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Estimation of Tannic Acid Bb RP-HPLC and Gallic Acid by HPTLC Method in the Methanolic Extract of *Carica papaya* Linn. Leaves and its Formulation

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ABSTRACT

After a through phytochemical screening of the Carica papaya Leaf extract for the constituents present, the tannins namely tannic acid and gallic acid were analyzed and quantified in the crude methanolic extract and herbal formulations. Both the RP-HPLC and HPTLC methods were developed and validated according to the ICH Q2 (R1) guidelines. The tannic acid was quantified by the RP-HPLC method and gallic acid was quantified by the HPTLC method. The linearity of tannic acid and gallic acid was found to be in the range of 20-50 µg/ml (y=2121.99 x-29648.1) and 400-1200 ng/spot (y=6.4325 x-2016) respectively with a correlation coefficient of 0.999 for both calibration graphs. The % RSD values calculated for the intraday, inter day precision repeatability of measurement, repeatability of sample application and accuracy studies which was found to be below 2. The LOD and LOQ were found to be 1 and 5 µg/ml respectively for tannic acid. The LOD and LOQ were found to be 100 and 300 ng/band respectively for gallic acid. The percentage recovery of tannic acid was found to be between 95 to 97% and between 94-97% for gallic acid at 80%, 100% and 120% level of recovery. The amount of tannic acid and gallic acid quantified in the herbal formulation was found to be 11 µg/ml and 0.378 µg/ml respectively. Both the chromatographic methods are simple, precise, accurate and sensitive which can be applied for the routine analysis of the two constituents in the Carica papaya leaf extract.

Keywords: *Carica papaya* Linn. Leaf extract, Tannic acid, Gallic acid, RP-HPLC, HPTLC.

INTRODUCTION

Medicinal plants are of great importance to health due to the presence of phytoconstituents. The most important of these constituents are alkaloids, glycosides, tannins, flavonoids, and phenolic compounds.

Carica papaya Linn. (Caricaceae) is a fast-growing, semi woody tropical herb reaching 3-10 m in height. *Carica papaya* contains many biologically active compounds [1]. Tannic acid, a kind of polyphenols, possesses the numerous phenolic hydroxyl groups in the structure, which make it possess excellent physical and chemical properties and remarkable biological and pharmacological activities. Tannic acid can interact with metals, proteins, alkaloids, and polysaccharides and perform several physiological and ecological effects [2].

Gallic acid is found in a wide variety of vegetables, fruits, tea, coffee and wine. It occurs in plants in the form of free acids, esters, catechin derivatives and hydrolysable tannins. Gallic acid has been reported to elicit various biological activities such as antibacterial, anti-fungal, antiviral, anti-inflammatory, antioxidant, anticancer, anti-diabetic etc. Gallic acid (GA) is a phenolic compound. It is chemically known as 3, 4, 5-trihydroxybenzoic acid [3].

The literature reveals that a number of methods have been reported for the isolation and estimation of constituents like alkaloids, flavonoids, phenolic compounds present in the *Carica papaya* leaf extract [4-12]. However, none of the methods were reported for the quantification of tannic acid and gallic acid present in the *Carica papaya* leaf using chromatographic methods based on HPLC or HPTLC. Hence the area of research for tannins compounds were considered and carried out.

MATERIALS AND METHODS

Analytical instruments

Shimadzu Prominence UFLC (Shimadzu Corporation, Kyoto, Japan) equipped with LC-20AD pump, SPD-M20A diode array detector, DGU-20A_3 degasser, SIL-20AC autosampler, CTO-10ASVP column oven (LC solutions chromatographic software, version 3.41.324) with the stationary phase Shim-pack GIST C_{18} (4.6 mm id × 150 mm, 5 µ particle size) Column, (Shimadzu Corporation, Kyoto, Japan) was used for the RP-HPLC method.

For the HPTLC method Camag Linomat 5 Applicator, TLC Scanner 3 controlled by winCATS - planar chromatography manager, version 1.2.6 (Camag, Muttenz, Switzerland) and sampling application on Merck TLC plates coated with silica gel 60F_{254} on aluminium sheet were used as stationary phase (Merck chemicals Ltd., Darmstadt, Germany).

The pH measurement was carried out using the Elico pH meter (Elico Ltd., Hyderabad, India). The extracts were sonicated using Leelasonic Ultrasonic Cleaner (Leelasonic, Mumbai, India). The sample weighing was done using Shimadzu Electronic Balance (ShimadzuCorporation, Kyoto, Japan). The filteration of solution was by the Gelman Sciences Vacuum Pump, (Pall Pharma lab Filtration Pvt. Ltd., Mumbai, India).

Chemicals and Reagents

The solvents such as water, acetonitrile, methanol, toluene, ethyl acetate and potassium dihydrogen phosphate were sourced from Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India. The biomarkers tannic acid and gallic acid were procured from Loba Chemie Pvt. Ltd and Himedia.

RP-HPLC Method

Preparation of mobile phase: Mobile phase consisted of mixture of two solvents A and B. The buffer made up of 10 mM potassium dihydrogen phosphate, with pH 4.7 was prepared using water as the solvent. The phosphate buffer was used as the solvent A. A mixture of acetonitrile and methanol in the ratio of 2:8 was used as the solvent B. All solutions were degassed by ultrasonication and filtered through 0.45 µm Nylon filter.

Preparation of stock solutions: An accurately weighed quantity of 10 mg of tannic acid was transferred into a 10 ml volumetric flask and dissolved using methanol. The final volume was made up to the mark to obtain a stock solution of tannic acid. The stock solution of 1000 µg/ml of tannic acid was used for further dilutions.

Preparation of working standard solutions: A working standard solution consisting of tannic acid (100 µg/ml) was also prepared with methanol as the diluent. The working standard solution prepared was further diluted using methanol for the linearity range, precision studies, standard solution used for accuracy appropriately to get the required concentration.

Preparation of extracts of *Carica papaya* leaf: Three different solvents were selected for the extraction of the tannic acid with varying polarity of highly polar aqueous solution to less polar chloroform as the solvent. *Carica papaya* leaf powder is extracted in three solvents, water: ethanol (50:50); methanol and chloroform.

An accurate weight of 25 g of dried leaf powder of *Carica papaya* was weighed and transferred to a 100 ml volumetric flask. The weighing was repeated three times into three different flasks. To each flask containing the leaf powder, 10 ml of solvent selected was added individually and thoroughly shaken. The volume was made up using the respective solvent and mixed well for the uniform distribution of the powder. The volumetric flask was kept at room temperature protected from light for the efficient maceration of the leaf powder for 72 hours. The solution is sonicated for 15 min and filtered using Whatman filter paper. The solvents of the filtrates were evaporated and reconstituted with methanol for use.

Preparation of solution for the formulation: The Caripill tablet crushed to powder was transferred into a beaker; 10 ml of methanol was added, shaken thoroughly to dissolve, left to stand for 72 hours at ambient temperature protected from light and contamination. The extract obtained was sonicated for 15 min and filtered using Whatman filter paper. The filtrate was evaporated to get a concentrated solution which was filtered through 0.2 µm syringe filter and used.

Validation of HPLC method

After achieving the optimal chromatographic separations, the method was subjected to validation process. The proposed method was validated as per ICH guidelines Q2 (R1).

Linearity: The working standard solution of 100 µg/ml was used to prepare the test solutions. Linearity test solutions containing tannic acid at seven concentration levels of 20, 25, 30, 35, 40, 45, 50 µg/ml were prepared using methanol as the diluent.

The calibration curve was drawn by plotting peak areas of tannic acid versus its corresponding concentrations. The linearity was evaluated by linear regression analysis, which was calculated by least square regression method. The correlation coefficient, slope and y - intercept of the calibration curve were obtained from the calibration graph.

Precision: The precision of the method was demonstrated by repeatability, intraday and interday precision studies.

Intra-day precision: The intra-day precision for the standard drug was investigated by injecting six individual preparations of 40 µg/ml of tannic acid within the same day. The chromatograms recorded were studied for the peak area and evaluated.

Inter-day precision: The Inter-day precision of the method was verified by repeating the same method in three consecutive days in the same laboratory under similar experimental conditions. The results were noted and tabulated.

Repeatability of the sample application: Repeatability of the sample application was also verified with the standard solution of tannic acid in the concentration of 40 µg/ml by injecting six times. The peak areas were noted for each injection and were statistically analysed for the % RSD.

Accuracy: The pre-analyzed tablet of the leaf powder was evaluated for the recovery of the spiked standard of tannic acid. The accuracy was carried out in triplicate at three concentration levels of 80%, 100% and 120%. The amount of standard added and the % recovery were calculated which were subjected to statistical analysis of % RSD.

Limit of detection (LOD) and limit of quantification (LOQ): A series of diluted solutions at known concentrations was injected to find out the LOD and LOQ below the linearity range observed. The detectable limit with an acceptable peak area was studied. The concentration limit that can be quantified was observed and noted.

System suitability: The system suitability studies were carried out by analyzing the chromatographic characteristics of the peak eluted. The peak characters analyzed were the peak area, plate number, resolution and tailing factor. The standard tannic acid solution at a concentration of 40 µg/ml was used for the studies.

Specificity: The method developed was checked for its specificity. This parameter is investigated by injecting blank solvent methanol under the fixed experimental conditions. The retention time and peak area were noted. This test is carried out to make sure that the analytes is eluted without any interference from the solvent used for the work.

Stability of solution: The stability of the solutions prepared and used for the studies were evaluated. The stability is determined by injecting the standard solution at various time intervals and their resulting chromatograms were recorded. The peak characteristics were studied for the evaluation of stability.

Application of the developed method to the crude Carica papaya leaf extract

The HPLC method developed and validated for the standard tannic acid was applied to the methanolic extract of the leaf powder. Tannic acid content was determined in *Carica papaya* leaf extract.

The crude extract of the leaf powder prepared as mentioned earlier in various individual solvents such as water-ethanol mixture (50:50), methanol, chloroform, were injected under similar experimental conditions applied for the standard for triplicate times. Well separated peaks were identified with acceptable peak characteristics in the methanol extract. The peaks were eluted till 15 minutes and the chromatograms were studied for the retention time and spectral match with the standard tannic acid. From the recorded peak areas, the amount of tannic acid present in the crude methanolic extract of *Carica papaya* was quantified.

Assay of marketed formulation: The HPLC method developed and validated for the standard tannic acid was applied to the tablets. The methanol extract prepared from the tablet was injected under similar operating conditions as that of the standard tannic acid and the peaks were eluted for the time period of 15 minutes. The recorded chromatograms were analysed for the

peaks and studied for the quantification of tannic acid. Determination of tannic acid content present in the herbal tablet is quantified.

Development and validation of High Performance Thin layer Chromatographic method for the estimation of gallic acid in the Carica papaya leaf extract and its formulation

Preparation of stock solutions: A stock solution of standard was prepared by dissolving an accurately weighed quantity of 10 mg of gallic acid in the selected diluent methanol. The solution is made up to the mark of 10 ml of the volumetric flask with methanol to obtain the gallic acid solution of concentration (1000 µg/ml). The prepared stock solution is used for the further dilutions.

Preparation of working standard solutions: A working standard solution gallic acid (100 µg/ml) was prepared by diluting the stock solution of gallic acid with methanol as diluent. The working standard solution prepared was further diluted using methanol for the linearity range, precision studies, standard solution used for accuracy appropriately to get the required concentration.

Preparation of sample solution (leaf extract): The crude *Carica papaya* leaf powder was extracted using three different solvents. These solvents were selected for the extraction of the gallic acid with varying polarity of highly polar aqueous solution to less polar chloroform as the solvent. The leaf powder is extracted in three individual solvents, water: ethanol (50:50); methanol and chloroform.

An accurate weight of 25 g of dried leaf powder of *Carica papaya* was weighed and transferred to a 100 ml volumetric flask. The weighing was repeated three times into three different flasks. To each flask containing the leaf powder, 10 ml of solvent selected was added individually and thoroughly shaken. The volume was made up using the respective solvent and mixed well for the uniform distribution of the powder. The volumetric flask was kept at room temperature protected from light for the efficient maceration of the leaf powder for 72 hours. The solution was sonicated for 15 min and filtered using Whatman filter paper. The solvents of the filtrates were evaporated and reconstituted with methanol for use.

Preparation of sample solution (formulation): The Caripill tablet was crushed to powder and transferred into a beaker; 10 ml of methanol was added, shaken thoroughly to dissolve, left to stand for 72 hours at ambient temperature, sonicated for 15 min and filteredusing Whatman filter paper. From this stock solution, working standard solution was prepared in diluent for the determination of gallic acid in the formulation.

Method validation: After achieving the optimal chromatographic condition, the method was subjected to the validation process in order to prove its reliability and suitability for its intended use. The proposed method was validated as per ICH guidelines Q2 (R1).

Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of the analyte in the sample.

For linearity study, aliquots of 100 µg/ml gallic acid working standard solution 4 µl, 6 µl, 8 µl, 10 µl and 12 µl was applied on pre-coated TLC plate to obtain the concentrations of 400, 600, 800, 1000 and 1200 ng/band. TLC plates were developed under the fixed chromatographic condition and scanned.

The calibration curve was drawn by plotting peak areas of gallic acid versus its corresponding concentrations. The linearity was evaluated by linear regression analysis, which was calculated by least square regression method. The correlation coefficient, slope and y - intercept of the calibration curve were obtained from the calibration graph.

Precision: The precision of the method was demonstrated by repeatability, intraday and interday precision studies.

Intra-day precision: The intra-day precision for the standard drug was investigated by injecting six individual preparations of 800 ng/band of gallic acid within the same day. The densitograms recorded were studied for the peak area and evaluated. The precision of the proposed method was obtained by calculating the % RSD values.

Inter-day precision: The Inter-day precision of the method was verified by repeating the same method in three consecutive days in the same laboratory under similar experimental conditions. The results were noted and tabulated and the precision of the proposed method was obtained by calculating the % RSD values.

Repeatability-Sample application and Sample measurement

Repeatability of sample application: It was evaluated by applying gallic acid (600 ng/band) six times on pre-coated TLC plate. This plate was developed, scanned and % RSD of peak areas was calculated.

Repeatability of sample measurement: It was evaluated by applying gallic acid (600 ng/band) on pre-coated TLC plate. After development the same spot was scanned six times without changing the position of plate and % RSD of peak areas was calculated.

Accuracy: The accuracy of this method was evaluated in triplicate at three concentrations levels of 80%, 100% and 120% of spiked standard and the percent recoveries were calculated. This method was carried out by mixing the known quantity of standard drug with pre analyzed formulation and then the contents were assayed for the gallic acid. The amount of standard added and the % recovery were calculated which were subjected to statistical analysis of % RSD.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of gallic acid were estimated by spotting the series of standard solutions of concentration below the linearity range selected. The lowest concentration that can be detected was noted and the concentration that can be quantified with the acceptable peak area was identified.

System suitability (Chromatographic performance): The system suitability parameters like asymmetry factor and tailing factor were evaluated by six replicate injections of the standard gallic acid solution at concentration of 800 ng/band.

Specificity/selectivity: The specificity and selectivity of the developed method was investigated by injecting the blank solution methanol to evaluate the interferences in the R_f value of analyte peak in the standard and sample solution. The peak purity of gallic acid was assessed by comparing the UV spectra at three levels, i.e., peak start (S), peak apex (M), and peak end (E) position of spot.

Stability study of the plate: When the developed chromatographic plate is exposed to atmospheric condition, the analyte is likely to decompose. The stability of the analyte on plate was studied at specific time intervals of up to 8 hours and peak areas were compared with the peak area of freshly scanned plate.

Application of the developed HPTLC method for crude Carica papaya leaf extract

The HPTLC method developed and validated for the standard gallic acid was applied to the methanolic extract of the leaf powder. Gallic acid content was determined in *Carica papaya* leaf extract.

The crude extract of the leaf powder prepared as mentioned earlier with the selected diluent methanol, was injected under similar experimental conditions applied for the standard in triplicate.

Well separated peaks were identified with acceptable peak characteristics in the methanol extract. The densitograms recorded were studied for the R_f and spectral match with that of the standard gallic acid. From the recorded peak areas, the amount of gallic acid present in the crude methanolic extract of *Carica papaya* was quantified.

Assay of marketed formulation

The developed method was applied for the analysis of marketed formulation of *Carica papaya* leaf extract. The methanol extract prepared from the tablet as mentioned earlier was injected under similar operating conditions as that of standard gallic acid. The densitograms recorded were studied for the R_f and spectral match with that of the standard gallic acid. From the recorded peak areas, the amount of gallic acid present in the herbal tablet formulation was quantified.

RESULTS

RP-HPLC method for the determination of tannic acid in the carica papaya leaf extract and its formulation

Selection of wavelength: The wavelength selected for the HPLC method was based on the UV spectra recorded. Tannic acid showed two wavelength maxima at 214 nm and 276 nm. So, the wavelength of 276 nm was selected as detection wavelength (Figure 1).

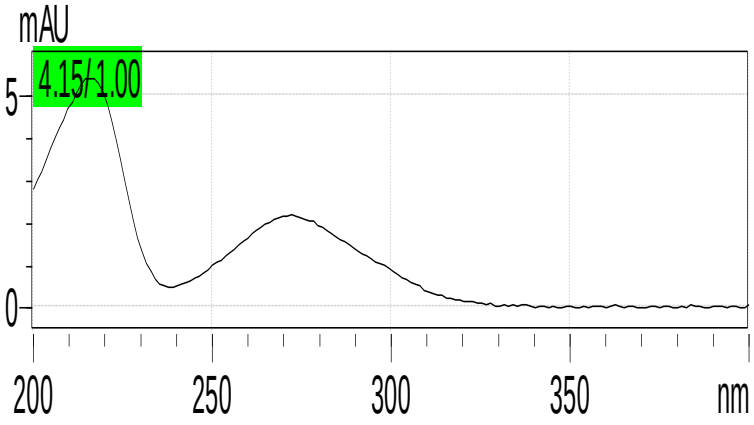

Figure 1: UV spectrum of Tannic acid.

Fixed chromatographic conditions: The column used for the work is RP – C18 column shimpack; 150 mm × 4.6 ID; 5micron particle size optimized at a wavelength of 276 nm with the mobile phase composition (75: 25, v/v) of 10 mM potassium dihydrogen phosphate buffer and methanol-acetonitrile mixture in 8:2 ratio at a flow rate of 1 ml/min and injection volume of 20 µl and operating temperature of 30°C.

Method validation: The validation study was performed to check the suitability of the method for routine analysis of tannic acid.

Linearity: Linear calibration plot for standard tannic acid is established over a calibration range of seven concentrations (20 to 50 µg/ml). The regression equation from the plot was y =2121.99 × -29648.1 and the correlation coefficient value was found to be 0.999 for tannic acid. The above results showed good correlation between the peak areas and concentrations of tannic acid. The representative chromatogram of the standard tannic acid is shown in Figure 2. The calibration graph and data for the linearity of tannic acid are given in Figure 3 and Table 1.

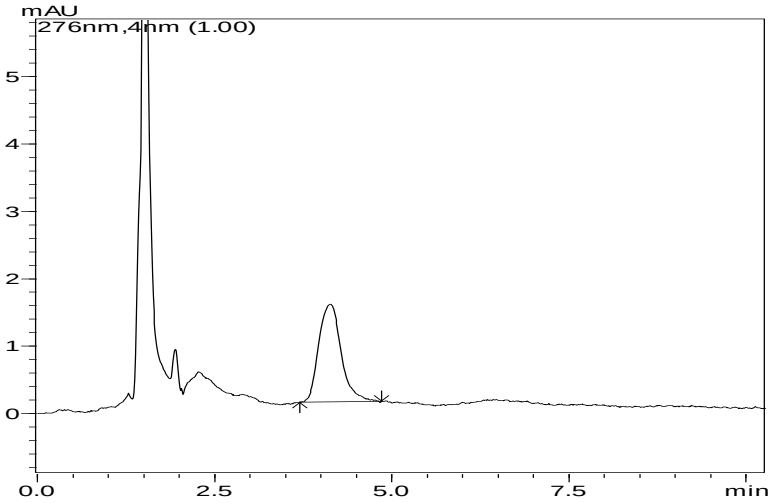

Figure 2: Chromatogram of tannic acid 30 µg/ml

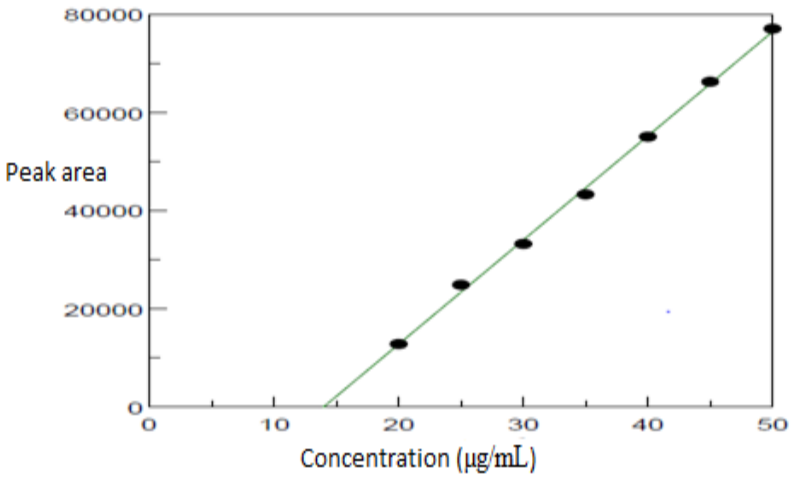

Figure 3: Calibration graph of tannic acid (20-50 µg/ml).

Table 1: Calibration data for tannic acid.

Conc. (µg/ml)	Peak area
20	12768
25	24889
30	33225
35	43255
40	55028
45	66198
50	76987

Precision: The RSD (%) values for inter- and intra- day precision and repeatability of sample application for the standard was found to be within 2%, confirming the precision of the developed method.

Accuracy: The recovery (%) of tannic acid was found to be between 95% to 97% confirming the accuracy of the developed method. The recovery (%) values for tannic acid are given in Table 2.

Table 2: Data for the recovery studies.

Level (%)	Conc. of standard added (µg/ml)	% recovery	Amount of standard found	RSD (%)*
80	450	95.9	431.55	1.1
100	550	94.8	521.4	1.2
120	650	97	630.5	1.4
*Average of 3 determinations				

Limit of detection (LOD) and limit of quantification (LOQ): The LOD of tannic acid was 1 µg/ml (Figure 4) and their LOQ value was found to be 5 µg/ml (Figure 5).

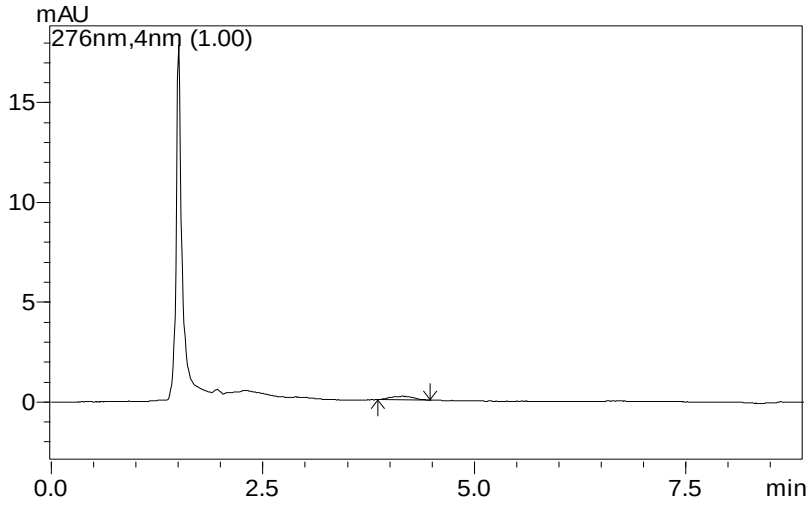

Figure 4: Chromatogram of LOD (1 µg/ml).

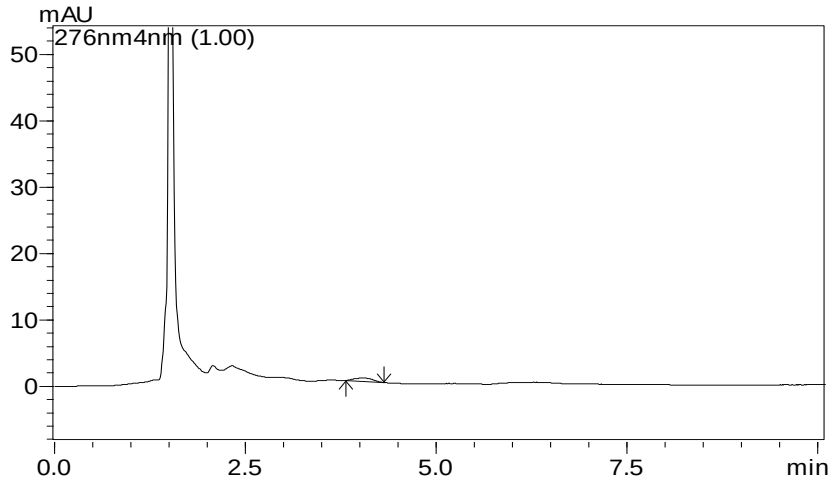

Figure 5: Cromatogram of LOQ (5 µg/ml)

System suitability (chromatographic performance): USP defines parameters that can be used to determine system suitability prior to analysis. These parameters include plate number (N), tailing factor (T_f), capacity factor (k) and relative standard deviation (RSD) of peak area or peak height for repetitive injections. The data are represented in Table 3.

Table 3: System suitability studies data.

Compound	%RSD* peak area	N	T_f	k
Tannic acid	0.6	2129	1.1	1.6
* Mean of six determinations				

Specificity: Under specificity, the ability of the analytical method to measure the analyte response in the presence of potential impurities and degradants was studied. No interference was observed, peaks of analytes were spectrally pure (peak purity index value = 1) and there were no co-eluting peaks and no difficulty in peak integration. Therefore, the developed method is specific for the determination of tannic acid. The blank chromatogram is shown in Figure 6.

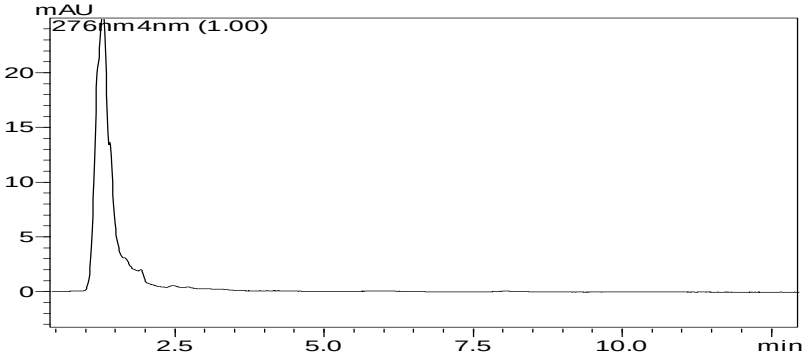

Figure 6: Chromatogram of blank.

Stability of solution

At room temperature: The standard tannic acid solution prepared and used for the study at room temperature was tested for its stability. Stability test was carried out by injecting the standard solution of tannic acid at different time intervals upto 8 hours and their peak areas were evaluated.

Refrigerated conditions: The solution of tannic acid stored under refrigerated conditions was tested for its stability. The stability of solution was evaluated by injecting them for 5 consecutive days and their peak areas were studied.

Application of the developed method to the crude *carica papaya* leaf extract: Sample solution was injected, chromatogram was recorded. From the chromatogram of *Carica papaya* leaf extract, the peak areas were evaluated to estimate the tannic acid content present in the extract. The amount of tannic acid present the methanolic leaf extract was found to be 15.2 mcg/ml. The procedure was carried out for three replicate times. The chromatogram of the *Carica papaya* leaf extract is shown in the Figure 7.

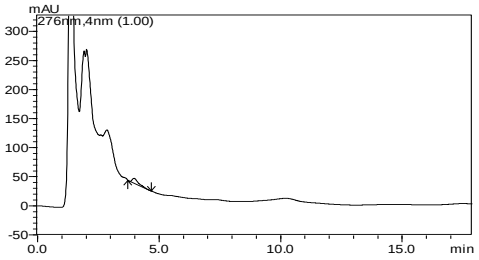

Figure 7: Representative chromatogram of the *Carica papaya* leaf extract.

Assay of marketed formulation: The developed method was applied for determination of tannic acid content in formulation. A sample solution was injected, densitograms were recorded. The results of formulation analysis are shown in Table 4 and Figure 8.

Table 4: Analysis of formulation.

Formulation	Amount of tannic acid found	%RSD*
Caripill	11 µg/ml	1.96
*RSD of 6 determinations		

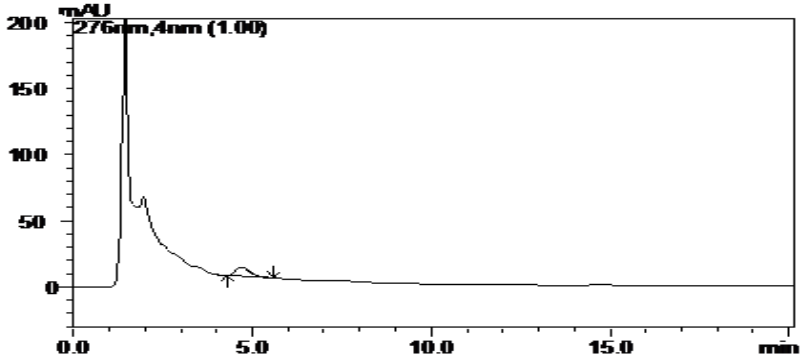

Figure 8: Chromatogram of caripill formulation.

Development and validation of High Performance Thin layer Chromatographic method for the estimation of gallic acid in the Carica papaya leaf extract and its formulation

Fixed chromatographic condition: The stationary phase used for the HPTLC method is Pre-coated silica gel $60F_{254}$ on aluminum sheets and the mobile phase consists of toluene: ethyl acetate: methanol [5:2:3, v/v/v] with 20 minutes as the chamber saturation time, 8 cm as the migration distance with a band width of 6 mm with detection of sample at 273 nm (Figure 9). The R_f value was found to be 0.29 ± 0.03**.**

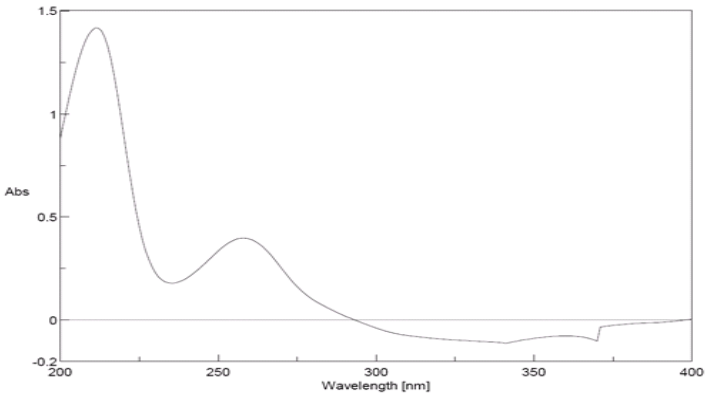

Figure 9: UV Spectrum of gallic acid.

Method Validation

Linearity: For linearity study, the calibration graph was plotted over concentration range of 400 to 1200 ng/band. Regression equation from the plot was y = 6.4325 × -2016 and the correlation coefficient value were found to be 0.999. The above result showed good correlation between the peak areas and concentrations of gallic acid. The densitograms of representative gallic acid standard is shown in Figure 10. The calibration data and graph is shown in Figure 11 and Table 5.

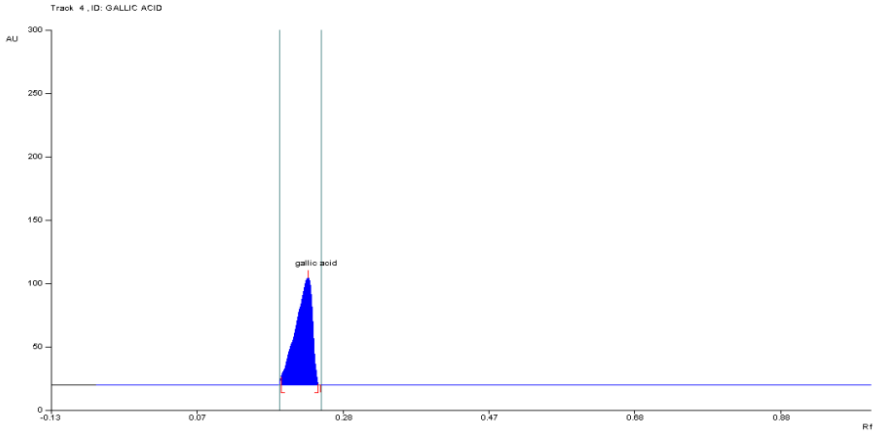

Figure 10: Densitometric chromatogram of standard gallic acid (600 ng/band).

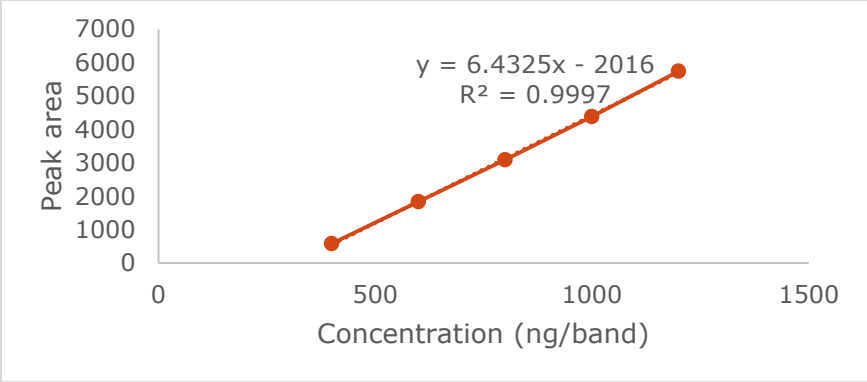

Figure 11: Calibration graph for gallic acid (400 – 1200 ng/band).

Table 5: Calibration data for gallic acid.

Conc. (ng/band)	Peak area
400	585
600	1840
800	3094
1000	4387
1200	5744

Precision: The % RSD values for inter- and Intra-day precision, repeatability of sample application and sample measurement for gallic acid were found to be within 2% confirming good precision of the developed method.

Accuracy: The percentage recovery of gallic acid was found to be between 94% to 97% confirming the accuracy of the developed method. The % recovery values for gallic acid are given in the Table 6.

Table 6: Recovery data for gallic acid.

Level	Conc. of standard added (ng/band)	Amount of standard recovered	% recovery	%RSD*
80%	432	406.08	94%	0.92
100%	540	518.61	96.04%	0.46
120%	648	622.08	96%	0.29
*Average of 3 determinations				

Limit of detection (LOD) and limit of quantification (LOQ): The LOD and LOQ were found to be 100 and 300 ng/band respectively. This result shows that the developed method is sensitive. The densitograms of the LOD and LOQ are shown in Figure 12 and Figure 13.

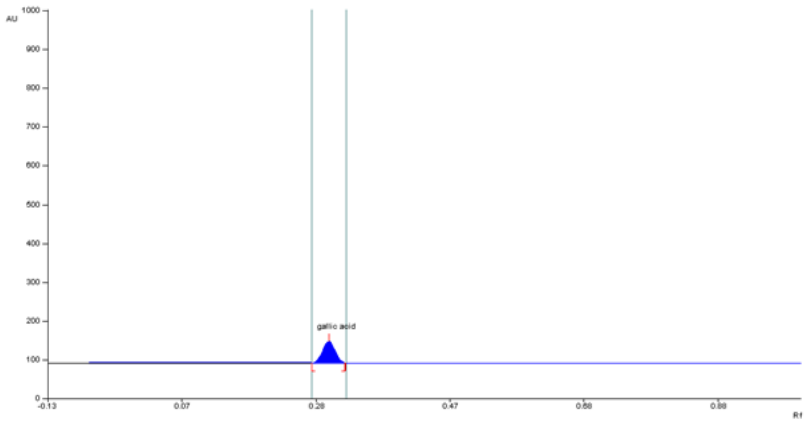

Figure 12: Densitogram of LOD (100 ng/band).

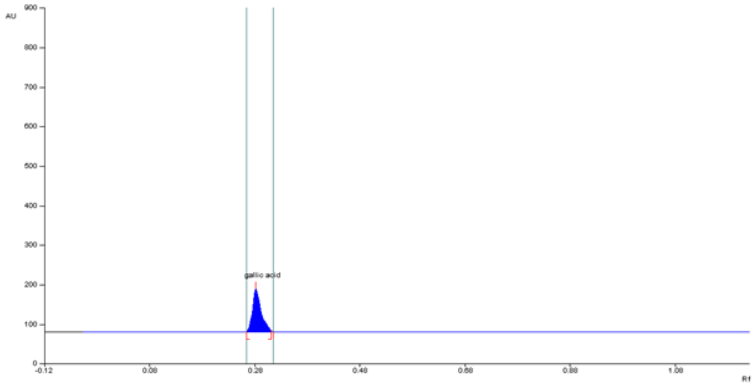

Figure 13: Densitogram of LOQ (300 ng/band).

System suitability: The Asymmetry factor and tailing factor were evaluated by six replicate injections of the standard gallic acid solution at concentration of 800 ng/band (Table 7).

Table 7: System suitability data.

Tailing factor	Asymmetry factor
2	1.2

Specificity/Selectivity: The peak purity of gallic acid was assessed by comparing their UV spectra at peak start (s), peak apex (m), peak end (e) position of the spot. Good correlation was obtained among spectra acquired at start (s), apex (m) and end (e) of the peaks [{correlation r (s, m) = 0.9998 and r (m, e) = 0.9987. Hence it can be concluded that analytes peak was spectrally pure and there were no co-eluting peaks. Therefore, the developed method is specific for determination of gallic acid.

Stability studies: The % RSD of assaying gallic acid during plate stability experiment was within 1%. No significant changes were observed in content of gallic acid during the plate stability experiments. The results of plate stability experiment confirm that developed plate was stable upto 8 hours.

Application of the developed method to crude *Carica papaya* leaf extract: The developed method was applied for determination of gallic acid content in *Carica papaya* leaf extract. A sample solution of *Carica papaya* leaf extract prepared as mentioned earlier was injected under same experimental conditions as that of standard.

The densitograms of the sample were recorded and their peak areas were noted. From the peak areas of the sample, the amount of gallic acid present in them was quantified. The amount of gallic acid present in the *Carica papaya* leaf extract was found to be 0.540 µg/ml. The densitogram of the *Carica papaya* leaf extract is shown in Figure 13 and the densitogram of gallic acid in *Carica papaya* leaf extract is shown in Figure 14.

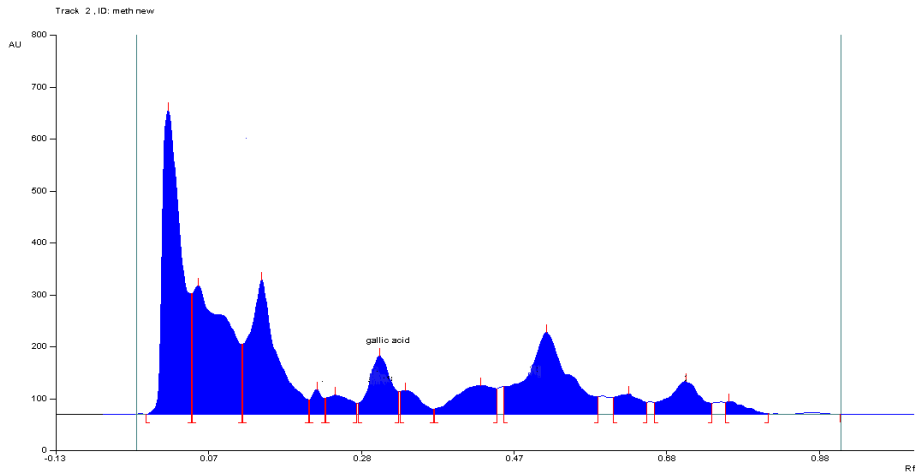

Figure 13: Densitogram of crude *Carica papaya* leaf extract.

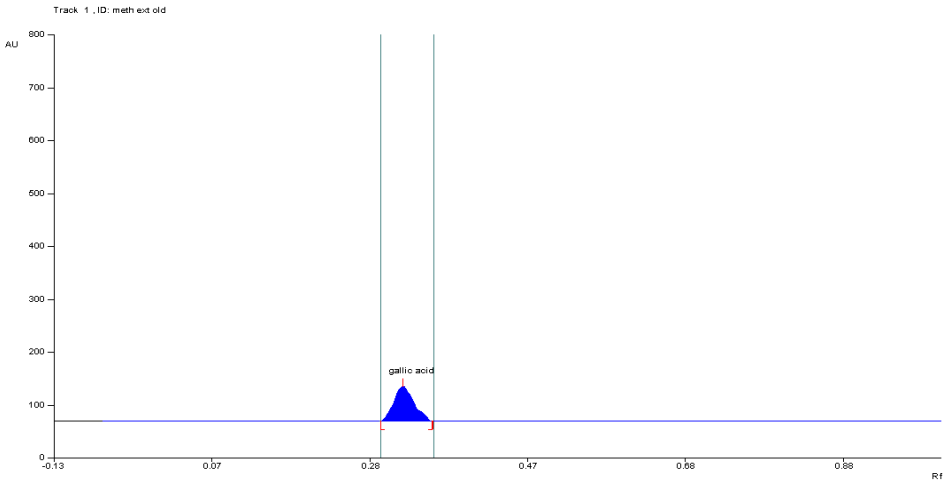

Figure 14: Densitogram of gallic acid in *Carica papaya* leaf extract.

Assay of marketed formulation: The developed method was applied for determination of gallic acid content in formulation. A sample solution of the formulation was injected, densitograms were recorded and shown in the Figure 15 and the densitogram of gallic acid in formulation is shown in Figure 16. The results of formulation analysis are shown in Table 8.

Table 8: Analysis of formulation.

Formulation	Amount of gallic acid found	%RSD*
Caripill	0.378 mcg/ml	1.96
*RSD of 6 determinations		

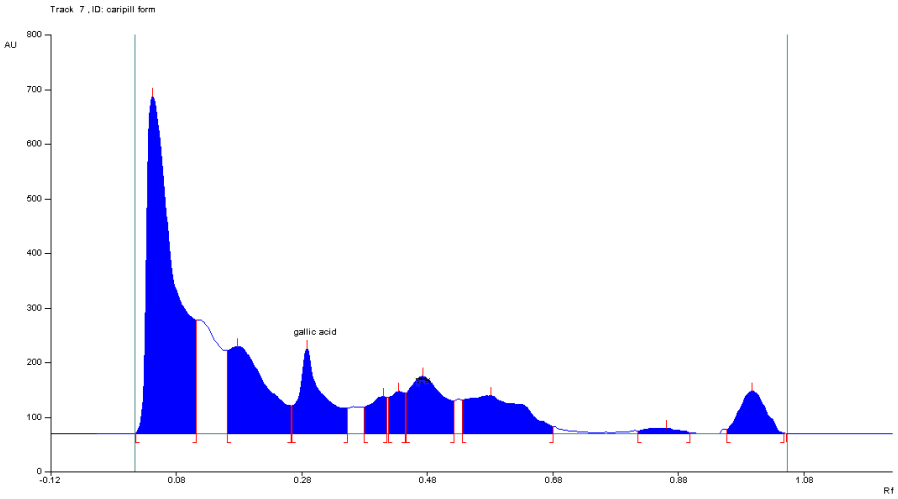

Figure 15: Densitogram of herbal tablet formulation

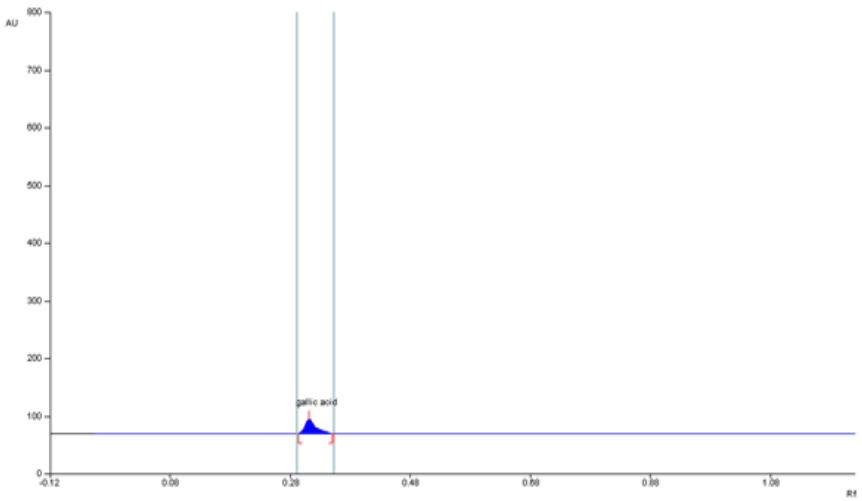

Figure 16: Densitogram of gallic acid in formulation

DISCUSSION

RP-HPLC method for the determination of tannic acid in the carica papaya leaf extract and its formulation

The RP-HPLC method has been developed and validated as per ICH guidelines Q2 (R1). The RP-HPLC method used C_{18} shim pack column as stationary phase and 75:25 v/v of 10 mM potassium dihydrogen phosphate buffer and methanol-acetonitrile mixture in 8:2 ratio. The solutions were injected with the flow rate of 1 ml/min and injection volume of 20 µl.

The Linearity was observed in the concentration range of 20-50 µg/ml with the correlation coefficient value of 0.999. The % RSD values for inter-day, intra- day precision and repeatability of sample application for the standard was found to be within 2%. The percentage recoveries of the standard tannic acid were found to be between 95 to 97%. The LOD and LOQ values were found to be 1 µg/ml and 5 µg/ml. A low RSD value for precision studies shows that the developed method is precise. Good recovery values show that the method is free from interferences. Results of the system suitability and specificity studies confirms that the developed method could generate accurate and precise results.

The developed HPLC method was successfully applied to the *Carica papaya* leaf extracts and its formulation. From the recorded peak areas, the amount of tannic acid present in the crude methanolic extract of *Carica papaya* and the herbal tablet formulation was quantified. The amount of tannic acid presents the methanolic leaf extract and its formulation was found to be 15.2 µg/ml and 11 µg/ml.

High Performance Thin layer Chromatographic method for the estimation of gallic acid in the Carica papaya leaf extract and its formulation

The High Performance Thin Layer Chromatography method has been developed and validated as per ICH guidelines Q2 (R1) guidelines. The HPTLC method used Pre-coated silica gel $60F_{254}$ on aluminum sheets as stationary phase and Toluene: ethyl acetate: methanol [5:2:3, v/v/v] as the mobile phase. The peak of the standard was achieved with an R_f value of 0.29± 0.03.

Linearity was observed in the concentration ranging between 400 to 1200 ng/band with the correlation coefficient value of 0.999. The % RSD values for inter-day, intra- day precision and repeatability of sample application for the standard was found to be within 2%. The percentage recoveries of the standard gallic acid were found to be between 94% to 97%. The LOD and LOQ values were found to be 100 and 300 ng/band.

CONCLUSION

The validation results demonstrate that the method is accurate, repeatable, precise, and specific. A result of the system suitability studies confirms that the developed method could generate accurate and precise results. The developed HPTLC method was successfully applied to the *Carica papaya* leaf extracts and its formulation. From the recorded peak areas, the amount of gallic acid present in the crude methanolic extract of *Carica papaya* and the herbal tablet formulation was quantified. The amount of gallic acid present the methanolic leaf extract and its formulation was found to be 0.540 µg/ml and 0.378 µg/ml.

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REFERENCES

[1]. Zunjar, V., Mammen, D., Pharmacognostic, physicochemical and phytochemical studies on *Carica papaya* Linn. leaves. *Pharmacognosy Journal*, **2011**.3 (20):5-8.

[2]. Fu, Z., Chen, R., Study of Complexes of Tannic Acid with Fe (III) and Fe (II). *JAnal Methods Chem*, **2019**.

[3]. Naira, N., Asdaq, S.M.B., Heba, S., Gallic Acid: A Promising Lead Molecule for Drug Development. *J Appl Pharm*, **2016**.8 (2):1-2.

[4]. Azmi, S.M.N., Parveen, J., Azura, A., Purification of Xanthine oxidase inhibitor from *carica papaya* leaves using reversed phase flash column chromatography (RPFCC)-high performance thin layer chromatography (HPTLC*). Aust J Basic & Appl Sci*, **2012**.6 (1):117-122

[5]. Sutar, R.C., Gangwar, S.S., Thakur, R.N., et al. HPTLC finger printing analysis of the tannins from *Holoptelea integrifolia* (Roxb.) Planch Leaves. *J Pharmacogn Phytochem*, **2016**.5 (6):199-204.

[6]. Michael, R.A., Paolo, R.B., Raymond, O.C., Safety and Preliminary Effect of the Anti-CD20 Monoclonal Antibody 1B8 in B Cell Non-Hodgkin's Lymphoma: Results of An Expanded Access Program. *IJPPR*, **2016**.6 (1):34-40.

[7] Prawez, A., Hasan, S.Y., HPTLC method for simultaneous determination of ascorbic acid and gallic acid biomarker from freeze dry pomegranate juice and herbal formulation. *Saudi Pharma J*, **2019**. 7 (6): 975-980

[8]. Mradu, Gupta., Sasmal, S., Majumdar, S., et al. HPLC profiles of standard phenolic compounds present in medicinal plants. *IJPPR*, **2012**. 4 (3):162-167.

[9]. Faten,M., Hanen, F., Riadh, K., et al. Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant Limonium delicatulum. *J Taibah Univ Sci*,**2014**.8:216-224.

[10]. Surya, P.G., Gopal, G., Quantitative estimation of gallic acid and tan. *Der Pharm Lett*,2014.6 (2):31-36

[11]. Kamal, K., Nilesh, G., Bhavna, S., et al. RP-HPLC Method Development and Validation of Gallic acid in Polyherbal Tablet Formulation. *J Appl Pharm*, 2013.3 (5):37-42.

[12]. Sangeetha, R.K., Thengungal, K.R., Gandhimathi, M., Development of UV spectroscopy and HPTLC method for the analysis of dofetilide and its degradation products. *Eur J Pharm Med Res*, **2017**.4 (3):520-523.