8238.2006.00259.x.
15. Blume HP, et al. Soil science. 1st ed. Vol. 1. Berlin, Heidelberg: Springer; 2016. p. 154-68.

Author for Correspondence: Prof.Dr.S.Manimaran