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PARALLEL QUANTITATIVE ESTIMATION OF GALLIC ACID IN AQUEOUS EXTRACT OF EMBLICA OFFICINALIS AND POLY-HERBAL DOSAGE FORM (CAPSULE) BY RP-HPLC TO ASCERTAIN THE AUTHENTICITY OF THIS INGREDIENT IN THE DEVELOPED FORMULATION.

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ABSTRACT

In the present study Gallic acid is Parallelly quantified by optimized RP-HPLC method in pharmaceutically developed poly-herbal dosage form (capsule) and aqueous extract of *Emblica officinalis* fruit to examine the authenticity of this ingredient in finished dosage form. The Chromatographic analysis was performed on Shimadzu 10AVP HPLC System using Phenomenex Luna C18 reverse-phase column (4.6 x 250mm, 5µ particle size). The mobile phase comprised of Water: Acetonitrile : Acetic acid(90 : 10 : 0.2 v/v) at a flow rate of 1.0 ml/min and 272nm as detection wavelength. The regression equation showed good linearity in the range of 0.5-50µg/mL for Gallic acid (R^2 > 0.9997) between the peak areas of each marker and concentration. The retention time was 4.31min and the amount of gallic acid in aqueous extract and poly-herbal formulation of *Emblica officinalis* was found to be 14.17%w/w and 1.95%w/w respectively.

KEYWORDS: *Emblica officinalis*, Gallic acid, HPLC.

INTRODUCTION

Emblica officinalis is effective in the treatment of amlapitta viz., peptic ulcer.[1,2] The fruits exhibit hypolipidaemic and antiatherosclerotic effects in the rabbits and rats.[3,4] The extract of amla also has antimicrobial properties.[5,6] Amla is an antioxidant with free radical scavenging properties.[7] Hepatoprotective,[8] adaptogenic,[9] antimutaginic,[10] cytoprotective

and antitumor[11] antifungal,[12] were also exhibited by Amla. The Gallic acid is the basis for the quality control of *Emblica officinalis* (aqueous extract of fruit) and other plant-derived drugs from the herb. It is difficult to identify a particular component in a poly-herbal formulation containing more than two herbs or a mixture of different herbal powder or extracts. Gallic acid usually found as a chemical constituent (poly-phenolic) in medicinal herbs (citrus fruits) viz. *Emblica* officinalis, *Terminalia chebula, Terminalia bellirica* etc. The poly-herbal formulation under study contains *Emblica officinalis* together with other three plants ingredients. The main aim of the present study is to estimate gallic acid by RP-HPLC in ingredient as well as in finished formulation and to verify its presence (qualitative & quantitative) and ascertain the Presence of Emblica *officinalis* in the pharmaceutically developed poly-herbal dosage form (capsule).

MATERIALS AND METHODS

Plant material and preparation of extract: The aqueous extract of dry fruit of *Emblica officinalis* (authenticated by voucher specimen (LIH No. 6934) was procured from an authentic supplier and the standard operating procedure to process it is as follows: The dry raw material was ground into coarse powder using a high-speed blender. This coarse powder was extracted with 60 liters of de-mineralized water by heating for 2 hours at 80 ^{o}C. Aqueous layer was decanted after cooling and the residual marc was extracted three times more using 45 liters of water every time. All the extracts were combined and filtered. Filtrate was concentrated under vacuum at 70-80 ^{o}C for 3 to 4 hours. Finally this extract was dried in vacuum tray drier at 70-80 ^{o} C for 14 to 16 hours. The dried extract was milled, sieved and packed in polythene bags for further use. The herb: extract ratio was found to be 25:10 and the yield was 40% on dried basis.

Chemicals and reagents: Gallic acid of 98.5 % purity was procured from M/s Natural Remedies Pvt. Ltd., Bangalore. Acetonitrile, methanol, toluene, ethyl acetate, formic acid, HPLC grade water of analytical grade were purchased from M/s. Rankem Ltd. and double-distilled water was used in all experiments.

Sample preparation of *Emblica officinalis* extract: To prepare the sample of *Emblica officinalis*, 150 mg of plant extract was taken and crushed in mortar Pestle. From that, accurately weighed 100mg powder transferred to 25mL standard flask. Volume is made up to the mark with water: Acetonitrile: Acetic acid(90 : 10 : 0.2 v/v), sonicated for 10 min. It was filtered with 0.22µ filter to obtain sample stock solution. Aliquot of 0.5ml from this sample

stock solution is transferred to 10mL standard volumetric flask. Volume is made up to the mark with water: Acetonitrile: Acetic acid (90: 10: 0.2 v/v). Then it is filtered with 0.22µ filter. Prepared sample solution was analyzed.

Sample preparation of poly-herbal formulation: To prepare the sample of poly-herbal formulation, 20 capsules were taken, opened and crushed in mortar Pestle. From that, accurately weighed 584mg powder transferred to 25mL standard flask. Volume is made up to the mark with water: Acetonitrile: Acetic acid(90 : 10 : 0.2 v/v), sonicated for 10 min. It was filtered with 0.22µ filter to obtain sample stock solution. Aliquot of 0.5ml from this sample stock solution is transferred to 10mL standard volumetric flask. Volume is made up to the mark with water: Acetonitrile: Acetic acid(90 : 10 : 0.2 v/v). Then it is filtered with 0.22µ filter. Prepared sample solution was analyzed.

Table 1: Chromatographic Conditions of HPLC for Gallic acid.

Mobile Phase	Water : Acetonitrile : Acetic acid(90 : 10 : 0.2 v/v)
Stationary Phase	Phenomenex Luna C18 (4.6 x 250mm, 5µ particle size)
Wavelength	272 nm
Run time	8 min
Flow Rate	1 mL/min
Injection Volume	20 µL
Temperature	Ambient
Mode of Operation	Isocratic elution

Preparation of Calibration Curve for HPLC: To prepare the calibration curve for HPLC, aliquots of 0.1, 0.3, 0.5mL standard stock solution (1000µg/mL) was transferred to 10mL of volumetric flasks and made up to the mark with water : Acetonitrile : Acetic acid(90 : 10 : 0.2 v/v) to get concentration of 10, 30, 50µg/mL. Aliquot of 0.1, 0.2, 0.5, 1mL from 50µg/mL was transferred to 10mL of volumetric flasks and made up to the mark with water: Acetonitrile: Acetic acid(90 : 10 : 0.2 v/v) to get concentration of 0.5, 1, 2.5, 5µg/mL respectively. The calibration curve was plotted using peak area versus drug concentration as given in Figure1& Table 2.

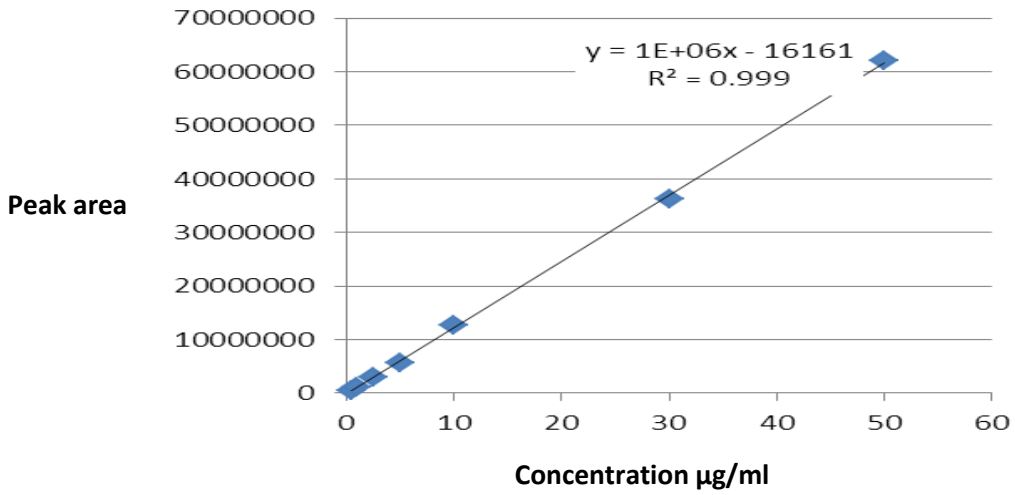

Figure 1: Linearity Graph of Gallic acid for HPLC.

Table 2: Linearity of Gallic acid, for assessing the linearity, the least square regression equation was adopted

Concentration (µg/mL)	Peak Area	Slope	Intercept	R^2
0.5	604490	1236331	161618	0.999773
1	1123993			
2.5	2890614			
5.0	5770210			
10.0	12597730			
30.0	36303160			
50.0	61975314			

Statistical analysis: Statistical calculations were carried out with the Microsoft Excel 2007 for Windows software package. Average, Sum, Standard Deviation (STDEV), Regression (RSQ) for Statistical Calculation, and Scattered Chart were used for Linearity; P values > 0.05 were considered to be significant.

RESULT AND DISCUSSION

Determination of Gallic acid in aqueous extract of *Emblica officinalis* and compound (Capsule) formulation: The amount of gallic acids in *Emblica officinalis* and in formulated Capsule were analyzed using optimized chromatographic method. The standards of different strengths, samples & compound formulation were injected in the HPLC column and peak areas were used for analysis of content by the regression equation. The developed mobile phase gave optimal separation, with well-defined and well-resolved sharp peaks in both standard and sample (Figure 2-10) at Retention time 4.31-4.34 for gallic acid,

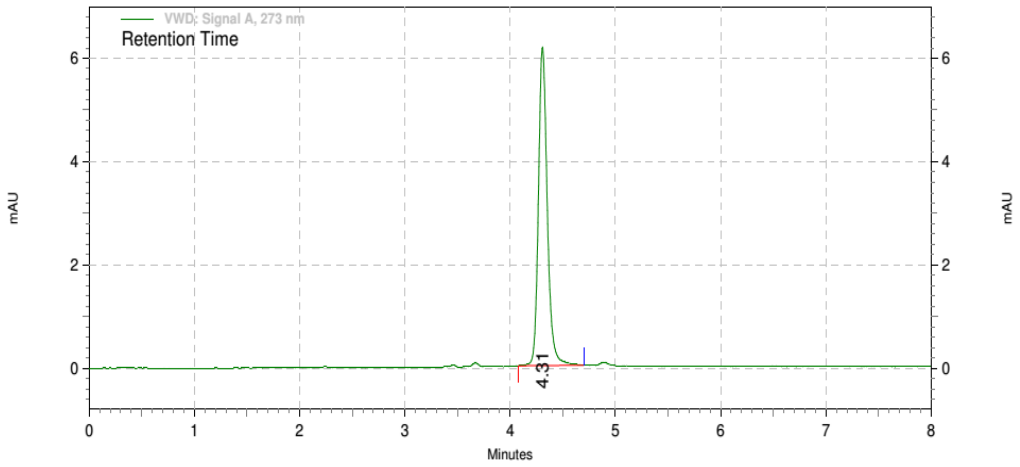

Figure 2: Chromatogram of Gallic acid Standard 0.5µg/ mL

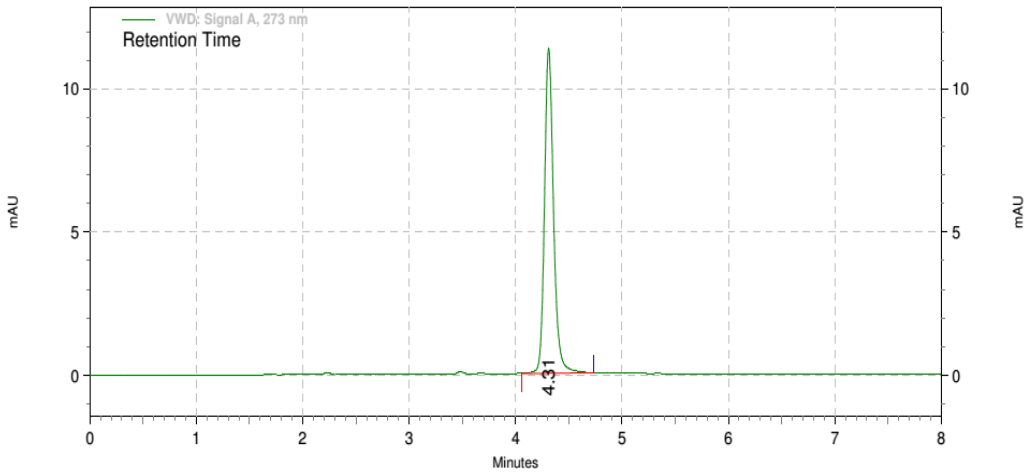

Figure 3: Chromatogram of Gallic acid Standard 1.0µg/ mL

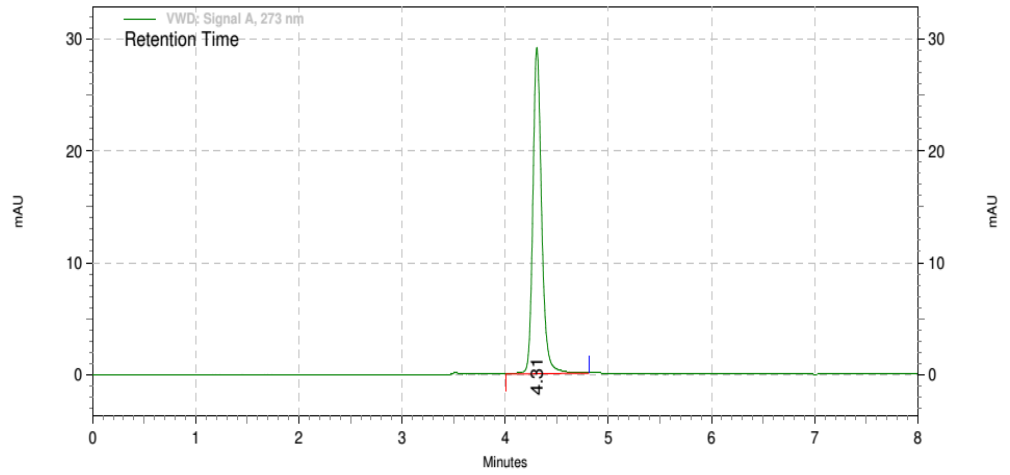

Figure 4: Chromatogram of Gallic acid Standard 2.5µg/ mL

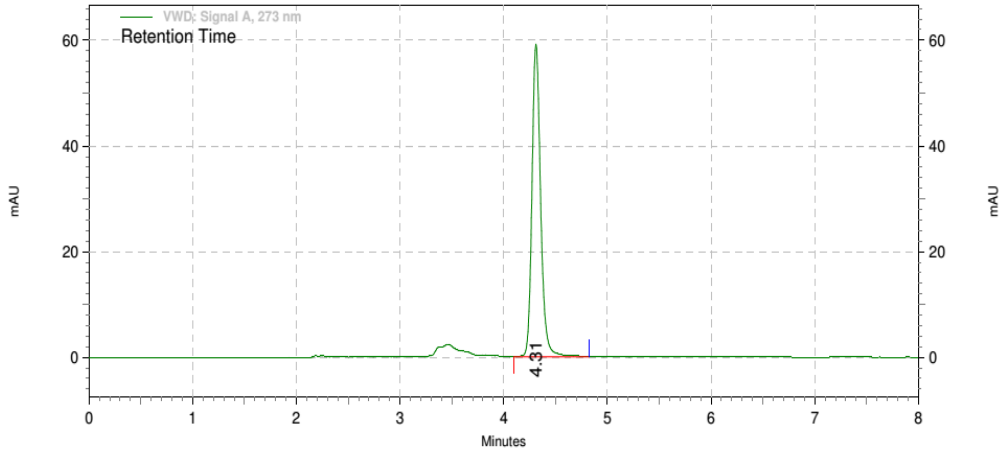

Figure 5: Chromatogram of Gallic acid Standard 5.0µg/ mL

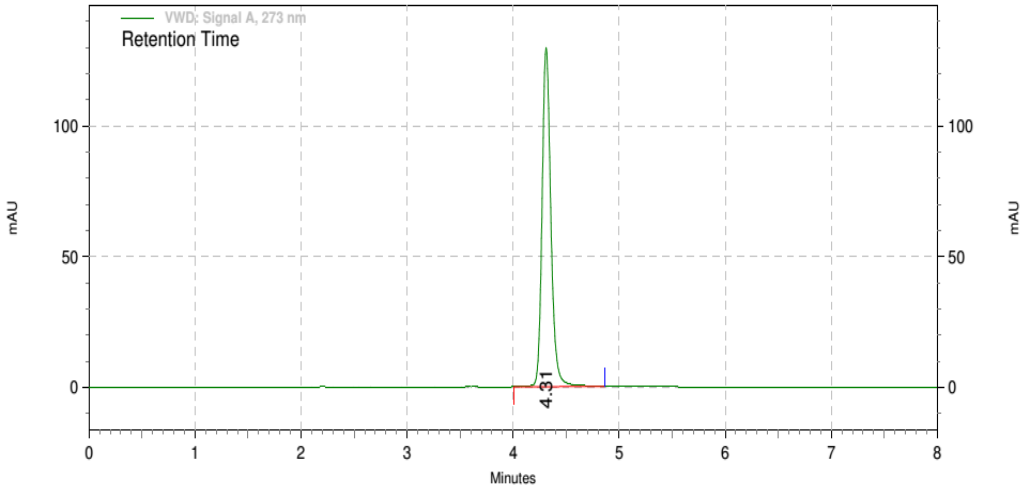

Figure 6: Chromatogram of Gallic acid Standard 10.0µg/ mL

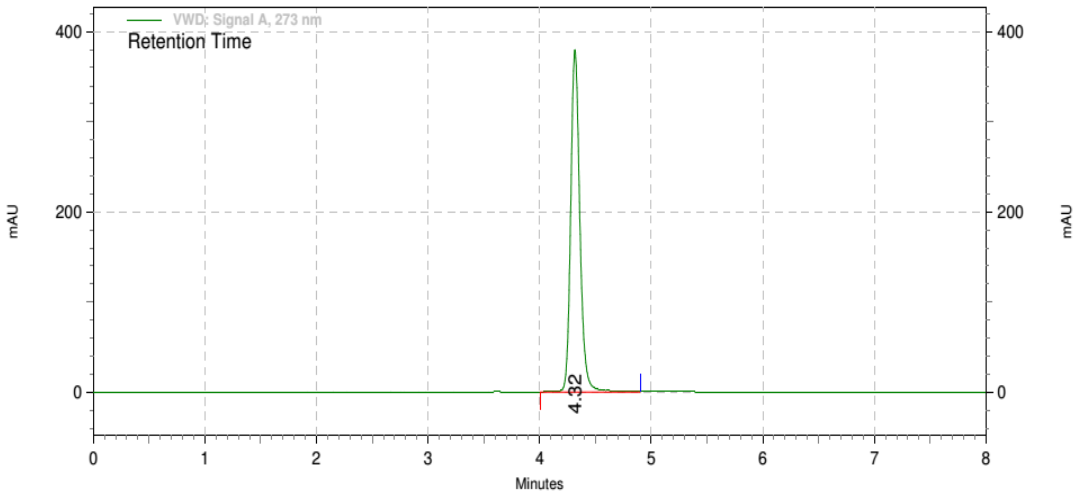

Figure 7: Chromatogram of Gallic acid Standard 30.0µg/ mL

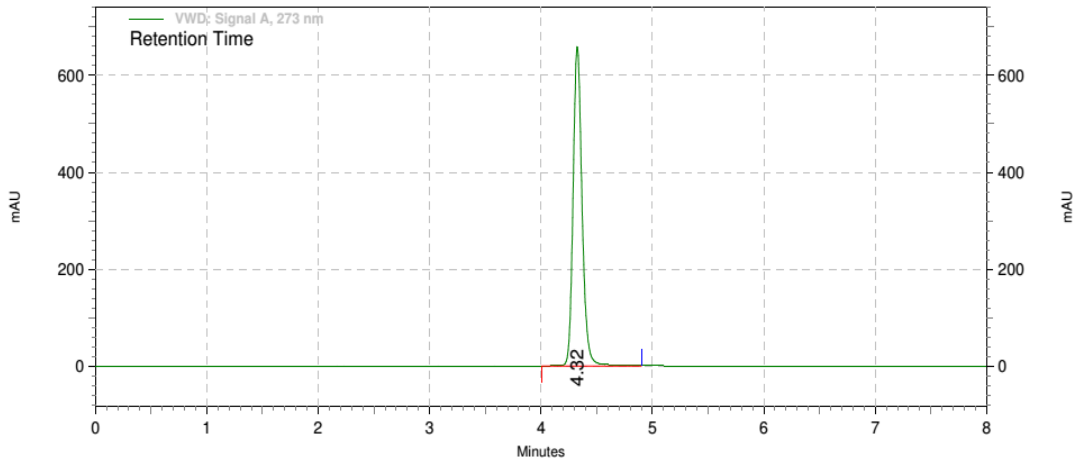

Figure 8: Chromatogram of Gallic acid Standard 50.0µg/ mL

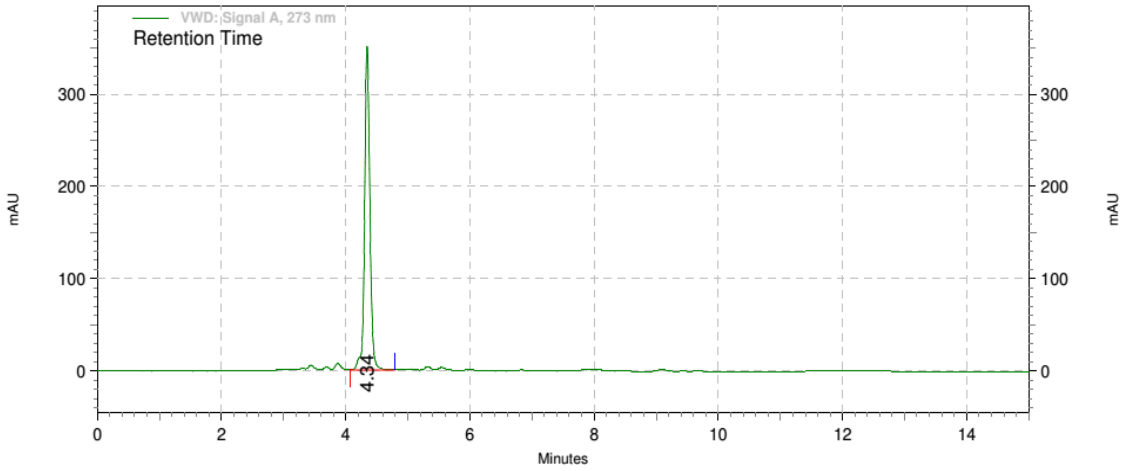

Figure 9: Chromatogram of Water extract of *Emblica officinalis* (dried fruit pericarp).

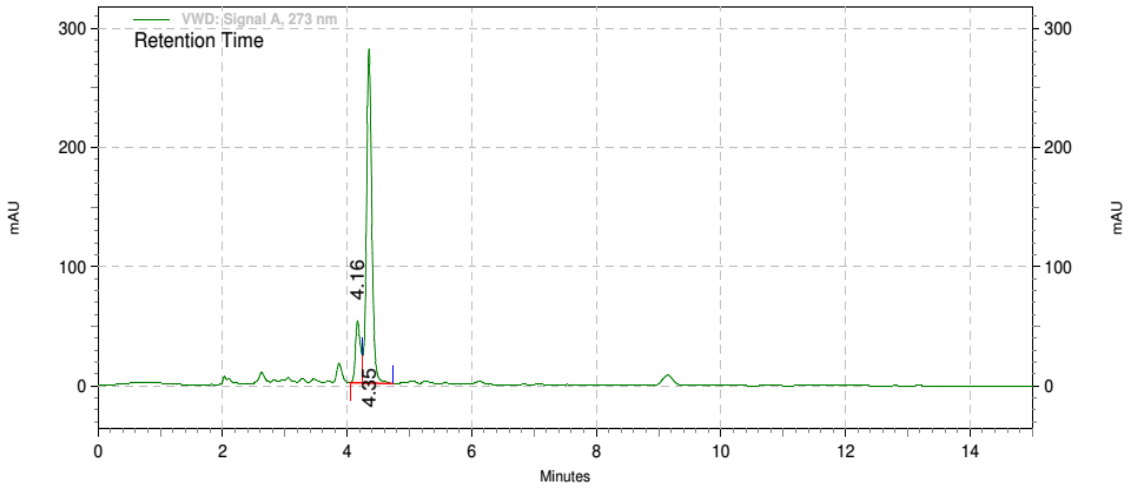

Figure 10: Chromatogram of developed formulation.

As the amount of gallic acid in Water extract of *Emblica officinalis* (dried fruit pericarp) reported in API is NLT 5.5%.[13] This raw material as such is used as one of the ingredient together with other three in the dosage form under study. The gallic acid in ingredient i,e, aqueous extract of *Emblica officinalis* was 14.17% w/w which complied the prescribed limit as mentioned in pharmacopoeias.

The amount of Gallic acid present in *Emblica officinalis* raw drug and extract reported in several official pharmacopoeias viz.

Pharmacopoeias	**Gallic acid in *Emblica officinalis* fruit**
Ayurvedic Pharmacopoeia of India(API)[13]	NLT 0.8% raw drug & NLT 7.5 (Water extract)
QSIMP [14]	0.015-.022%(in fresh fruit)
Indian pharmacopoeia(IP)[15]	NLT 1.0 per cent w/w (dried fruit pericarp)

However it is found 1.95% w/w in polyherbal formulation as analyzed by HPLC. Since the other ingredients except *Emblica officinalis* of the formulation do not or contain gallic acid in traces hence the gallic acid in this poly-herbal drug has driven from *Emblica officinalis* ingredient only.

CONCLUSION

This study authenticate the presence of *Emblica officinalis* ingredient on the basis of gallic acid in the the pharmaceutically developed poly-herbal dosage form (capsule). The amount of gallic acid in polyherbal formulation was found 1.95% w/w and 14.17% w/w in ingredient i,e, aqueous extract of *Emblica officinalis* obtained by RP-HPLC method.

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