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Simultaneous Estimation of Quercetin and Rutin in *Aganosma Cymosa by* High Performance Liquid Chromatography Method

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Abstract:

In order to measure quercetin and rutin in Aganosma cymosa at the same time, a straightforward, specific, accurate, and exact HPLC technique was created. A chromatographic separation was accomplished using a mobile phase consisting of acetonitrile:25mM ammonium acetate pH 3 (40:60 v/v), a C18 column with dimensions 150 x 4.6mm i.d. and a 5µ Hibar Lichrospher. At 259 nm, the absorbance was measured, and the flow rate was 1 ml/min. Rutin had a retention duration of 1.71 minutes and quercetin of 4.30 minutes.

The analytical parameters that were used to verify the proposed technique were established according to the recommendations set forth by the International Conference on Harmonisation (ICH). These parameters include accuracy, linearity, precision, robustness, and limit of detection (LOD) and limit of quantification (LOQ). There was a linear relationship between the detector response and the concentrations of quercetin (1–5 µg/ml) and rutin (0.1–0.5 µg/ml).

Both components of Aganosma cymosa were accurately and concurrently estimated using the suggested approach. This work developed a quantitative approach to extracting rutin and quercetin from Aganosma cymosa at the same time.

Keywords: *Aganosma cymosa*, Simultaneous estimation, Flavonoids, Quercetin, Rutin..

INTRODUCTION

The plant world is teeming with flavonoids, a class of polyphenolic chemicals. About 300 different types of flavonoids have been identified so far. A number of pharmacological actions, including as anti-allergic, anti-inflammatory, and anti-oxidant effects, are shown by flavonoids, a key active component [1-3]. Rutin and quercetin are antioxidants that lower the oxidation of low density lipoproteins [4].

Famous flavonoids with anti-inflammatory, anti-allergic, anti-thrombotic, hepatoprotective, anti-spasmodic, and anti-cancer effects include quercetin and rutin [5, 6].

The main components of marker-based standardisation are the identification of distinctive and important plant compounds as markers and the development of analytical methods for tracking these

compounds [7]. In addition to their antihepatotoxic, antifungal, antibacterial, and diuretic effects, many plants that contain flavonoids are antispasmodic or have diuretic qualities themselves [8].Due to its ease, sensitivity, precision, and adaptability for high throughput screening, high performance layer chromatography (HPLC) has lately been the analytical instrument of choice for identification and quantification of marker chemicals in herbal medications. For the purpose of estimating the chemical components found in plant materials, the HPLC technique is an appropriate approach [9–12]. In accordance with the recommendations made by the International Conference on Harmonisation (ICH) [13], the suggested approach has been fine-tuned and verified.

There was no mention of simultaneously estimating the flavonoid components of Aganosma cymosa in the reports that were found in the literature review. Current research relies on quantitative measurement of these substances, which necessitates a range of methodologies. This work revealed the quantification of key flavonoids that are typical of Aganosma cymosa. For the purpose of simultaneously estimating quercetin and rutin in the hydro alcoholic and methanolic extract of Aganosma cymosa, a sensitive, accurate, and specific HPLC technique was devised and validated.

Materials and Methods

Materials and reagents

Sigma-Aldrich of Bangalore, India, supplied 96% of the quercetin and 98% of the rutin. Qualigens Fine Chemicals of Mumbai, India, supplied the HPLC-grade methanol and acetonitrile. Analytical and HPLC grade chemicals and reagents were used throughout. Purified water was extracted using the Milli Q RO system.

Plant Material

In March of 2020, the whole Aganosma cymosa plant was gathered. Dr. Gunasekaran, a field botanist from India, verified and certified the plant's identification. We used a tray drier set at 500C for 48 hours to dry the chopped plants. For the investigation, the powdered materials were used after they were dried.

Preparation of standard solution

The quercetin and rutin standard stock solutions (1 mg/ml) were made by diluting the compounds in methanol. Lightproof containers were used to store these stock solutions. The stock solution was used to generate diluted standard solutions of quercetin and rutin, with concentrations ranging from 1–5 µg/ml and 0.1–0.5 µg/ml, respectively, for the purpose of creating a calibration curve for the two substances.

Preparation of sample solution

The powdered sample, weighing about 50 g, was extracted using the chosen solvents using a Soxhlet apparatus for a duration of 24 hours. We used a rotary evaporator (BUCHI Rota vapour) to dry the filtrate after collecting and filtering the extract. The drying process took place at 50°C under decreased pressure. We dissolved the dried extract (1 mg/ml) in the mobile phase. The extract was injected immediately after passing it through Whatmann filter paper No.42.

Instrumentation and Chromatographic conditions

A Shimadzu liquid chromatographic system (Shimadzu technologies, Japan) was used to measure quercetin and rutin at the same time. The system had a number of useful components, including an LC-2010AT VP solvent delivery system (pump), SPD M-10A photodiode array detector, Rheodyne 7725i injector with a 20 µl loop volume, and

a Class VP 6.01 data station for data collection and processing. A 1 ml/min flow rate was used to pump the mobile phase, which consisted of acetonitrile and ammonium acetate in a ratio of 40:60. At 259 nm, the elution was seen. Comparison of spectra and retention times verified peak identification. At room temperature, all of the tests were run.

Results and Discussion

Method development and validation

Figure 1 shows that quercetin and rutin both exhibited well distinct peaks when the proposed approach was used. Aganosma plant extracts included quercetin and rutin. The quantitative analysis showed that the hydro alcoholic extract of A. cymosa had the highest concentration of rutin (72.08 + 0.18 mg/g), while the methanolic extract had a slightly lower concentration of quercetin (4.87 + 0.16 mg/g) and 3.60+0.20 mg/g, respectively. Figures 2 and 3 show the chromatograms of the hydroalcoholic and methanolic extracts, which included rutin and quercetin, respectively. The International Conference on Harmonization's recommendations for the validation of analytical techniques include ensuring the method's robustness, precision, accuracy, detection limit, and linearity.

Linearity and range of the developed method

Five solutions ranging from 1 to 5 µg/ml of quercetin and 0.1 to 0.5 µg/ml of rutin were examined for the linearity research. Triplicates of each concentration were prepared and examined. An equation for linear regression and the value of the correlation coefficient were constructed using the peak regions measured against each analyte concentration (Table 1). The linear regression equations for quercetin (Y= 88980x- 5306) and rutin (Y = 41670x+ 333.33), where x is the analyte concentration in µg/ml and Y is the peak area, showed good linearity across the range described earlier. For quercetin, the correlation coefficient was shown to be 0.997, while for rutin, it was 0.995. Over the concentration range that was tested, the findings show that the approach is linear (Figure 4, Figure 5).

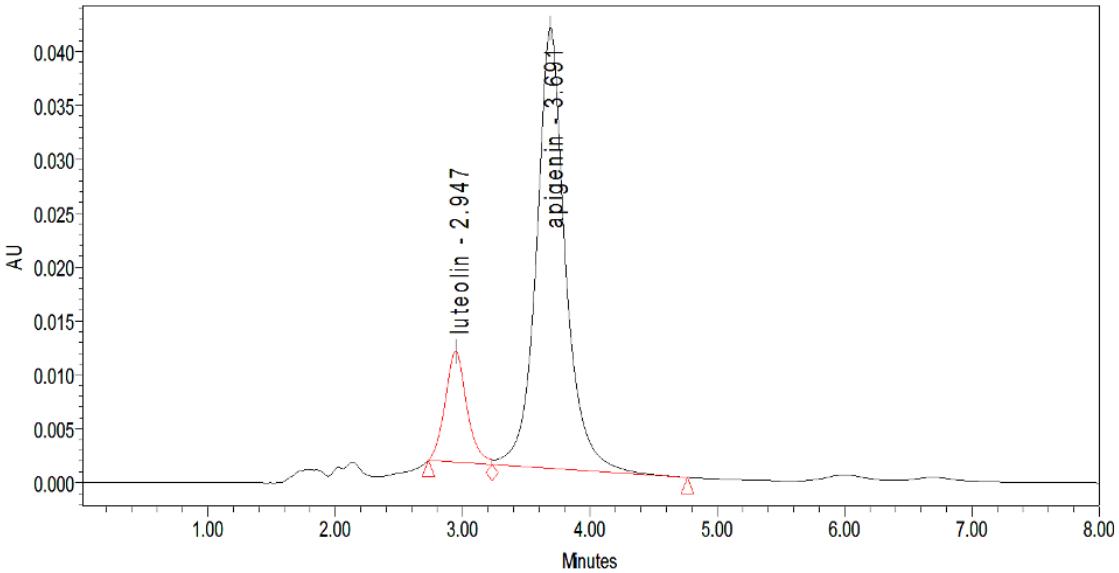

Fig. 1: Typical HPLC Chromatogram of Quercetin and Rutin standard solutions

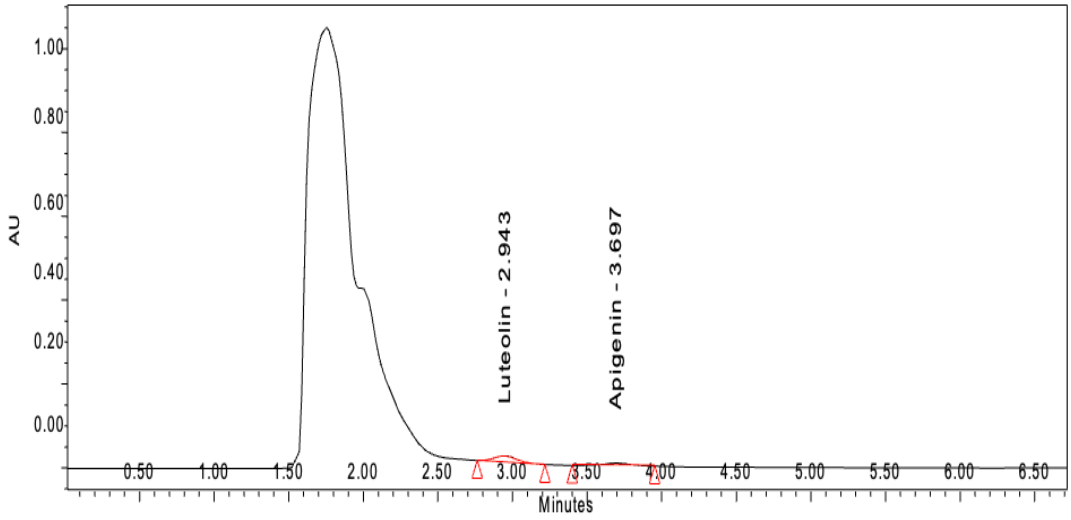

Fig. 2: Typical HPLC Chromatogram of hydro alcoholic extract containing Quercetin and Rutin

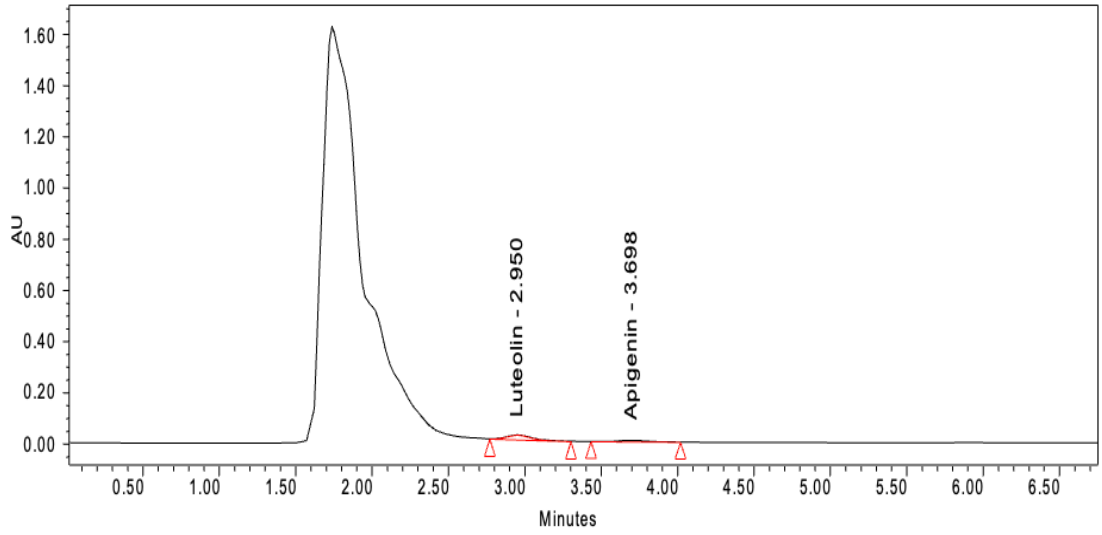

Fig. 3: Typical HPLC Chromatogram of methanolic extract containing Quercetin and Rutin

Table 1: Linearity and range for quercetin and rutin by HPLC

Sl.No.	Concentration of Quercetin (µg/ml)	Concentration of rutin (µg/ml)	Peak area	
			Quercetin	Rutin
1	01	0.1	88915	4167
2	02	0.2	154416	9334
3	03	0.3	262559	13501
4	04	0.4	355976	16668
5	05	0.5	440992	20835

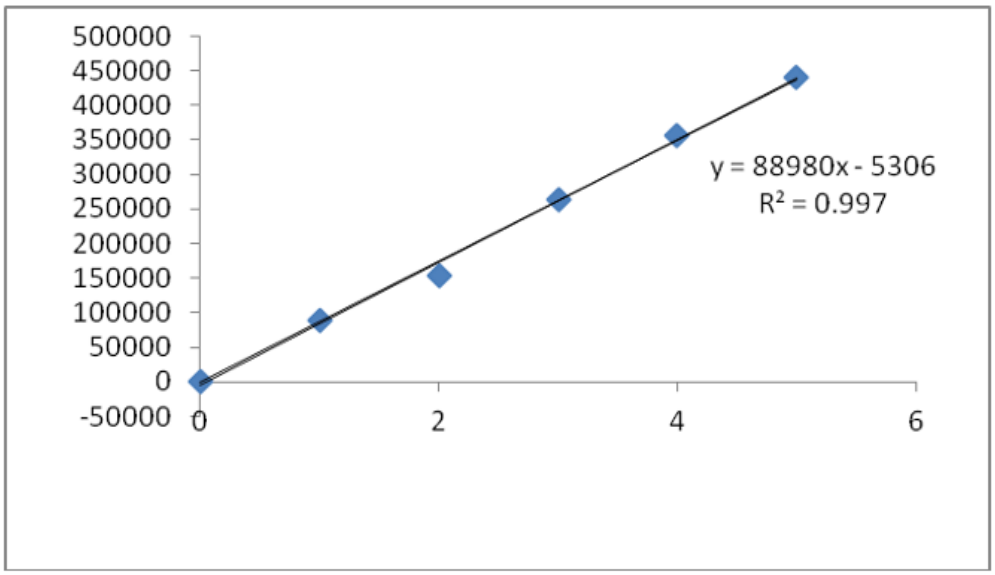

Fig. 4: Calibration curve of Quercetin by HPLC

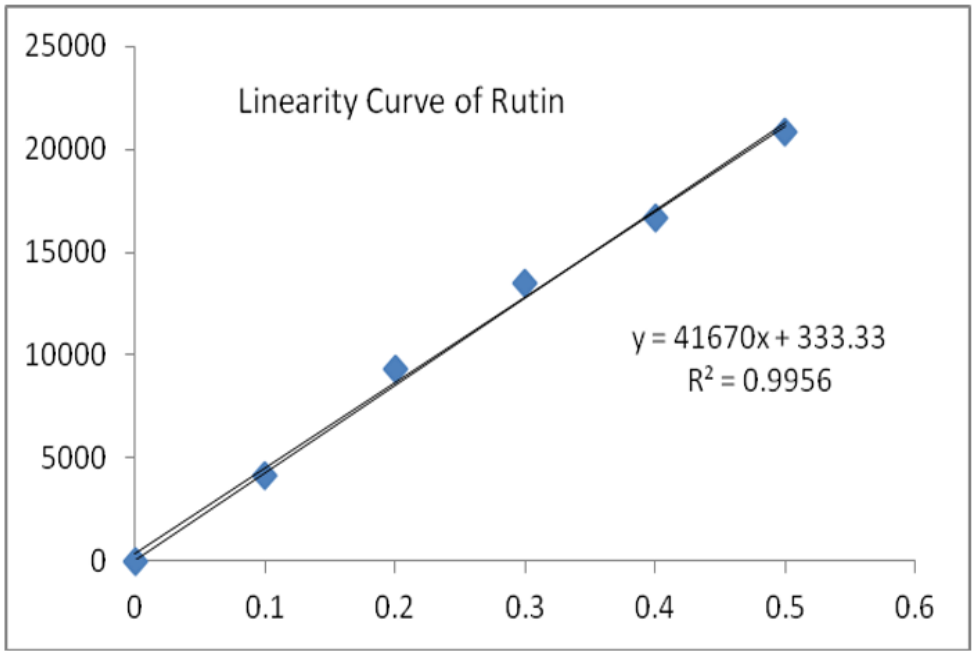

Fig. 5: Calibration curve of Rutin by HPLC

To conduct this experiment, the placebo solution was infused with known concentrations of quercetin and rutin. Concentrations of 1, 2, and 3 µg/ml for quercetin and 0.1, 0.2, and 0.3 µg/ml for rutin, respectively, were used to make three different solutions. Results showed that quercetin had a recovery range of 99.3% to 101.1% and rutin of 101.3% to 102% (with a limit of 98-102%). Table 2 shows that the relative standard deviations for quercetin and rutin, respectively, were 0.185 to 0.529 and 0.054 to 0.075 percent.

Table 2: Recovery and accuracy data

	Recovery		
Compounds	**Amount Added (µg/ml)**	**Recovery (%)**	**RSD (%)**
	1	99.3	0.185
Quercetin	2	100.3	0.44
	3	101.1	0.529
	0.1	101.7	0.054
Rutin	0.2	102.0	0.075
	0.3	101.3	0.06

Precision of the developed method

The relative standard deviation (RSD) was calculated for six concentration measurements on the same day under the same experimental circumstances to study repeatability. The concentrations were roughly 1 mg/mL. Calculations were made using the relative standard deviation for the findings of quercetin and rutin measurements in the working standard solution (Table 3). Evaluation of analytical variance between laboratory runs on several days is an integral part of intermediate precision research. For quercetin, the RSD value was 0.97 and for rutin, it was 1.268%.

Table 3: Precision studies for Quercetin and Rutin

Compound	Conc. (µg/ml)	N	Inter day		Intra day	
			Mean	%RSD	Mean	%RSD
	1		0.99	0.925	1.00	0.525
Quercetin	3	6	3.01	0.512	3.08	1.419
	5		5.12	1.376	5.02	0.932
	0.1		0.10	1.309	0.10	0.834
Rutin	0.3	6	0.30	1.447	0.29	1.410
	0.5		0.48	1.630	0.50	1.132

Limit of detection and quantification

LOD were calculated by using the following equations.

LOD = 3.3 x SD/S and LOQ = 10 x SD/S,

where SD = the standard deviation of the response, S = Slope of the calibration curve.

The LOD value was found to be 100 ng/ml and the LOQ value was found to be 300 ng/ml for the simultaneous estimation of quercetin and rutin.

Robustness of the developed method

The robustness of the proposed method was evaluated by deliberately changing the chromatographic conditions such as solvent ratio, flow rate and absorbance. The results showed that varying the chromatographic conditions had no appreciable effects on the chromatographic parameters (Table 4).

Table 4: Robustness study of the proposed HPLC method

Parameter	Conditions	Retention Time	
		Quercetin	Rutin
	0.9	4.85	1.93
Flow Rate	1.0	4.30	1.71
(ml/min)	1.1	3.95	1.60
	65:35	6.00	3.00
Mobile Ratio (v/v)	60:40	4.30	1.71
	55:45	3.65	2.60
	264	4.33	1.74
Absorbance λ-max	259	4.30	1.71
(nm)	254	4.29	1.75

Conclusions

The method's validity was confirmed when concurrently determining quercetin and rutin using this approach. The analytical approach that has been suggested for the simultaneous measurement of quercetin and rutin in Aganosma cymosa extracts is reliable, repeatable, accurate, linear, robust, and falls within the specified range. According to the findings, Aganosma cymosa has a high flavonoid content, suggesting that it may be useful as a food additive or medical supplement due to its high concentration of antioxidants. This work developed a quantitative approach to extracting rutin and quercetin from Aganosma cymosa at the same time.

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