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Original Research Article

Phytochemical Analysis of Quercetin, Catechol and Tannic Acid in Ethanol Extract of *Barleria Prionitis Linn* Leaf by RP-HPLC Technique

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ABSTRACT

Barleria prionitis Linn is an herbal plant from Acanthaceae family used in treatment of wide variety of diseases and disorders. Different phyto constituents like quercetin, catechol and tannic acid are expected to be present in the plant leaf. However, to date, there is no analytical method development or validation pertinent to for simultaneous quantification of quercetin, catechol and tannic acid in *Barleria prionitis Linn* leaf extract**.** The objective of this work was to develop asensitive RP-HPLC method for simultaneous estimation of quercetin, catechol and tannic acid in 70% ethanol extract of *Barleria prionitis Linn* leaf. The analytes were separated by a 5 µm C_{18} column (4.6×150mm), methanol: 0.1% orthophosphoric acid (pH: 2.4) (60:40 v/v) mobile phase and analyzed at 40±0.5°C. The method had a flow rate of 0.5 ml min^{-1} and the three phytochemicals were detected at 275 nm using a PDA detector. The method was validated by ICH Q2 R1 guidelines. A linear relationship of peak area was established with the concentration range of 0.1-100 µg/ml. The method was found to be specific, accurate and precise (%RSD <2%). The method showed negligible changes in the responses when the chromatographic conditions were altered and thus proved to be robust. Thus, the novel RP-HPLC method was proven to be quite effective, reliable and can be applied for simultaneous estimation of quercetin, catechol and tannic acid in *Barleria prionitis Linn* leaf extract.

***Keywords*:** *Barleria prionitis Linn*, Quercetin, Catechol, Tannic acid, RP-HPLC, Method validation.

Introduction

Barleria prionitis Linn is an herbal plant that belongs to the Acanthaceae family mainly found in the tropical regions of South Africa, Sri Lanka and India. [1] The plant is popular by several names like Common yellow nail dye (English), Vajradanti (Hindi), Kantajati (Bengali), Piyabaasa (Unani), Karunta (Sanskrit) and many more. The plant is important to the medicinal field due to its contribution to treat a wide variety of diseases and disorders. It is a branched shrub thatgrows up to 1.8 to 2m in height. The stem is 1.8 mm thick and erect with greyish to light dark in color with 3 to 4 divaricate spines that are present at the center of the leaf. The flowers have 4 cm long yellow-orange blooms with dilating stalks. The leaf of the herbal plant is oval and about 10 cm long and 4 cm wide. It is highly reticulate and has costa. The seeds have a plush hair-like appearance which is enveloped in a case and is 8mm long and 5mm wide. [2]

Whole parts of the plant are considered to have medicinal value according to the traditional culture of medicine.[3] Several parts of the plant have the presence of different phytochemicals which possess different medicinal benefits.

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The leaf of the plant contains alkaloids, flavonoids, saponins, tannins, phytosterols, terpenoids, sterol(stigmasterol) and polyphenolic compounds whereas the aerial parts and the flower contain glycosides, terpenoid (lupeol), flavonoid and neohesperide.[4,5]. Different phytoconstituents like quercetin, catechol and tannic acid are expected to be present in the plant leaf and thus this study was intended to estimate these constituents. Quercetin (Que) is a flavonoid isolated from the leaves of *Barleria prionitis Linn* with proven pharmacological action. Quercetin is known for its anticancer activities and also can be used in several other ailments related to the heart and sometimes blood vessels. [6] Quercetin is a powerful antioxidant present in fruit juices and vegetables. It is the aglycone of several glycosides and possesses several other activities like anti-inflammatory and anti-diabetic.[7]

Catechol (Cal) is used to treat different CNS and ANS disorders. It is a natural polyphenol. Catechol chemically is a dihydroxy benzene compound having a molecular weight of 110.11. It appears to be white to brown with crystalline in nature.[8] The other compound that can be identified in the leaf extract is tannic acid (Tn) which is a polyphenolic compound with anticancer potential. The tannin is yellowish and can be used against ovarian, lung, liver and breast cancer. It is an anti-tumor agent and has a wide application against cancer cells. [9] The herbal plants are effective against various diseases and disorders but their application is limited due to the lack of quality in the product.[10] Several analytical methods are applied to identify or quantify phytoconstituents present in herbal products like HPLC (High-Performance Liquid Chromatography), HPTLC (High-Performance Thin Layer Chromatography), GC (Gas Chromatography) and electrophoresis. Other techniques like NMR and IR can also be used for compound fingerprint and functional group region detection.[11] The herbal drugs' quality must be controlled and thus new analytical methods must be developed and validated to increase the reliability of

the herbal drugs to the consumers.[12] This present study aimed todeveloping a suitable method to separate and quantify the three different phytoconstituent that may be present in the extract of *Barleria prionitis* Linn. The plant has antioxidant potential and is therapeutically used against cancer cells and CNS disorders, but there is a lack of specification for the quality of the raw materials of the plant which is required for the production as well as the registration of the phytomedicinal products. Thus, this study is performed to develop a method to simultaneously estimate quercetin (1), catechol (2) and tannic acid (3) in the plant extract as no method is present which can estimate the three phytochemicals simultaneously. The method is also validated according to ICH guidelines which will prove reliability in the method. This method will prove scientific evidence for the quality control of the plant extract which is obtained from *Barleria prionitis Linn* leaves. The method was also validated with the help of different parameters like specificity, linearity, sensitivity, accuracy, precision, robustness and system suitability. The stability of the analytes in solution were determined up to 72 hours.

Materials and Methods

Plant material

The leaves of the *Barleria prionitis Linn* plant were collected from the Herbal Garden of JSS College of Pharmacy, Mysuru, India. The collection was done during June 30th and July 1st and plant leaves were identified and authenticated by Dr. J. Suresh, Professor and Head, Department of Pharmacognosy, JSS College of Pharmacy, Mysuru (JSSCP/PCOG/55).[13]

Preparation of the extract

The leaves of the plant (1 kg) were washed with distilled water and contaminants were removed. The leaves were dried in shade at room temperature(25^{0}C). 250 gm of powder was taken and packed in a Soxhlet column. The extract was concentrated under reduced pressure (bath temperature 50^{0}C) to get a concentrated crude extract (24 gm) which was stored in an airtight container in a refrigerator below 10^{0}C until analysis.[14]

Chemicals and reagents

Milli-Q water was obtained from Millipore Direct-Q® 3 UV water purification system (filter >0.22 µm). HPLC grade methanol and acetonitrile were obtained from Merck, India and orthophosphoric acid was procured from Spectrochem Pvt Ltd, Mumbai, India which were used in the separation analysis. The pure samples used in this study like quercetin and tannic acid were purchased from SD Fine Chem Ltd., India and catechol from Sigma Aldrich – Merck, India.

Instrumentation and chromatographic conditions

Shimadzu 2010 A HPLC system (Shimadzu, Japan) was used for the analysis of the analytes. The HPLC system is equipped with a quaternary pump and also and an autosampler. A PDA detector (SPD-M20A) was used for analyte detection. LC solution software was used for the analysis and data acquisition of the analytes. The signal outputs of the analytes were monitored and integrated using Lab solutions software. Weighing balance (ME204/AD4, Mettler Toledo, Switzerland) was used for sample weighing and the sonication of the samples was done using GT SONIC ultrasonic device (Antech, India). Lab India pH meter was used in this study to measure the pH of the mobile phase.

The analytes were separated using a Shimadzu Shim-pack Solar C_{18} column (4.6×150mm, 5 µm). The total run time of the analysis was 12 minutes which was run at a rate of 0.5ml min^{-1}. The mobile phase, methanol:0.1% orthophosphoric acid (pH: 2.4) in the ratio 60:40 (v/v) respectively was used in this study. The analytes were injected at a volume of 10µl by keeping the column oven temperature at 40±0.5°C. PDA detector was used for the detection of the analytes at 275nm. These conditions were applied to simultaneously estimate quercetin, catechol and tannic acid and also to quantify the three phytoconstituents that may be present in the leaf extract of *Barleria prionitis Linn.*

Preparation of standard solutions

10 mg of the three standards (Que, Cal and Tn) was weighed accurately and transferred into three 10 ml of volumetric flasks respectively, dissolved and put together up to mark with methanol to obtain stock solution of 1000 µg ml^{-1} concentration. The standard solutions were further diluted up to six concentrations individually and in combination with the stock solution with methanol to get a concentration range of 0.1-100 µg ml^{-1}. The standard solutions of the six concentration levels were sonicated for 15 mins for degassing.[15] The solutions were filtered usingsyringe filters (0.22µm) and kept in HPLC vials.

Preparation of sample solution

The sample solution was prepared by taking 1 mg of the crude extract in a volumetric flask and dissolved with 1 ml of methanol. The sample solution of 1000 µg ml^{-1} concentration was obtained was sonicated for about 15 mins for degassing and was filtered using syringe filters (0.22µm) to remove particulate matter. The filtered solution was kept in HPLC vials for analysis.

Method validation

The analytical method was developed and then validated according to ICH Q2 R1 guidelines. [16] The method was employed for the simultaneous quantification of quercetin, catechol and tannic acid in the ethanol extract of the *Barleria prionitis Linn* leaf. The validation of the method was performed increase the reliability, to check the ability of the method to determine the three phytoconstituents simultaneously. The developed method was validated for different parameters like specificity, linearity and range, system suitability, accuracy, precision, sensitivity (LOD, LOQ) and robustness.

Specificity: Specificity of the analytical method is the ability to separate and identify the analyte in the presence of different components which may be present in the sample. Several components like degradants or impurities may be present in the analytes which can give an interference peak in the result, but if the method is specific, it is able to identify the three phytoconstituents separately without any interference peak.[17] Specificity was determined by filling an HPLC vial with methanol and then injecting it as a blank. The standard and sample solution were also injected following the blank injection to analyze the presence of any interference peak. The resolution of the three standard peaks (Que, Cal and Tn) that were expected to be found in the leaf extract was then integrated and determined by Lab solutions software.

Linearity and range: The linearity of the standards was established for six different concentrations. The linearity relationship was determined between the peak area and the concentrations of the three standards. The concentration of Que, Cal and Tn was in between the range of 0.1-100 µg ml^{-1} where six different points were considered 0.1, 0.5, 1, 10, 50 and 100 µg ml^{-1}. Linear regression analysis was carried out between peak areas of all three standards with their concentration ranges.

System suitability: The reproducibility of the analytical responses in terms of retention time (RT), peak area response, theoretical plates (N), tailing factor (T), and resolution (R_s) was determined where the standards (Que, Cal and Tn) were injected six times (n=6). The relative standard deviation (%RSD) of the responses to the standards was estimated after the analysis.[18]

Accuracy: Accuracy is defined as the characteristic of the method to give expected results. It is the ability to give results that are close to the true values. The accuracy was determined by triplicate injections (n=3) of three different levels of the three standards that are lower (1 µg ml$^{-1)}$, intermediate (10 µg ml^{-1}) and highest (100 µg ml^{-1}) concentrations. The accuracy results were assessed as the % mean recovery of the triplicate injections of the three different concentration levels. The % recovery of the standard samples was calculated by: the amount recovered/amount injected×100. The relative standard deviation (%RSD) of the results was also determined from the

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response. Additional support to the accuracy results was provided by employing the standard addition method where different concentrations of the standard Que, Cal and Tn (1,5,10 µg ml^{-1}) were added to a pre-analyzed known sample. The % recovery of the drug was then calculated with the formulae;

% recovery = $[(C_f-C_s)/C_i] \times 100]$

where C_f is the final concentration of the three phytoconstituents after the standard addition to the samples; C_s is the pre-analyzed sample concentration of the three phytoconstituents; and C_i is the initial concentration of the standards before addition to the sample.[19]

Precision: Precision refers to an analytical method's capacity to produce repeatable results. It is the measurement of how consistent the method is to give similar results over some time. Precision was estimated with parameters like repeatability (intraday precision) and intermediate precision (inter-day precision). The intraday precision was carried out by injection of intermediate concentration (10 µg ml^{-1}) of the standards six times (n=6) within the same day whereas the inter-day precision was determined by six injections of the same concentration of the standards on different days.[20] The deviation of the responses of the three standards Que, Cal and Tn was calculated concerning relative standard deviation (%RSD).

Sensitivity: Sensitivity is referred to the detection potential of the method and also to determine the concentration of the analyte. The limit of detection (LOD) is the least concentration level of the analyte that the method can able to detect whereas the limit of quantification (LOQ) is the minimum level of analyte that the method can able to quantify. Sensitivity was determined from the calibration curve of Que, Cal and Tn where standard deviation and the slope are taken into consideration. LOD and LOQ were estimated by employing these equations: [21]

LOD = 3.3×SD/S
LOQ = 10×SD/S

Where SD is the standard deviation and S is the slope of the calibration curve.

Robustness: The analytical method's robustness was tested by varying the developed condition's mobile phase ratio and flow rate.[22] The ability of a developed method to deliver unaffected responses despite changes in chromatographic conditions is referred to as robustness. The mobile phase ratio [methanol (MeOH: 0.1% orthophosphoric acid (OPA), 62:38 and 58:42] and also the flow rate (± 0.1 ml min^{-1}) were changed to estimate any change in the response with respect to peak area repeatability (n=6), theoretical plate (N), retention time (RT), tailing factor (T) and resolution (R_s).

Stability study
The stability of the solution was estimated for the three standard solutions and also the sample solution at acidic conditions (0.1N HCl), alkaline conditions (0.1N NaOH), Oxidative conditions (15% H_2O_2) and by storing the solutions in the autosampler vials at an ambient temperature (25°C) and in the refrigerator at a temperature of 4-8°C. The different concentration levels (1, 10, 50 µg ml^{-1}) of standard Que, Cal and Tn solutions were considered in this stability study along with the pre-analyzed sample solution of the ethanol leaf extract of *Barleria prionitis Linn*. The study was done for 72 hours and the % drug degradation was determined by comparing with the initial concentration of the analytes with the concentration obtained after 24, 48, and 72 hours.[20]

Results and Discussion

The HPLC method developed to simultaneously estimate the three phytoconstituents, Que, Cal and Tn present in the 70% ethanol extract of *Barleria prionitis Linn* leaf. Initially, we employed solvent system 0.1% orthophosphoric acid: acetonitrile (90:10) for analysis. Milli-Q water was used as a diluent. The results obtained describe a solubility issue in the case of Tn as no peak of standard Tn was found. The diluent was changed to methanol and the methanol: 0.1% orthophosphoric acid solvent system was used instead. The optimization of the chromatographic conditions was done by changing the mobile phase ratio and flow rate to get an optimized chromatographic condition which could simultaneously estimate the three standards with better resolutions and better peak shape.
The detecting wavelength was choosen from standard results by setting 200-400nm wavelength range in PDA detector. Que had shown maximum absorbance at a range of 270-330nm whereas Cal was showing its maximum absorbance at 272-300nm range. Tn on the other hand was giving the best peak response in the range of 250-300nm and so a specific wavelength of 275nm was selected for detection, giving the best result for all the three standards and also provided stability in the determination.[23]
The optimized chromatographic condition was developed where three distinct standard peaks of (1) quercetin, (2) catechol and (3) tannic acid with a retention time of 3.311 min, 4.702 mins and 8.553 mins respectively in the total runtime of 12 mins as shown in Figure 2.
The developed method was validated according to ICH Q2 R1 guidelines to increase the reliability of the method.
The devised analytical approach was found to be quite specific in analyzing the standards (Que, Cal and TN). Specificity was determined by analyzing the blank along with the standard and sample solution. The standard chromatogram obtained for the three standards showed three distinct peaks where quercetin was eluted at 3.311 min, catechol at 4.702 min and tannic acid at 8.553 min as shown in Figure 2. There was no interference peak observed at the place of the analyte peaks and the peaks showed a good resolution (R_s) which was more than 4. The linear response of the standards mainly depends upon the concentrations of the three standards. The responses obtained for the three standards demonstrated that there is a linear relationship between the peak area response and the concentration as with the increase in concentration there was a linear increase in the responses. Linearity was determined by estimating the correlation coefficient (r) from the calibration curves. The linear relation was obtained for all three standards as shown in Figure 4 where the r^2 values for Que, Cal and Tn were 0.9975, 0.9996 and 1 respectively (table 1.).

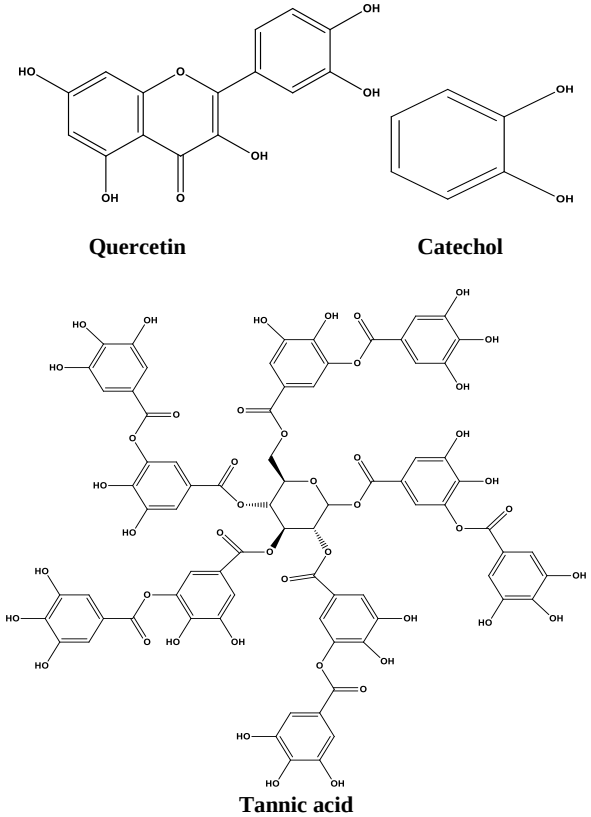

Figure 1: Quercetin (Que), Catechol (Cal) and Tannic acid (Tn)

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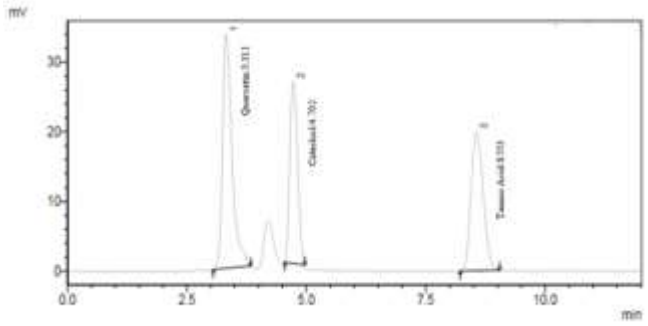

Figure 2: Standard chromatogram of 1. Quercetin (3.311 min), 2. Catechol (4.702 min) and 3. Tannic acid (8.553 min)

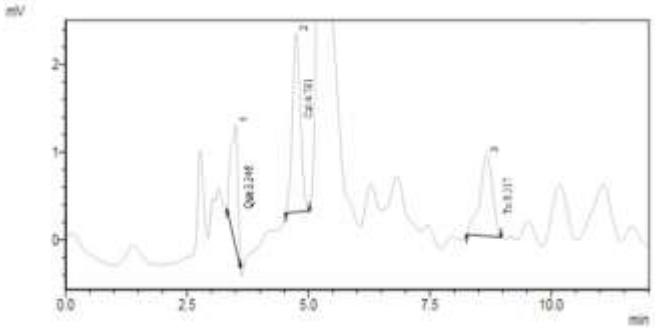

Figure 3: Sample chromatogram of 1. Quercetin (3.346 min), 2. Catechol (4.791 min) and 3. Tannic acid (8.517 min)

The correlation coefficient values for all three standards were found to be more than 0.99. The total analysis of this study was within 10 min as tannic acid was having the highest retention time of 8.553 min. A system suitability test was applied in this method to confirm the responses obtained were well within the limit of ICH guidelines. The column efficiency was measured in terms of theoretical plates (N) for quercetin, catechol and tannic acid which was found to be more than 2000 for all three standards. The tailing factor (T) was also within the limit of less than 2.0 and also the resolution was more than 2.0 for the three standards. The % RSD (Relative standard deviation) obtained from the peak area responses were 3.127, 2.289 and 2.241 for Que, Cal and Tn respectively. All these results assure the adequacy of the proposed HPLC method for routine analysis of Que, Cal and Tn. (Table 2.) The accuracy was determined by % recovery of the standard solution and also standard addition samples solutions of Que, Cal and Tn. All the accuracy data obtained after the analysis are presented in tables 3 and 4. The method was proved to be quite accurate as the % recovery results of all three phytoconstituents (Que, Cal, Tn) were found to be well within the limit (98-102%). The method was giving expected and repeatable results as the %RSD for all the responses was less than 2%. The method was precise as it was giving repeatable results for Que, Cal and Tan standards. The precision was evaluated using % RSD and the method showed %RSD less than 2% which proves it to be precise and was giving un-deviated results. The results obtained in repeatability (intraday precision) and intermediate precision (inter-day precision) are shown in table 5. and it demonstrates the method is precise. The sensitivity was determined from the calibration curve and with the help of regression analysis. The LOD values for Que, Cal and Tn were found to be 0.086 µg/ mL, 0.092 µg/ mLand 0.055 µg/ mL respectively whereas the LOQ values were 2.964 µg/ mL, 3.594 µg/ mL and 1.681 µg/ mL respectively as shown in the table1.

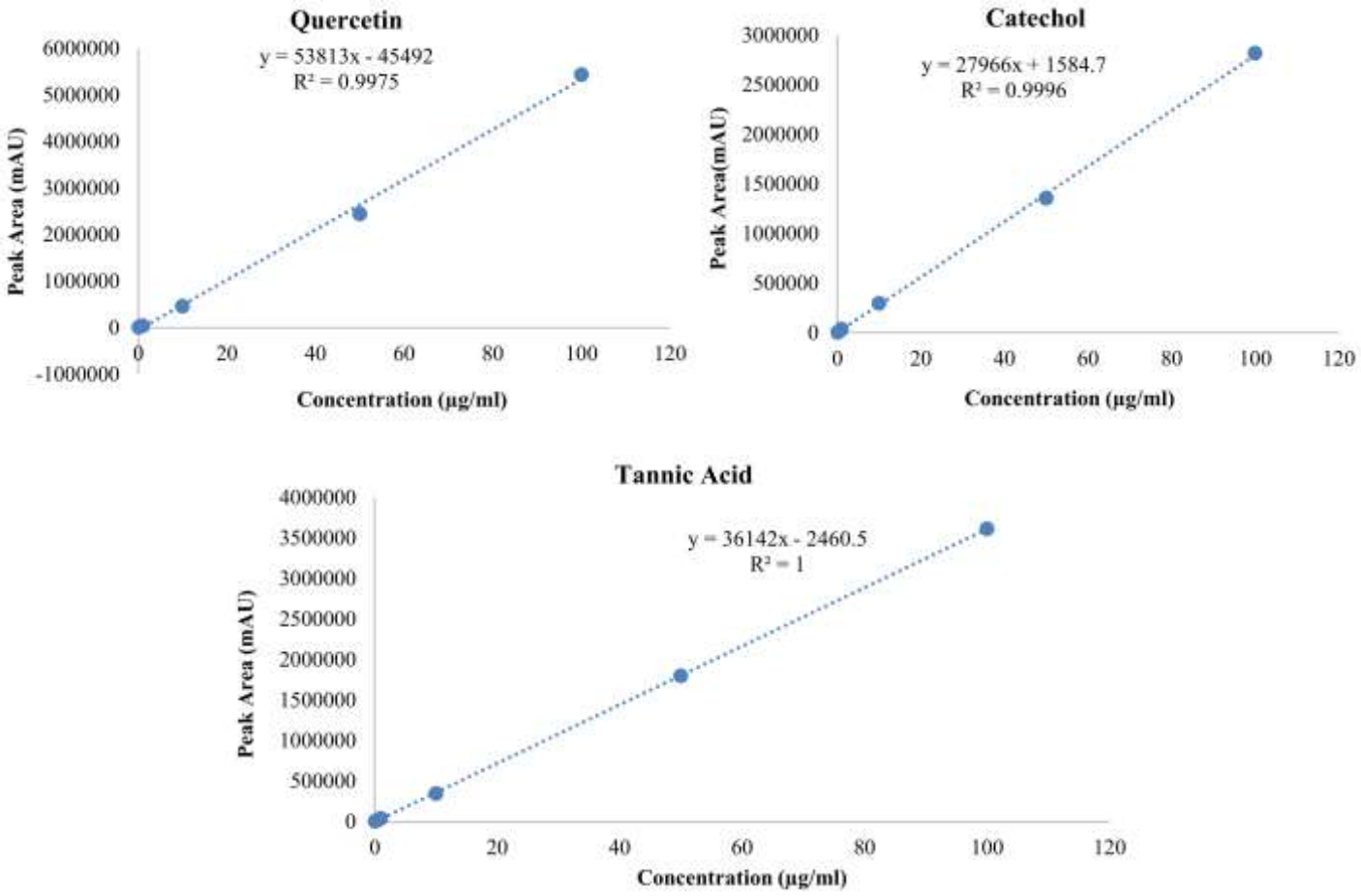

Figure 4: Calibration curve of Quercetin, Catechol and Tannic acid

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Table 1: Calibration curve parameters, LOD and LOQ for Quercetin, Catechol and Tannic acid

Compound	Calibration curve equation	Correlation coefficient (r^2)	Linearity range (µg ml^{-1})	LOD (µg ml^{-1})	LOQ (µg ml^{-1})
Quercetin	y = 53813x - 45492	0.9975	0.1-100	0.086	2.964
Catechol	y = 27966x + 1584.7	0.9996	0.1-100	0.092	3.594
Tannic acid	y = 36142x - 2460.5	1.00	0.1-100	0.055	1.681

Table 2: System suitability results of Quercetin, Catechol and Tannic acid

Compound (10 µg ml^{-1})	Mean Retention time (RT) ± %RSD (min)	Mean Peak Area ± %RSD	Mean Theoretical Plate (N) ± %RSD	Mean Tailing Factor(T) ± %RSD	Mean Resolution (R_s) ± %RSD
Quercetin	3.329 ± 0.143	479221 ± 3.127	2316 ± 2.764	1.782 ± 0.789	0
Catechol	4.752 ± 0.059	294609 ± 2.289	3885 ± 4.633	1.224 ± 0.952	4.250 ± 1.019
Tannic Acid	8.519 ± 0.196	346242 ± 2.241	5098 ± 3.260	1.210 ± 1.118	9.645 ± 1.925

N = 3

Table 3: Accuracy results of standard Quercetin, Catechol and Tannic acid

Compound	Amount of standard added (µg ml^{-1})	Amount recovered (µg ml^{-1})	Recovery (%)	Mean (%)	RSD (%)
		1.013	101.3		
	1	1.013	101.3	101.3	0.028
		1.014	101.4		
		10.009	100.0		
Quercetin	10	10.005	100.0	100.0	0.028
		10.012	100.1		
		101.513	101.5		
	100	101.504	101.5	101.5	0.0006
		101.561	101.5		
		1.021	102.1		
	1	1.019	101.9	101.9	0.136
		1.017	101.7		
		10.139	101.3		
Catechol	10	9.996	99.9	100.7	0.582
		10.082	100.82		
		101.616	101.6		
	100	101.742	101.7	101.9	0.198
		102.626	102.6		
		1.0140	101.4		
	1	1.0136	101.3	101.7	0.486
		1.0243	102.4		
		10.009	100.0		
Tannic acid	10	10.059	100.5	99.8	0.716
		9.889	98.8		
		100.274	100.2		
	100	100.642	100.6	100.3	0.035
		100.218	100.2		

N = 3

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Table 4: Accuracy results of standard addition of Quercetin, Catechol and Tannic acid to sample

Compound	Amount of standard added (µg ml^{-1})	Amount recovered (µg ml^{-1})	Recovery (%)	Mean (%)	RSD (%)
		1.965	98.2		
	1	1.944	97.2	98.2	0.403
		1.983	99.1		
Quercetin (Conc. Measured		6.162	102.7		
in sample = 1 µg ml^{-1})	5	6.078	101.3	101.7	0.683
		6.070	101.1		
		10.808	98.2		
	10	10.786	98.0	97.9	0.307
		10.729	97.5		
		1.812	100.6		
	1	1.779	98.8	99.7	0.7366
		1.794	99.6		
		5.890	101.5		
Catechol (Conc. Measured	5	5.677	97.8	100.5	1.934
in sample = 0.8 µg ml^{-1})		5.935	102.3		
		10.896	100.8		
	10	11.073	102.5	101.6	0.6713
		10.951	101.4		
		1.539	102.6		
	1	1.525	101.7	101.0	1.5634
		1.483	98.9		
		5.688	103.4		
Tannic acid (Conc.	5	5.504	100.0	101.0	1.6730
Measured in sample = 0.5		5.479	99.6		
µg ml^{-1})		10.392	98.9		
	10	10.496	99.9	99.1	0.6532
		10.332	98.4		

N = 3

Table 5: Repeatability and the intermediate precision results of Quercetin, Catechol and Tannic acid

Compound (10 µg ml^{-1})	Repeatability Mean (µg ml^{-1})	Mean drug in %	RSD (%)
Quercetin	9.660	96.610	1.3054
Catechol	10.358	103.584	1.6378
Tannic acid	9.9155	99.1592	1.604034

Compound (10 µg ml^{-1})	Intermediate precision Mean (µg ml^{-1})	Mean drug in %	RSD (%)
Quercetin	9.736	97.370	0.9073
Catechol	10.611	106.122	1.4439
Tannic acid	9.954	99.543	0.86870

N = 3

The findings proved that the approach can detect and quantify Que, Cat and Tn effectively and with sufficient sensitivity. The robustness of the analytical method ensured that little change in the chromatographic conditions will not affect the responses. The robustness was evaluated by considering retention time, peak area response, theoretical plate, tailing factor and resolution of the three standard peaks (Que, Cal, Tn). The results obtained are shown in table 6. and which state the method is insensitive and gives expected results to slight changes like mobile phase ratio and flow rate in the developed chromatographic conditions. The stability of the analytes in a solution that is the standard and the sample (Que, Cal, Tn) were investigated for acidic conditions (0.1N HCl), alkaline conditions (0.1N NaOH), Oxidative conditions (15% H_2O_2) and temperature (ambient temperature 25°C and refrigeration(4-8°C). Quercetin was unstable at basic conditions with 26.2% degradation, Catechol was unstable at basic conditions with 26.2% degradation. Tannic acid was unstable at basic and oxidative conditions with 39.6% and 35.1% degradation (Table 7a) The stability study was evaluated up to 72 hours and the % of drug degradation was considered in this study. The stability study demonstrated that the analyte solutions were quite stable up to the period of study of 72 hours. The standard solutions were found to have a % drug degradation of less than 10% whereas the sample solutions were found to have less than 9% degradation in the case of the solutions stored at ambient temperature (Table 7b). The analytes stored in the refrigerator showed more stability as the % drug degradation was found to be less than 4% and 3% for standard and

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sample solutions respectively (Table 7c). These results proved that the analytes in the solution were stable for up to 72 hours. The three phytoconstituents (Que, Cal, Tn) were quantified simultaneously by this method which was expected to be present in the 70% ethanol extract of *Barleria prionitis Linn* leaf as an application of the method. The sample chromatogram was obtained after the quantitative method was applied to the sample solution which is shown in Figure 3.

The results obtained state that the sample solution of concentration 1000 µg ml^{-1} had 1 µg $ml^{-1}$of quercetin, 0.8µgml^{-1} of catechol and 0.5 µg ml^{-1} of tannic acid which is 0.1%, 0.08% and 0.05% respectively. The developed method was found to be sensitive and effective in the quantification of the phytoconstituents in the leaf extract of *Barleria prionitis Linn.*

Table 6: Robustness results of Quercetin, Catechol and Tannic acid

Compound (10 µg ml^{-1})	Mean Retention time (RT) ±%RSD (min)	MeOH: OPA Mean Peak Area±%RSD	(62:38)Mean Theoretical Plate (N) ± %RSD	Mean Tailing Factor(T) ±%RSD	Mean Resolution (R_s) ± %RSD
Quercetin	3.277 ± 0.210	478239 ± 2.925	2419 ± 2.189	1.710 ± 0.598	0
Catechol	4.629 ± 0.129	306921 ± 3.159	3687 ± 3.996	1.245 ± 1.121	4.026 ± 1.490
Tannic Acid	7.966 ± 0.510	357014 ± 2.966	4888 ± 2.962	1.248 ± 1.035	8.775 ± 1.925

Compound (10 µg ml^{-1})	Mean Retention time (RT) ±%RSD (min)	MeOH: OPA Mean Peak Area±%RSD	(58:42) Mean Theoretical Plate (N) ±%RSD	Mean Tailing Factor(T) ±%RSD	Mean Resolution (R_s) ± %RSD
Quercetin	3.375 ± 0.288	471780 ± 1.896	2905 ± 1.228	1.584 ± 0.964	0
Catechol	4.853 ± 0.283	298332 ± 2.918	3732 ± 2.007	1.237 ± 0.536	4.715 ± 2.019
Tannic Acid	9.657 ± 0.623	350176 ± 2.966	5679 ± 1.339	1.172 ± 1.899	11.572 ± 2.399

Compound (10 µg ml^{-1})	Mean Retention time (RT) ± %RSD (min)	Flow Rate: Mean Peak Area ± %RSD	0.6 ml min^{-1} Mean Theoretical Plate (N) ± %RSD	Mean Tailing Factor(T)± %RSD	Mean Resolution (R_s) ± %RSD
Quercetin	2.766 ± 0.318	480297 ± 1.896	2312 ± 2.031	1.754 ± 0.110	0
Catechol	3.939 ± 0.094	273739 ± 2.951	3626 ± 3.258	1.228 ± 0.072	4.221 ± 1.852
Tannic Acid	7.120 ± 0.104	345284 ± 3.009	4964 ± 3.391	1.181 ± 0.830	9.557 ± 0.579

Compound (10 µg ml^{-1})	Mean Retention time (RT) ±%RSD (min)	Flow Rate: Mean Peak Area ± %RSD	0.4 ml min^{-1} Mean Theoretical Plate (N) ±%RSD	Mean Tailing Factor(T) ± %RSD	Mean Resolution (R_s) ± %RSD
Quercetin	4.373 ± 0.265	522695 ± 3.074	2167 ± 1.099	1.792 ± 0.323	0
Catechol	5.900 ± 0.289	273739 ± 2.951	4186 ± 1.904	1.181 ± 0.20	4.595 ± 0.707
Tannic Acid	10.694 ± 0.743	388899 ± 2.879	5722 ± 2.973	1.199 ± 0.463	10.306 ± 0.951

N = 3

Table 7a: Stability study results of Quercetin, Catechol and Tannic acid

S/N	Conditions	Standard area	Results		
			% Mean Difference	Tailing factor	Theoretical plates
	Quercetin (40mcg)	2107028			
01	0.1N_HCl	464419	19.87799	1.810	2077
02	0.1N_NaOH	635520	26.23237	1.224	4106
03	15%H_2O_2	2050716	2.616096	0.898	3267
	Catechol(40mcg)	1120225			
01	0.1N_HCl	338114	29.654	1.329	5725
02	0.1N_NaOH	151568	13.06521	2.864	3268
03	15%H_2O_2	198204	17.97825	1.095	7338
	Tannic Acid (40mcg)	1443220			
01	0.1N_HCl	163161	13.81707	1.206	8665
02	0.1N_NaOH	489483	39.6822	1.180	9645
03	15%H_2O_2	313771	35.12737	1.207	9224
				NMT 2.0	NLT 2000

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Table 7b: Stability study results of Quercetin, Catechol and Tannic acid at ambient temperature (25°C)

		0 hour		
Concentration (µg ml^{-1})		**% Drug degradation of Quercetin**	**% Drug degradation of Catechol**	**% Drug degradation of Tannic acid**
	1	3.002	2.866	2.792
Standard	10	2.496	2.372	0.756
	50	2.732	3.113	0.007
Sample		0.383	1.067	1.467
		24 hrs		
Concentration (µg ml^{-1})		**% Drug degradation of Quercetin**	**% Drug degradation of Catechol**	**% Drug degradation of Tannic acid**
	1	5.008	2.866	3.794
Standard	10	3.160	2.703	2.128
	50	4.972	5.216	0.673
Sample		0.557	2.064	2.229
		48 hour		
Concentration (µg ml^{-1})		**% Drug degradation of Quercetin**	**% Drug degradation of Catechol**	**% Drug degradation of Tannic acid**
	1	6.004	7.494	6.674
Standard	10	4.886	3.059	4.543
	50	5.408	7.409	1.425
Sample		0.673	3.791	4.349
		72 hour		
Concentration (µg ml^{-1})		**% Drug degradation of Quercetin**	**% Drug degradation of Catechol**	**% Drug degradation of Tannic acid**
	1	6.81	7.238	9.775
Standard	10	6.137	3.924	6.285
	50	7.618	8.405	3.026
Sample		2.81	4.753	8.193

N = 3

Table 7c: Stability study results of Quercetin, Catechol and Tannic acid at refrigerator temperature (4-8°C)

		0 hour		
Concentration (µg ml^{-1})		**% Drug degradation of Quercetin**	**% Drug degradation of Catechol**	**% Drug degradation of Tannic acid**
	1	1.282	0.954	1.028
Standard	10	2.283	1.368	2.149
	50	1.596	0.964	0.529
Sample		0.426	0.637	0.253
		24 hour		
Concentration (µg ml^{-1})		**% Drug degradation of Quercetin**	**% Drug degradation of Catechol**	**% Drug degradation of Tannic acid**
	1	1.426	1.007	1.281
Standard	10	2.374	1.509	2.793
	50	1.708	1.826	2.029
Sample		0.593	0.937	0.826

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Concentration (µg ml^{-1})		48 hour		
		% Drug degradation of Quercetin	% Drug degradation of Catechol	% Drug degradation of Tannic acid
Standard	1	2.096	1.967	2.429
	10	2.829	2.029	2.819
	50	2.173	1.967	2.793
Sample		0.429	1.170	0.937

Concentration (µg ml^{-1})		72 hour		
		% Drug degradation of Quercetin	% Drug degradation of Catechol	% Drug degradation of Tannic acid
Standard	1	3.186	2.863	2.723
	10	2.908	3.529	3.964
	50	2.829	2.017	2.901
Sample		1.729	1.829	2.012

N = 3

Conclusion

The developed HPLC method was validated and found to be specific, accurate, precise, robust and effective for simultaneous estimation of quercetin (Que), catechol (Cal) and tannic acid (Tn) in 70% ethanol extract of *Barleria prionitis Linn* leaf.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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