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Phytochemical Profiling Of Curcumin, Quercetin, And Catechin In Plant Extracts Using A Novel Hplc-Dad Technique

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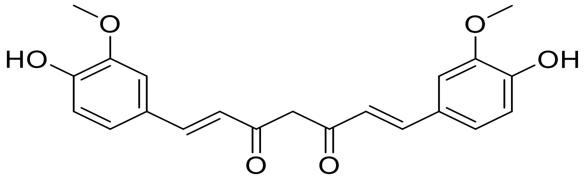
Abstract:
Background: *Curcumin, a compound of Curcuma longa, quercetin from Allium cepa, and catechin from Acacia catechu were measured simultaneously. Curcumin, quercetin, and catechin were obtained through the development and validation of a reverse-phase high-performance liquid chromatography (RP-HPLC) technique.*
Objective: *To develop a sensitive, precise, and ICH-compliant HPLC method for simultaneously determining curcumin, quercetin, and catechin in plant extracts.*
Methods: *HPLC system (Agilent 1100) that consists of a Diode Array Detector (DAD) and the chromatographic separation was carried out using Inertsil C18 column (250 x 4.6 mm, 5 The mobile phase consisted of methanol and phosphate buffer in the ratio of 80:20 v/v and the the flow rate was 1.0 mL/min. The detection wavelength was 280 nm for curcumin and 254 nm for quercetin, catechin, and 274 nm. The method was linearity-validated, precision-validated, accuracy-validated, limit of detection (LOD), and limit of quantification (LOQ) according to ICH Q2(R2) requirements.*
Result: *Curcumin, quercetin, and catechin showed retention times of 17.1, 3.1, and 6.1 minutes, respectively. The analysis procedure was very precise (%RSD < 2%), excellent linearity (R 2 > 0.980), and recovery rates of 100-102%. LODs of curcumin, quercetin, and catechin were 0.004, 0.020, and 0.003ug/mL, respectively.*
Conclusion: *The developed RP-HPLC is simple, robust, and reliable for the simultaneous determination of curcumin, quercetin, and catechin in herbal preparations. It can be used in quality control, standardization, and phytopharmaceutical studies.*

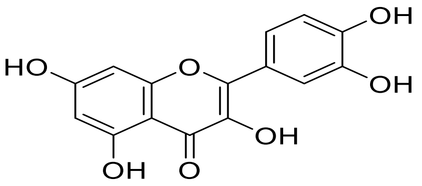
Keywords: *HPLC, curcumin, quercetin, catechin, method validation, and quantification.*

INTRODUCTION:

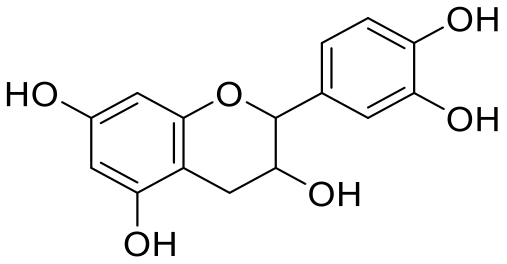
In recent decades, herbal medicines have gained popularity worldwide due to their effectiveness in treating various ailments. Plants with medicinal value can be used as a reference drug in pharmacological studies, as a source of medicinal chemicals, or simply as a raw material in drug research and development. Analytical scientists major challenge in performing quality monitoring of plant medications due to their complexity and unclear methodology. Lack of the right quality control and standardization procedures in synthetic pharmaceuticals has rendered the distribution of medicinal plants worldwide hard to achieve (1). Determination of the active ingredients of medicinal plants that impart the therapeutic effect has been a problem because of the synergistic action of many ingredients, and the problem of consistency of quality between different batches. Qualitative and quantitative estimations of herbal medicines have increased largely over the past couple of decades (2). In order to standardize plant extracts, the European Medicines Agency (EMEA) and the United States Food and Drug Administration (USFDA) have stressed the necessity to assess botanical consistency. The World Health Organization (WHO) has endorsed a fingerprint method of qualitative and quantitative assessment of herbal medicines and stability. The tests are necessary in the determination of the consistency of the herbal preparation (3). It is now possible to purify and quantify each component in a mixture individually due to recent advancements in complex chromatographic separation modes, such as high-performance reverse-phase liquid chromatography (RP-HPLC) and liquid chromatography coupled with mass spectrometry (LC-MS) (4). The main active ingredient in *Curcuma longa* is curcumin. 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione is the structural formula for curcumin. It is a naturally occurring polyphenol that is extracted from *Curcuma longa* rhizomes. The powder has a bright yellow-orange color. Antioxidant, anti-inflammatory, anticarcinogenic, hypocholesterolemic, wound-healing, antispasmodic, anticoagulant, anticancer, and hepatoprotective properties are among the many pharmacological actions of curcumin. Many natural treatments for skin infections and inflammation contain curcumin. Amenorrhoea, joint dislocation, diarrhoea, diabetes, liver disease, bronchitis, ringworm infection, toothache, anaemia, and visual weakness are among the conditions it is used to treat therapeutically (5). The main active ingredient in *Allium cepa* is quercetin. It is a naturally occurring polyphenol that is extracted from the *Allium cepa* bulb. 3,3′,4′,5,7-pentahydroxyflavone is the structural formula for quercetin. The flavonoid quercetin (QUE) 3,3′,4′,5,7-pentahydroxyflavone is made up of three benzene rings and five hydroxyl groups. It is abundant in vegetables, fruits, flowers, wine, walnuts, tea, and bark roots. It is water-insoluble and a yellow, crystalline substance with an unpleasant taste. It has been demonstrated through several studies to have anti-inflammatory, anti-tumor, antioxidant, neuroprotective, antiallergic, and antibacterial properties. Because of these possible pharmacological effects, QUE was widely accepted and used in the pharmaceutical industry (6).(+) Catechin and its enantiomer represent the two steric forms of 3,3′,4′,5,7-pentahydroxyflavan catechin. The term "catechu" referring to the extract from *Acacia catechu*, is the source of its name. Recent years have seen numerous studies explore the pharmacological properties of extracts derived from the bark, heartwood, leaves, seeds, and pods of *Acacia catechu*. This extract exhibits a range of pharmacological characteristics, including immunomodulatory, anti-hyperglycemic, antinociceptive, antihyperlipidemic, and anti-ulcer effects. The flavonoids present in the bark of *Acacia catechu* have been employed for various medicinal purposes, particularly in Asia, and demonstrate antiviral, antibacterial, anti-inflammatory, cardiovascular, and anti-cancer properties (7).



Curcumin



Quercetin



Catechin

One of the most popular chromatography techniques is high-performance liquid chromatography (HPLC), which is simple, versatile, and requires low solution preparation. Besides, in contrast to gas chromatography (GC), HPLC is able to deal with thermolabile, non-volatile compounds in different matrices. HPLC can detect multiple analytes in a single sample and has the potential to support both qualitative and quantitative analyses, and can also be easily adjusted to alternative chromatographic conditions (8). The most commonly used type of UV/visible detectors in HPLC analysis is the diode array detectors (DAD). This feature enhances sensitivity because it makes it possible to identify the wavelength that provides the best fit (9). A variety of organizations have provided guidelines on the validation procedure that should be adopted (9). Several organizations have come up with guidelines describing the process of validation that must be adhered to. They are the International Conference on Harmonization (ICH), the Food and Drug Administration (FDA), and the WHO (10).

MATERIALS & METHODS:

2.1 Method development of HPLC:

2.1.1 Instruments:

The liquid chromatographic system was utilized using Open Lab CDS software. The machine was an Agilent (1100 series). The detector was a Diode Array detector, and the column was an Inertsil-C18 ODS (250 x 4.6 mm, 5 µ).

2.1.2 Chemicals:

Curcumin, Quercetin, and Catechin Samples were purchased from Yucca Enterprises. Trifluoroacetic acid (TFA), methanol, acetonitrile (ACN), HPLC-grade water, and potassium dihydrogen phosphate ($K_2H_2PO_4$) from MERCK labs in Mumbai.

2.2 Method Development:

2.2.1 Preparation of standard solution:

10 mg of the standards for curcumin, quercetin, and catechin were precisely weighed and poured into 10 ml of dry, clean volumetric flasks. Approximately 7 mL of acetonitrile was added, and the air was sonicated from the flasks. After cooling, diluent was added to bring the solution's volume up to par. The solution was then passed through a 0.45 µm syringe filter. We used the outcome as a standard solution (11–13).

2.2.2 Preparation of Sample Solution: About 7 mL of diluent was added to 10 mL clean, dry volumetric flasks containing the equivalent weight of extracts from *Curcuma longa*, *Allium cepa*, and *Acacia catechu*. The extracts were then sonicated to dissolve them completely. The same solvent was used to bring the volume up to par (14–16). The method development starts with initial chromatographic conditions using a liquid chromatographic system. WATERS: Inertsil-C18 ODS (250 x 4.6 mm, 5 µ) column, Diode Array Detector (DAD), 996 PDA detectors, software: Empower 2, Alliance 2695 separation module. To improve the process, several mobile phase compositions were used. Initially, different methanol: acetonitrile concentrations had been used to improve analyte separation. Using a column inertsil-ODS C18 (250 x 4.6 mm, 5µ), a methanol and buffer ratio of 80:20 V/V, a flow rate of l ml/min, and a detector wavelength of 280 nm, 254 nm, and 274 nm for the curcumin, quercetin, and catechin, the procedure was ultimately optimized. It was discovered that the retention periods for quercetin, catechin, and curcumin were 3.0 minutes each. These chromatographic conditions allowed for the good-resolution elution of both analytes. Additionally, the theoretical plates and retention times were adequate. Figure 1-6 represents the chromatogram. The developed methods of linearity, accuracy, precision, LOD, and LOQ were verified following the ICH criteria (17).

2.3 Method Validation:

2.3.1 System suitability:

Curcumin, quercetin, and catechin working standards were used to prepare a standard solution, which was then injected five times in triplicate into the HPLC system following the test procedure. Using standard chromatograms, the system's suitability parameters, such as theoretical plates and resolution, were assessed (6). Table 2 represents the results.

2.3.2 Precision and repeatability:

The test procedure was followed to produce the standard solutions, then injected five times into replicates to ensure precision and repeatability. Table 3 represents the calculated peak area, average, and relative standard deviation percentage. Six injections of the same concentrations of curcumin, quercetin, and catechin were made at the recommended dosage circumstances to obtain intermediate precision (intra- and inter-day) and repeatability of the samples to be analyzed. The analyte sample was produced and examined on three separate days (n=5) to ensure repeatability. Tables 4 & 5 represent the results.

2.3.3 Accuracy:

The accuracy of the suggested technique was assessed using curcumin, quercetin, and catechin recovery tests at three distinct concentrations (10, 20, and 30%). The target concentration was spiked in triplicate at each level, and the amount of each compound recovered was calculated (6). Table 6 shows the percentage recovery at each stage, which was calculated.

2.3.4 Linearity:

Curcumin, quercetin, and catechin working standards were used to develop a series of solutions with concentrations ranging from 10 ppm to 70 ppm of the desired concentration. After the injection of each sample solution into the HPLC equipment in replications, peak areas were determined. Peak areas versus concentrations were shown on a graph, and R^2 values were calculated (1). The findings are shown in Table 7 and in Figures 7, 8, and 9.

2.3.5 Limit of Detection and Limit of Quantification:

Based on the linearity results, the limit of detection (LOD) and quantification (LOQ) for curcumin, quercetin, and catechin have been determined using the following equations (6). Table 8 represent the results.

$\mathrm{LOD} = \frac{3.3\sigma}{S}$

σ = standard deviation of the response

S = slope of the analyte's calibration curve

$\mathrm{LOQ} = \frac{10\sigma}{S}$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte

RESULT:

3.1 HPLC method development

Absorption maxima (UV-1800, Shimadzu, Japan) in the 200–600 nm range were scanned against ACN as a blank for each drug standard (curcumin, Figure 1, quercetin Figure 3, and catechin, Figure 5), and their extracts from *Curcuma longa* Figure 2, *Allium cepa* Figure 4, *and Acacia catechu*, Figure 6 that were produced in ACN. The peak characteristics of the optimized chromatogram for curcumin, quercetin, and catechin were presented in Table 1.

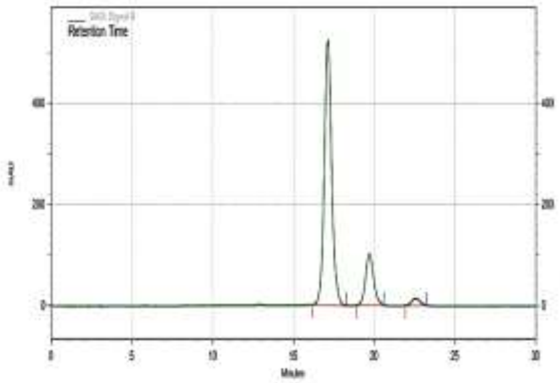


Figure 1 Curcumin standard

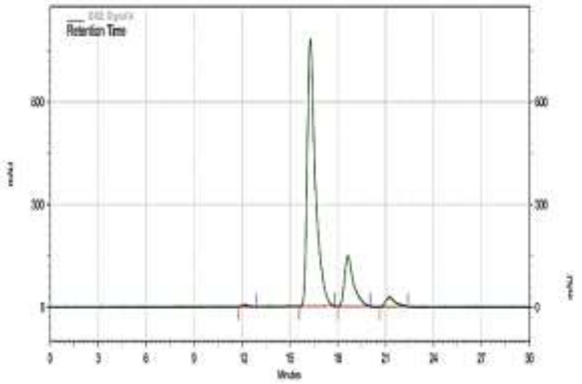


Figure 2 Curcuma longa sample

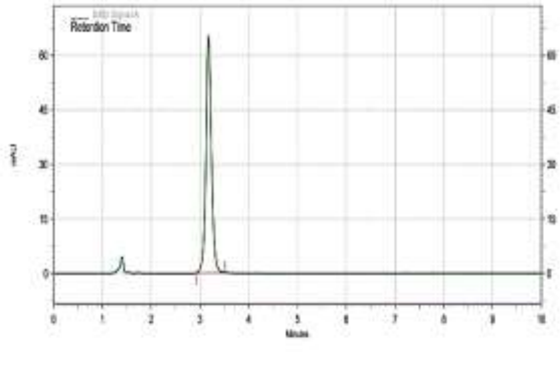


Figure 3 Quercetin standard

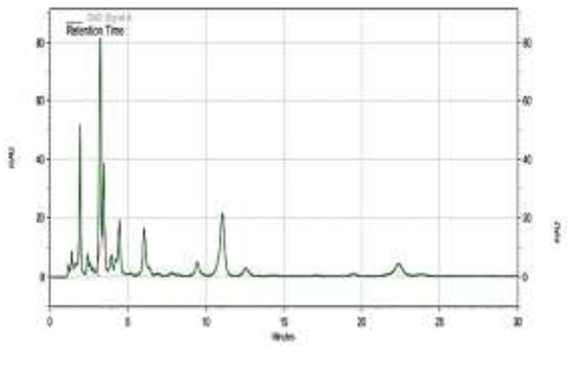


Figure 4 Allium cepa sample

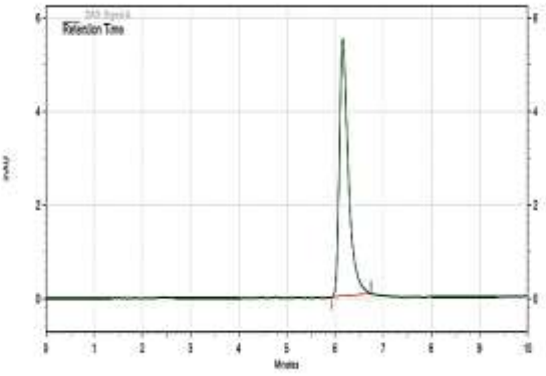


Figure 5 Catechin standard

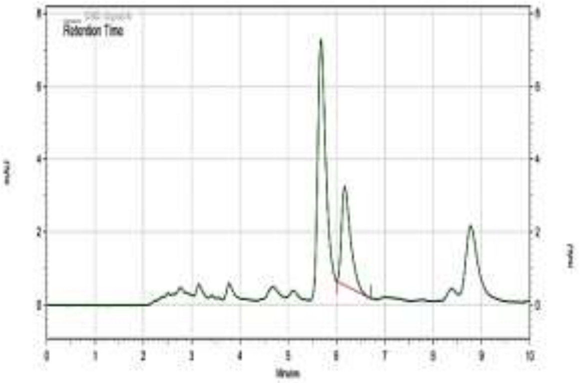


Figure 6 Acacia Catechu sample

Table 1 Peak characteristics of the optimized chromatogram for curcumin, quercetin, and catechin

Sr. No	**Drug**	**Retention time**	**Area**	**ESTD Concentration (%)**
1	Curcumin (sample)	17.14	12810659	3.67
	Curcumin (standard)	17.12	348667	
2	Quercetin (sample)	3.14	462957	32.48
	Quercetin (standard)	3.17	142536	
3	Catechin (sample)	6.17	2986895	2.52
	Catechin (standard)	6.16	1183615	

3.2 System suitability:

By replicating injections and parameters such as theoretical plates, system suitability studies were carried out. The observed values satisfy the criteria for approval.

Table 2 Data of System Suitability for Curcumin, Quercetin, and Catechin

	Curcumin			**Quercetin**			**Catechin**		
Injection	**Retention time**	**Peak Area**	**USP Plate count**	**Retention time**	**Peak Area**	**USP Plate count**	**Retention time**	**Peak Area**	**USP Plate count**
1	17.1	348665	1368	3.15	142533	4200	6.14	1183611	5170
2	17.09	348660	1188	3.16	142534	3611	6.12	1183607	4662
3	17.11	348662	1440	3.13	142531	4552	6.11	1183602	4888
4	17.12	348663	1304	3.16	142534	3611	6.13	1183610	5162
5	17.09	348660	1242	3.14	142535	3349	6.14	1183611	5170

3.3 Precision and repeatability:

Repeatability was performed in six replicate injections, and the % RSD of the peak areas was calculated. The results were observed in Table 3.

To determine repeatability and intra-interday cycles, precision studies were conducted. Tables 4 & 5 showed the percentage RSD values for intra-inter-day precision.

Table 3 Data of Repeatability for Curcumin, Quercetin, and Catechin

	Curcumin		**Quercetin**		**Catechin**	
Injection	**Peak Areas of Curcumin**	% Assay	**Peak Areas of Quercetin**	% Assay	**Peak Areas of Curcumin**	% Assay
1	348667	102.061	142534	102.855	1183614	100.941
2	348665	104.102	142533	100.689	1183612	102.203
3	348666	102.061	142531	99.608	1183613	100.942
4	348663	101.041	142533	100.689	1183615	99.680
5	348662	99.729	142533	101.772	1183612	103.465
6	348664	101.041	142531	98.742	1183611	102.203
Mean	348664.5	101.672	142532.5	100.726	1183612.833	101.572
SD	1870.828693		1224.744871		1471.960144	
%RSD	0.536569881		0.85927411		0.124361624	

Table 4 Precision studies (intra-day) of Curcumin, Quercetin, and Catechin

	Curcumin					Quercetin					Catechin				
Injection	Peak Areas of Curcumin	% Assay	Mean	SD	%RSD	Peak Areas of Quercetin	% Assay	Mean	SD	%RSD	Peak Areas of Curcumin	% Assay	Mean	SD	% RSD
1	348663	102.061	348668.2	4622.409	1.325733	142531	102.855	142536	3162.278	2.218582	1183628	100.941	1183626	4381.78	0.3702
2	348662	104.102				142538	100.689				1183620	102.203			
3	348669	102.061				142537	99.608				1183622	100.942			
4	348670	101.041				142540	100.689				1183626	99.680			
5	348672	99.729				142534	101.772				1183628	103.465			
6	348673	101.041				142536	98.742				1183632	102.203			

Table 5 Precision studies (inter-day) Curcumin, Quercetin, and Catechin

	Curcumin					Quercetin					Catechin				
Injection	**Peak Areas of Curcumin**	**% Assay**	**Mean**	**SD**	**%RSD**	**Peak Areas of Quercetin**	**% Assay**	**Mean**	**SD**	**%RSD**	**Peak Areas of Curcumin**	**% Assay**	**Mean**	**SD**	**%RSD**
1	348662	102.067	348669.3333	4926.120854	1.412834564	142533	102.858	142538.8333	3188.521078	2.23694905	1183630	100.941	1183628.333	4457.203907	0.376571241
2	348665	104.112				142540	100.685				1183623	102.203			
3	348670	102.069				142539	99.608				1183625	100.942			
4	348671	101.048				142542	100.689				1183626	99.680			
5	348673	99.739				142538	101.772				1183631	103.465			
6	348675	101.041				142541	98.742				1183635	102.203			

3.4 Accuracy:

Recovery studies were used to assess the method's accuracy. Following the target assay, three target concentrations 10%, 20%, and 30% were developed and injected into the HPLC system in triplicate. The average recovery rates for each spike level ranged from 100% to 102%, satisfying the acceptance criterion. The accuracy of the procedure is demonstrated by the recovery values. Table 6 shows the results.

Table 6 Data of Accuracy for Curcumin, Quercetin, and Catechin

		Curcumin			Quercetin			Catechin		
Concentration (%) of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Mean recovery	Amount found (ppm)	% Recovery	Mean recovery	Amount found (ppm)	% Recovery	Mean recovery
10% Injection 1	10	10.15	101.5	101.7	10.13	101.3	101.4	10.09	100.9	101.0

10% Injection 2	10	10.17	101.7		10.12	101.2		10.08	100.8	
10% Injection 3	10	10.19	101.9		10.18	101.8		10.12	101.2	
20% Injection 1	20	20.17	100.9	100.8166667	20.04	100.2	100.5	20.07	100.4	100.6
20% Injection 2	20	20.18	100.9		20.09	100.5		20.13	100.7	
20% Injection 3	20	20.14	100.7		20.14	100.7		20.15	100.75	
30% Injection 1	30	30.14	100.5	100.5	30.06	100.2	100.3	30.17	100.6	100.5
30% Injection 2	30	30.16	100.5		30.12	100.4		30.11	100.4	
30% Injection 3	30	30.15	100.5		30.1	100.3		30.16	100.5	

3.5 Linearity:

Drugs at different concentrations were injected into the HPLC system to test the linearity of the procedure. Peak area was plotted vs concentration on a graph, and the correlation coefficient was calculated. The R^2 values for the drugs were within the acceptable ranges, at 0.980, 0.993, and 0.995. Table 7 and Figures 7, 8, and 9 displayed the results, and the R^2 values validated the linearity of the technique.

Table 7 Linearity data of Curcumin, Quercetin, and Catechin

	Curcumin			**Quercetin**			**Catechin**		
Concentration (ppm)	**Average Area**	**Statistical Analysis**		**Average Area**	**Statistical Analysis**		**Average Area**	**Statistical Analysis**	
0	0	Slope	6667.5	0	Slope	7238.9	0	Slope	6977
10	128665	y-Intercept	44219	118665	y-Intercept	26719	98665	y-Intercept	15886
20	198660	Correlation Coefficient	0.980	178660	Correlation Coefficient	0.993	158660	Correlation Coefficient	0.995
30	258662			248662			218662		
40	328663			318663			298663		
50	378665			398665			378665		
60	428664			448664			438664		

70	498666		528666		488666	

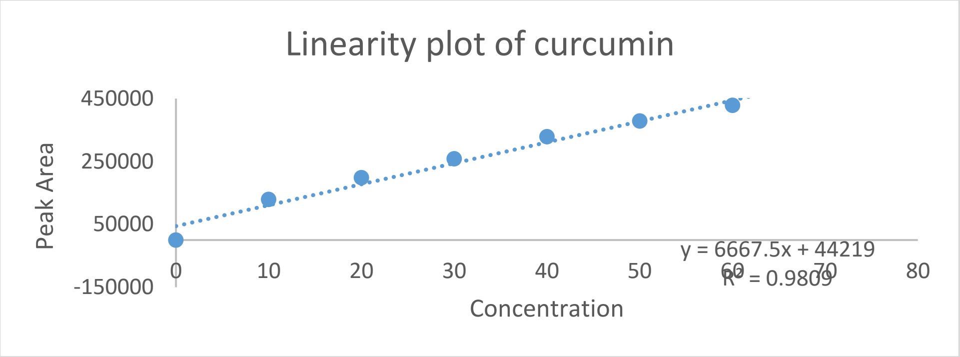


Figure 7 Linearity plot of curcumin

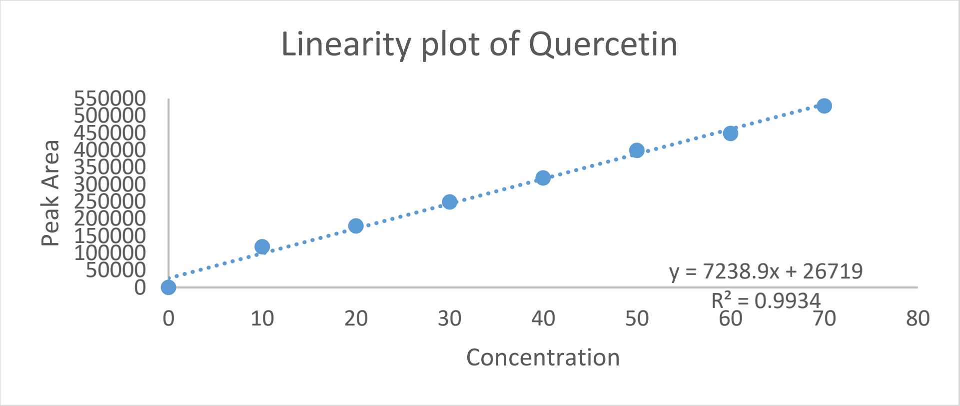


Figure 8 Linearity plot of Quercetin

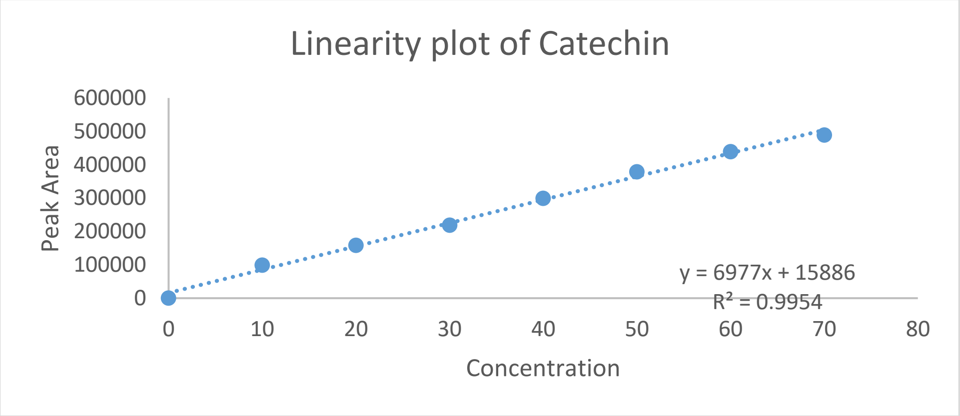


Figure 9 Linearity plot of Catechin

3.6 Limit of Detection and Quantification (LOD and LOQ)

The results showed that the LOQ values for curcumin, quercetin, and catechin were 0.014, 0.066, and 0.012, respectively, while the LOD values were 0.004, 0.020, and 0.003, respectively. Table 8 shows the results of LOD and LOQ.

Table 8 Limit of Detection and Quantification (LOD and LOQ)

	Curcumin	**Quercetin**	**Catechin**
LOD	0.00432374	0.020041395	0.003680172
LOQ	0.014412466	0.066804651	0.012267239

DISCUSSION

This study effectively illustrates the establishment and ICH-compliant validation of a reverse-phase HPLC technique for the concurrent quantification of curcumin, quercetin, and catechin from extracts of *Curcuma longa, Allium cepa*, and *Acacia catechu.* These phytoconstituents possess various pharmacological properties, such as antioxidant, anti-inflammatory, and anticancer activity, rendering their precise quantification essential for the quality control and standardization of herbal formulations (18).

The detection wavelengths were selected based on the maximum UV absorption of the individual compound: 280 nm for curcumin, 254 nm for quercetin, and 274 nm for catechin, thus enabling selective and sensitive detections (19).The method had an outstanding linearity within the concentration ranges tested, with correlation coefficients (R^2) greater than 0.980 for all the analytes, which is comparable or better than the previously reported techniques. Accuracy proves the method's repeatability with %RSD values of intra-day and inter-day less than 2. Accuracy was high, and the recovery ranged between 100-102 percent, which adheres to the acceptance criteria of ICH recommendations. Also, the low limits of detection (LOD) and quantification (LOQ) show that the method has superb sensitivity and can detect very low concentrations of analytes in the complex plant matrices (9).It is a reliable, one-run and simultaneous determination of all three target analytes and works out as a better use of time and resources than other reported analytical methods involving individual compounds or binary mixtures. More often in the past, complex gradient systems or long retention times were used, but in this case, a simple isocratic elution with respectable performance properties has been applied. The procedure is usable in routine analysis of raw materials and finished herbal products and in stability studies, pharmacokinetic studies, and regulatory submissions, where analytical validation is of utmost importance (19).

CONCLUSION

For this study, a validated RP-HPLC method was designed that could accurately measure curcumin, quercetin, and catechin levels in extracts of *Curcuma longa*, *Allium cepa*, and *Acacia catechu*. Because the method showed good linearity, precision, accuracy, and sensitivity, it is ideal for regular control and proper adjustment of herbal raw materials and their preparations. The simple and fast analysis process allows the method to be used widely in both schools and companies. As the method analyses data and relationships well, it could be used in other fields. It can be extended to the scientific studies on the kinetic behavior and access to these phytoconstituents in animals and humans are conducted, monitoring the stability and changes in herbal formulations kept under different forms of storage, evaluating the quality of polyherbal and nutraceutical products that are made from various active compounds found in plants.

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