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UV and HPTLC-Based Approaches towards Rutin Determination in Abutilon theophrasti Extract

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Abstract:

Background: The *Malvaceae* family of plants comprises the genus Abutilon, which include the ethnomedicinal plant *Abutilon theophrasti*.. It is important ingredient of several traditional herbal formulations. Rutin, also known as rutoside, has been investigated for a variety of pharmacological effects among them. Rutin is one of the active ingredients in numerous things, including tea leaves, apples, and many more.

Materials and Methods: Hence, we intended to develop a method for its quality control quickly and validate it using UV-visible spectroscopic and HPTLC methods for better therapeutic efficacy.

Results and Discussion: The determination was carried out in the ultraviolet (UV) region using the UV-visible spectroscopic absorbance mode at 203 nm and As the stationary phase, 20 cm × 10 cm HPTLC silica gel G60F254 plates with a fluorescent indicator were utilized. Ethyl acetate, formic acid, acetic acid, and water (13.4:1.4:1.4:3.6 v/v/v/v) made up the mobile phase. and the migration distance was 70 mm for the plate and chamber saturation. Rf value of rutin was determined to be 0.32, and a correlation coefficient of 0.9993 has been used to determine linearity in the concentration range of 200 to 1000 ng/spot.It was observed that the LOD and LOQ were 2.145 and 6.5, respectively. The method's accuracy, precision, and robustness also were validated.

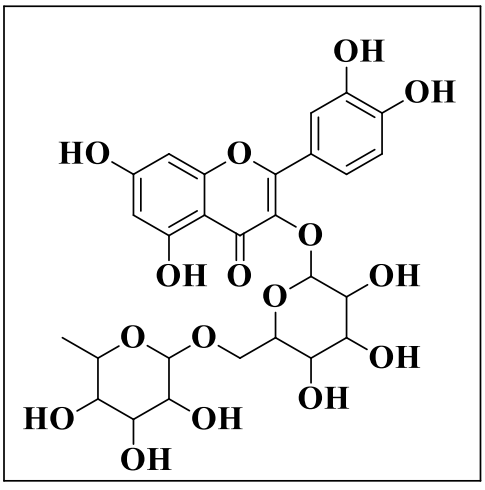
Conclusion: The results of the provided approach are highly reliable and repeatable. The method can be frequently used to concurrently identify rutin in *Abutilon theophrasti* extract. The recommended UV-visible spectroscopic and HPTLC method offers a quicker and more efficient quantitative control.

INTRODUCTION

Plant medicines have been utilized for thousands of years in traditional and folk medicine. Herbal raw resources are utilized in the modern global pharmaceutical industry to create medicines. The benefit of employing plant medicines is that they are well tolerated, have few side effects, and are used to treat a wide range of illnesses.[1] Different types of phytochemicals found in medicinal plants release a variety of pharmacological effects. Historically, plants have been utilized to treat a variety of contagious diseases. Plants include a variety of phytochemical substances such flavonoids, tannins, terpenoids, and alkaloids that help with antioxidant, antibacterial, anticancer, anthelmintic, and other biological functions.[2]

Abutilon theophrasti, a member of the Malvaceae family, has been utilized for millennia, due to its benefits on detoxication and dieresis. It is frequently used to treat venom, swelling, and ulcers. Additionally, it possesses analgesic and anti-inflammatory properties.[3-4] Studies on *Abutilon theophrasti*'s phytochemical components have been conducted, focusing mostly on flavonoids and phenolic compounds such rutin, naringenin, vanillic acid, caffeic acid, ferulic acid, syringic acid, and protocatechuic acid.[5-6] Researchers in the fields of chemistry, medicine, and nutrition are currently becoming more and more interested in the flavonoids found in the leaves of *Abutilon theophrasti*.[7] Fruits, vegetables, and plants contain flavonoids and phenols, which are thought to have a variety of pharmacological effects, including antibacterial, antioxidant, anti-inflammatory, anticancer, and hepatoprotective properties.[8]

Rutin (Figure 1) is a flavonol glycoside made up of the disaccharide rutinose and the flavonol quercetin. Fruits, vegetables, and beverages made from plants, including tea and wine, are the main sources of rutin consumption. It exhibits a variety of pharmacological actions such as antibacterial, antiviral, anti-inflammatory, antiallergic, and antiprotozoal effects due to its scavenging properties on oxidising species such as OH radical, peroxyl radical, and superoxide radical.[9-13]



Rutin

Chemical Formula: $C_{27}H_{30}O_{16}$

Exact Mass: 610.15

Molecular Weight: 610.52

Figure 1: 2D Structure of Rutin Flavanoid

One option for quality assessment is phytochemical evaluation, which involves marker compound analysis utilizing contemporary analytical techniques, preliminary phytochemical screening, and chemo profiling. UV-visible spectroscopic and High performance thin layer chromatography (HPTLC) has become a vital tool for the qualitative and quantitative phytochemical investigation of herbal medications and formulations over the past 20 years.[14-15].

Hence, we developed a quick qualitative and quantitative standardized technique for its quality control by using HPTLC and UV-visible spectroscopic methods for its improved therapeutic efficacy since this plant is a significant component of several traditional herbal formulations.

MATERIALS AND METHODS

Plant Material

Collection of Aqueous and Methanolic extract from Vital herbs Block-z,26/27 Commercial Enclave, Mohan Garden, Uttam Nagar, Delhi, 110059. And additionally, Rutin biomarker were procured from and Kshipra Biotech, Indore respectively

Chemicals and reagents

HPTLC plates with silica gel 60F254 were procured from Merck. Ethyl acetate, formic acid, acetic acid (all analytical grade) and methanol (all analytical grade) as blank for UV-visible spectroscopic.

Equipment

For this study, a CAMAG TLC system equipped with a Linomat-5 applicator, a CAMAG TLC scanner, and a Shimadzu single pan balance was used and for UV-visible spectroscopic Shimadzu UV-1900.

HPTLC method

Preparation of standard solution:

Rutin stock solution was obtained by dissolving 10 mg of precisely weighed rutin in 10 ml of methanol to a final concentration of 1 mg/ml.[16-17]

Preparation of sample solution:

To develop a sample stock solution Abutilon theophrasti extract, carefully weighed at 500 mg, was transferred to a 50 ml volumetric flask and the remaining volume was filled with methanol. The solution was then filtered under vacuum through a 0.45 m Millipore nylon membrane filter.[18]

Chromatographic conditions:

HPTLC silica gel G60F254 plates with a fluorescent indicator and a 20 cm x 10 cm size were used for the analysis. The plate was cleaned by predeveloping it with methanol to the top and then drying it for 5 minutes in an oven set at 105ºC. Linomat 5 automated spray-on applicator with a 100-µl syringe, settings for 6 mm band length, 4 l/sec application rate, 4 mm gap between bands, 6.5 mm distance from plate side edge, and 2 cm distance from plate bottom was used to apply bands representing the sample and standard zones to the layer. Ethyl acetate, formic acid, acetic acid, water (13.4:1.4:1.4:3.6 v/v/v/v) was used as mobile phase, plate and chamber saturation time was 30 min, migration distance was 70 mm. [19]

Validation of method:

The developed analytical method's linearity, repeatability, specificity, limit of detection, limit of quantification, precision, accuracy, and robustness were all validated in accordance with Q2 of the ICH guidelines. [20]

Linearity: The generated standard stock solution was diluted to create linearity standard solutions with rutin concentrations of 200–1000 ng/spot. There were three sets of this solution available, and each set was analysed in order to draw a conclusion. The calibration curves' standard deviation (SD), coefficient of determination (r2), slope, and intercept were determined in order to assess the method's linearity.

Repeatability: The repeatability of the technique was evaluated by scanning the same area seven times for rutin (1000 mg) and computing the coefficient of variance (% CV).

Specificity: The peak purity of the standard and test samples was used to gauge the method's specificity. To verify the location of standard in the sample, the Rf values and spectra of the separated bands were compared to those of the standards at three levels: peak start, peak apex, and peak end.

Limit of Quantification and Detection: Standard solutions in different rutin dilutions, as well as methanol and the blank, have been used to determine the limit of detection and limit of quantification, which were calculated using the signal to noise ratio.The calibration curve's slope, S, and the regression line's Y-standard intercept's deviation, SD, were used to determine the LOD and LOQ, respectively. The LOD was set to 3:1 (SD/s) and the LOQ to 10:1 (SD/S).

Precision: Three bands of both sample and reference solutions per plate on three plates (intra-day precision) and three consecutive days (inter-days precision) at three different concentrations of 600, 800, and 1000 ng/spot for rutin were evaluated to determine precision. All results were expressed as mean RSD, and intra-day (repeatability) and inter-day (intermediate precision) precisions were performed using these three distinct concentration levels of the standard and sample solutions (%).

Accuracy: The accuracy was assessed using recovery experiments. The pre-analyzed samples were externally injected with 20, 40, and 60% of standard rutin to conduct the recovery investigation. The procedure was repeated on a separate plate three times.

Robustness: By purposefully changing the wavelength and calculating the percent divergence from the original approach, the robustness of the method was assessed.

UV-visible spectroscopic method

Preparation of standard stock solution

Rutin in the specified amount (10 mg) was dissolved in 100 mL of methanol (100 g/mL). From this stock, 0.2–1.2 ml was pipetted, diluted with methanol (2–12 g/mL), increased to 10 ml, and measured between 200–400 nm. To confirm the medicines' maximum absorbance, a UV-Vis Specrophotometer (UV-1900, Shimadzu, Japan) was used to measure the maximum absorbance.

Validation of analytical method

The analytical performance characteristics which may be tested during methods validation: % Recovery, Precision, Ruggedness and sensitivity.[21-22-23]

RESULTS AND DISCUSSION

In the HPTLC method, a solvent system which showed the dense spots with significant Rf value was selected for this study. Ethyl acetate, formic acid, acetic acid, water (13.4:1.4:1.4:3.6 v/v/v/v) mobile phase was selected as it showed good resolution. After then, the TLC plate was developed in the solvent system for over 7 cm migration distance. Before carrying out the densitometric study, the plates were then dried. 0.32 ±0.004 was found to be the Rf value and chromatogram [Figure 2] and Method Validation Parameters for determination rutin has been performed [Table 1].

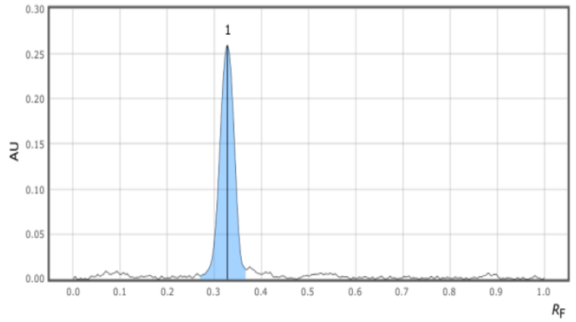


Figure 2: HPTLC chromatogram of test rutin (Rf 0.32 ±0.004).

Parameters	Rutin
Range	200-1000 ng/spot
Limit of Detection (ng/spot)	2.145
Limit of Quantification (ng/spot)	6.5
Repeatability (n=7) (%CV)	0.19
Specificity	Specific

Table 1 : Method Validation Parameters for determination of rutin by HPTLC

Parameters	Value (Rutin)
Linearity Range	200-1000ng/spot
Correlation of coefficient (According to area)	0.9993
Slope	0.0008

Linearity:

For the concentration range of 200-1000 ng/spot, the linear regression data demonstrated a strong linear relationship [Table 2]. The correlation coefficient R^2 was found to be 0.9993 and the curve [Figure 3].

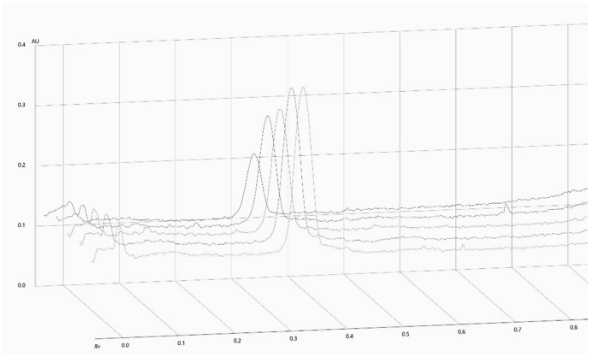


Figure 3 : linearity curve of HPTLC

Table 2 : Linearity Parameters for determination of rutin by HPTLC

Precision:

Three replications of each sample have been used to evaluate the precision of the approach. A precise method was determined to have a precision (% RSD) of less than two again for replications.Peaks of caffeine eluted on to the HPTLC plate were found to be pure [Table 3].

Recovery studies:

Rutin had indicated a percent recovery of 99.45, 99.38, 99.03, 99.91, 100.24% for 60, 70, 80, 90, 100ng/band respectively. The average percent recovery was observed to be 99.60 %.[Table 4]

	Intraday Precision			Interday Precision		
Biomarker	**Concentration (ng/spot)**	**Peak area ± SD (n=3)**	**%CV**	**Concentration (ng/spot)**	**Peak area ± SD (n=3)**	**%CV**
Rutin	600 ng/spot	0.006453 ± 1.52	0.24 %	600 ng/spot	0.006456 ± 1.52	0.26 %
	800 ng/spot	0.008246 ± 2.08	0.25 %	800 ng/spot	0.008243 ± 1.7	0.19 %
	1000 ng/spot	0.009963 ± 2.14	0.21 %	1000 ng/spot	0.009943 ± 3.05	0.31 %

Table 3: Intraday and Interday Precision of rutin

Biomarker	Nominal amt (ng/band)	Calculated amt (ng/band) Mean ± SD	%RSD	%Recovery	%Mean
Rutin	60	59.67 ± 0.59	0.99 %	99.45 %	99.60 %
	70	69.57 ± 0.54	0.78 %	99.38 %	
	80	79.23 ± 0.56	0.71 %	99.03 %	
	90	89.92 ± 0.23	0.26 %	99.91 %	
	100	100.24 ± 0.5	0.50 %	100.24 %	

Table 4: Recovery studies of rutin

Robustness of method:

The interval between the sample application and scanning spanned 0, 20, 40, and 60 minutes. Each parameter's standard deviation of peak areas was calculated, and the percent RSD was reported to be less than 3%.

In the UV multi-component spectral method , The solution of rutin in methanol was found to exhibit maximum absorption at 203 nm after scanning on the UV-Vis spectrophotometer which was reported as λmax in the literature and the procured drug sample of rutincomplies with the reference spectra (Figure 4).

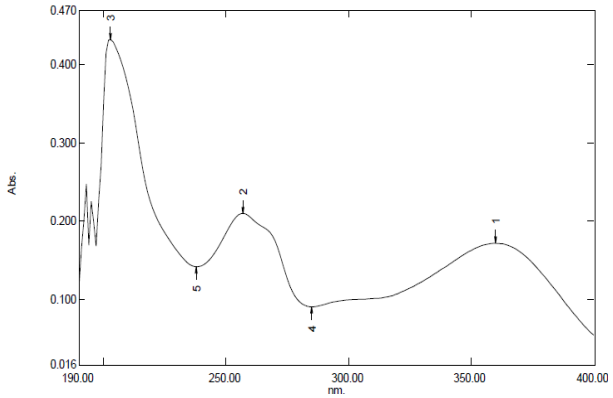


Figure 4: UV spectra of Rutin.

Linearity

Accurately weighted rutin(10 mg) was dissolved in 100 ml of methanol to obtain working standard of 100 µg/ml. Aliquots were pipetted from the stock solution of drug and were transferred to 10 ml volumetric flask, the final volume was adjusted with methanol so that concentration of 2-12 µg/ml could be made. Using a UV-Vis spectrophotometer (UV-1900, Shimadzu, Japan), the absorbance of the abovementioned concentration was taken at 203 nm in comparison to a blank solution made in the same way but without the inclusion of the drug. (Figure 5) shows the absorbance vs. concentration graph, and R^2 was estimated to be 0.999.

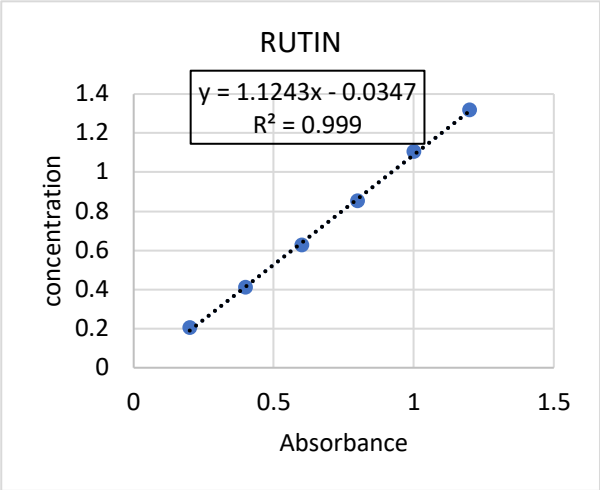


Figure 5: UV graph of absorbance vs concentration

Recovery

Recovery study is performed by standard addition method by adding the known amount of rutin (Working standard) at two different concentration levels i.e 80%, 100% of assay concentration and % recovery for all these drug were calculated. Result was reported in [Table 5].

Drug	Initial amount (µg/ml)	Added Amount (µg/ml)	% Recovery	% RSD (n = 3)
Rutin	2	2.1	100.25	0.06
	2	1.8	100.85	0.03

Table 5: Recovery parameters

Precision

Analysis for rutin at two different concentrations—2 mg/ml and 3 mg/ml—three times on the same day (n = 3) allowed for the estimation of intraday precision (Table 7). Over the period of one week (n = 3), the inter-day variability was evaluated using the three concentrations previously mentioned that have been evaluated on three different days (Table 6).

Drug		Intra - Day		Inter - Day	
	Concentration (µg/ml)	Mean ± SD	% RSD	Mean ± SD	% RSD
Rutin	2	2.0 ± 0.0016	0.05	2.0 ± 0.0014	0.05
	3	3.0 ± 0.0014	0.02	2.9 ± 0.0048	0.01

Table 6: Precision parameters

Ruggedness

Two analyzers used comparable operating and environmental circumstances to create and evaluate sample solutions containing rutin (2 g/ml) from stock solutions (Table 7) (n = 3).

% Amount Found			% RSD	
Drug	Analyst I	Analyst II	Analyst I	Analyst II
Rutin	100.44	100.89	0.01	0.03

Table 7: Ruggedness study

Sensitivity

The proposed method's sensitivity was evaluated using Limit of Detection (LOD) and Limit of Quantitation (LOQ) (Table 8).

Drug	*LOD*	*LOQ*
Rutin	0.29 ± 0.002	0.88 ± 0.016

Table 8: Sensitivity study

CONCLUSION:

The proposed UV and HPTLC spectrophotometric method was found very simple, rapid and economical. The method is validated in compliance with ICH guidelines is suitable for estimation of rutinwith excellent recovery, precision and linearity. Hence this analytical approach can be used for routine quality-control of *Abutilon theophrasti*.

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List of Abrrevations:
Limit of Detection (LOD) , Limit of Quantitation (LOQ) , Relative standard deviation (RSD) , Standard Deviation (SD), ultraviolet (UV), High performance thin layer chromatography (HPTLC).

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