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Estimation of rutin in *Nelumbo nucifera* leaves by HPTLC

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Abstract
The present research article emphasis on the estimation of rutin in *Nelumbo nucifera* leaves by High Performance Thin Layer Chromatography (HPTLC). *Nelumbo nucifera* is also called as Lotus. The leaves of plant contain many constituents; mainly it is rich in flavonoids. Rutin and quercetin are the flavonoids present in lotus leaves. Rutin is used for strengthening and increasing the flexibility in blood vessels, and also your arteries and capillaries. HPTLC is an advanced sophisticated analytical technique of thin layer chromatography. It has the advantages like accuracy, specific identification of compounds in the plants. We used aluminium back coated silica gel of 60F 254 stationary phase and mobile phase of n-Butanol: Glacial acetic acid: Water: 0.1% Formic acid (7:1:1:0.25v/v/v/v) with dosage speed of 20µL/sec and 5×5mm band length and width at 254nm detection wavelength. Rutin was developed by ascending mode and quantified by using JustTLC software. R_f value of rutin was found to be 0.68. Further the developed method was validated for system suitability, accuracy, linearity, precision, LOD, LOQ and robustness according to ICH guidelines. Finally conclude that estimation of rutin by HPTLC method was found to be simple, precise and accurate and can be carried for routine analysis.

Keywords: Rutin, *Nelumbo nucifera*, HPTLC

Introduction
Lotus leaf is the dry leave of water lily plant lotus (Nelumbo nucifera Gaertn), has another name lotus leaf, lotus root leaf in China, most of the region has more plantation and is extensively used in food and medicine, the kind that the second batch specified for the Ministry of Health "is food and medicine". Flavonoids such as rutin (Figure-1) and quercetin are primarily included in lotus leaves. The glycoside combining the flavonol quercetin and the disaccharide rutinose is a bioflavonoid, or plant pigment sometimes referred to as rutoside, quercetin-3-O-rutinoside and sophorin, Rutin. It is a citrus flavonoid present in a wide range of plants, including vegetables and citrus fruits. The apples are rutin-filled. Rutin is also found in buckwheat, most oranges, figs, and both black and green tea [1]. Rutin has strong antioxidant properties. It also helps to create collagen and vitamin C in the body. You can add rutin to your diet by eating foods that contain it or taking it in supplement form [2].

Materials
High Performance Thin Layer Chromatography instrument used was Aetron manufactured with Sample applicator, Documentation system and Just TLC software was used for quantification of compounds. Samples were applied by using Hamilton syringe. Soxhlet apparatus and Rotary film evaporator was used for extracting the rutin from *Nelumbo nucifera* leaves.

Preparation of standard solutions
Weigh accurately about 10mg of standard rutin in a 10mL volumetric flask and make up the volume with methanol (i.e., 1000µg/mL). From the above solution pipette out 0.5mL into 10mL volumetric flask and make up the volume with methanol (i.e.,50µg/mL). This is used as working standard solution for the estimation of rutin in *Nelumbo nucifera* leaves.

Preparation of sample solution
Nelumbo nucifera leaves were collected, air dried and powdered. Extraction was done by taking powder and was placed in the Soxhlet apparatus using ethanol and water combination for one week. The collected solvent was taken and evaporated by using rotary film evaporator under -10^{0}C and 62^{0}C bath temperature with a 30rpm rotating speed. The collected residue was dissolved in methanol for further analysis.

Method Development
Estimation of rutin in the lotus leaves by using HPTLC was done using different mobile phases with different dosing speed and in different concentrations.

Chromatographic conditions
Trail 1
Stationary phase: Aluminium back coated silica gel of 60F 254
Mobile Phase: Hexane: Glacial acetic acid: Methanol: Orthophosphoric acid (8:1:1:0.25v/v/v/v)
Dosing speed: 20µL/sec
Band Length and Width: 5×5mm
Injection Volume: 20µL/sec
Detection Wavelength: 254nm
Chromatogram of trail 1 was represented in Figure-2

Trail 2
Stationary phase: Aluminium back coated silica gel of 60F 254
Mobile Phase: n-Butanol: Glacial acetic acid: Methanol: Formic acid (6:2:2:0.25v/v/v/v)
Dosing speed: 20µL/sec
Band Length and Width: 5×5mm
Injection Volume: 20µL/sec
Detection Wavelength: 254nm
Chromatogram of trail 2 was represented in Figure-3

Trail 3
Stationary phase: Aluminium back coated silica gel of 60F 254
Mobile Phase: n-Butanol: Glacial acetic acid: Water: Formic acid (8:0.5:1.5:0.5v/v/v/v)
Dosing speed: 20µL/sec
Band Length and Width: 5×5mm
Injection Volume: 20µL/sec
Detection Wavelength: 254nm
Chromatogram of trail 3 was represented in Figure-4

Optimized conditions
Stationary phase: Aluminium back coated silica gel of 60F 254
Mobile phase: n-Butanol: Glacial acetic acid: Water: 0.1% Formic acid (7:1:1:0.25v/v/v/v)
Band length and width: 5×5mm
Dosing speed: 20µL/sec
Injection volume: 20µL/sec
Detection Wavelength: 254nm
Optimized chromatogram was represented in Figure-5

Method Validation
Method validation was done according to ICH guidelines and parameters are system suitability, linearity, accuracy, precision, LOD, LOQ and robustness.

Results and Discussion
System Suitability
It ensures that the method is suitable for carrying out in the system by adopting the suitable conditions. Six replicate injections were given and analysed according to the ICH acceptance criteria called relative standard deviation (%RSD). The data of system suitability was tabulated in Table-1.

Discussion: The system suitability results showed that the %RSD observed was within the acceptance limits.

Precision
The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. The data of interday and intraday precision was enlisted in Table-2 and Table-3.

Discussion: For the different parameters intraday and interday precision the %RSD observed was within the acceptance limits.

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 (amount) of analyte in the sample.The data and calibration curve of linearity was represented in Table-4 and Figure-6.

Discussion: The compound rutin was linear and regression found was 0.9998.

Detection Limit
The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Discussion: Limit of detection observed was 0.344 and acceptance range was within 3 therefore it is accepted.

Quantitation Limit
The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

Discussion
Limit of quantification observed was 2.084 and acceptance range was within 10 therefore it is accepted.

Accuracy
The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. The data of accuracy studies was given in Table 5.

Discussion: The accuracy results showed that the %recovery was within the acceptance limits.

Robustness
The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters like

A. Change in mobile phase of Decreasing - n-Butanol: Glacial acetic acid: Water: 0.1% Formic acid (6.5:0.5:1:0.25v/v/v/v) and Increasing - n-Butanol: Glacial acetic acid: Water: 0.1% Formic acid (7.5:1.5:1:0.25v/v/v/v).
B. Change in Dosage Speed of Decreasing - 16µL/sec and Increasing - 25µL/sec.
C. Change in Band Width of Decreasing – 6mm and Increasing -12mm.

The data of robustness with variation in parameters like Mobile Phase, Dosage Speed and Band Width were represented in Table-6, Table-7 and Table-8.

Discussion
For the parameters like mobile phase, dosage speed and band width the %RSD observed was within the acceptance limits.

Table 1: Data of System suitability

S. No	Concentration (µg/mL)	Area
1.	300	4160
2.	300	4125
3.	300	4155
4.	300	4107
5.	300	4163
6.	300	4158
Average		4144.667
SD		23.071
%RSD		0.56

Table 2: Data of Interday precision

S. No	Concentration (µg/mL)	Area
1.	300	4162
2.	300	4150
3.	300	4105
4.	300	4138
5.	300	4143
6.	300	4166
Average		4144
SD		21.918
%RSD		0.53

Table 3: Data of Intraday precision

S. No	Concentration (µg/mL)	Area
1.	300	4165
2.	300	4153
3.	300	4145
4.	300	4127
5.	300	4153
6.	300	4108
Average		4141.833
SD		20.789
%RSD		0.50

Table 4: Linearity of Rutin

S. No	Concentration (µg/mL)	Area
1.	100	1402
2.	200	2800
3.	300	4160
4.	400	5446
5.	500	6807

Table 5: Data of Accuracy

S. No	Level	Concentration (µg/mL)	Area	%Recovery	%Average Recovery
1.	50	100	2801	99.97	99.47
		100	2899	98.72	
		100	2890	99.72	
2.	100	300	4160	99.60	99.77
		300	4167	99.97	
		300	4163	99.73	
3.	150	500	6807	99.98	99.92
		500	6814	99.92	
		500	6810	99.86	

Table 6: Robustness data for variation with Mobile Phase

Mobile Phase (Decreased)			Mobile Phase (Increased)		
S. No	Concentration (µg/mL)	Area	S. No	Concentration (µg/mL)	Area
1.	300	3205	1.	300	4140
2.	300	3263	2.	300	4238
Average		3234.5	Average		4189.5
SD		41.01	SD		69.296
%RSD		1.27	%RSD		1.65

Table 7: Robustness data for variation with Dosage Speed

Dosage Speed (16µl/sec)			Dosage Speed (25µl/sec)		
S. No	Concentration (µg/mL)	Area	S. No	Concentration (µg/mL)	Area
1.	300	2160	1.	300	5218
2.	300	2115	2.	300	5315
Average		2137.5	Average		5266.5
SD		31.820	SD		68.589
%RSD		1.49	%RSD		1.30

Table 8: Robustness data for variation with Band Width

Band Width (6mm)			Band Width (12mm)		
S. No	Concentration (µg/mL)	Area	S. No	Concentration (µg/mL)	Area
1.	300	2100	1.	300	6251
2.	300	2145	2.	300	6132
Average		2122.5	Average		6191.5
SD		31.820	SD		84.146
%RSD		1.50	%RSD		1.36

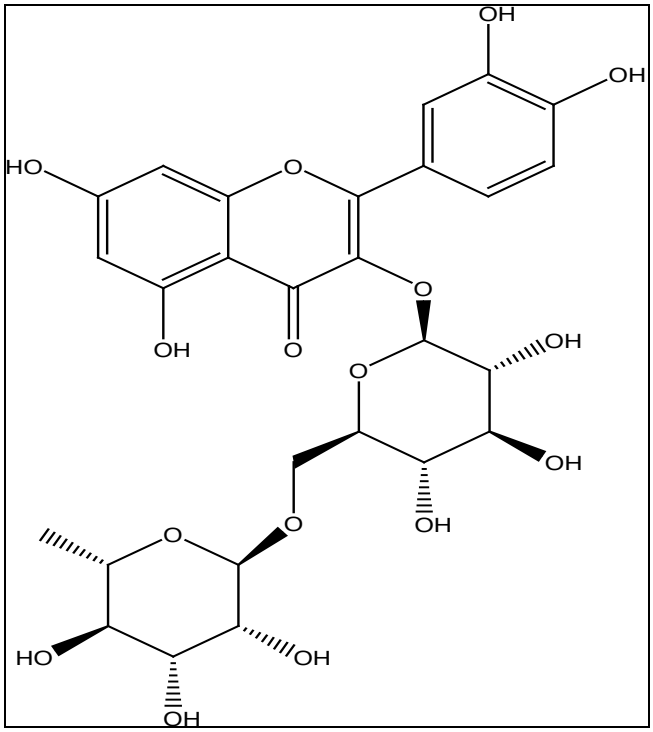

Fig 1: Structure of Rutin

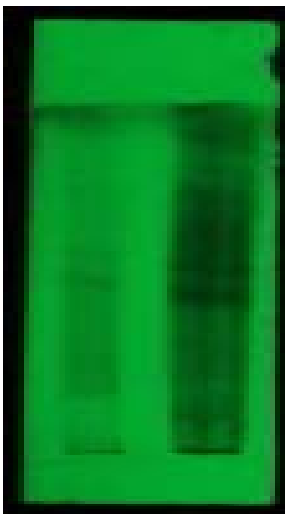

Fig 2: Chromatogram of Trial 1

Fig 3: Chromatogram of Trial 2

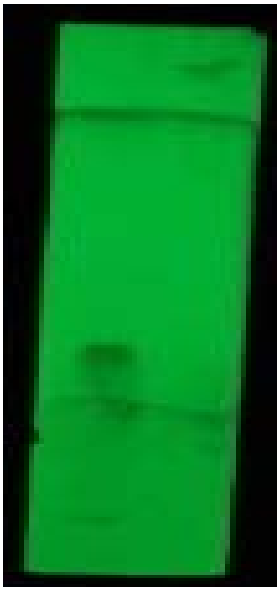

Fig 4: Chromatogram of Trial 3

Fig 5: Optimized Chromatogram

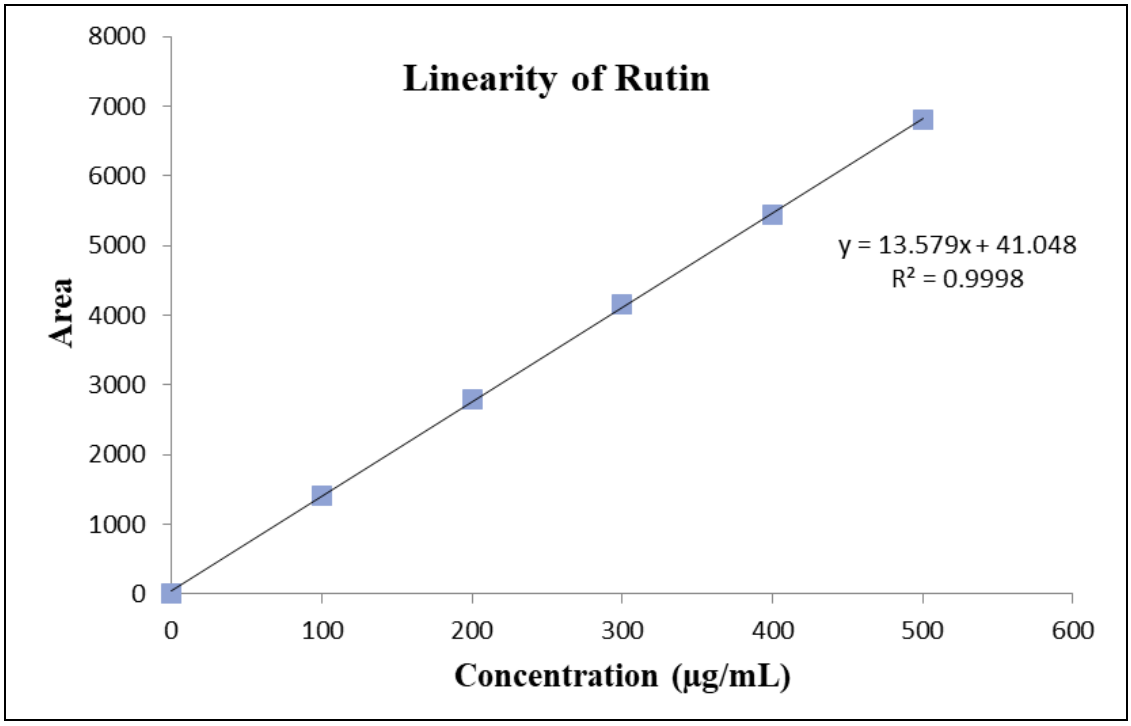

Fig 6: Calibration curve of Rutin

Conclusion

For the estimation of rutin from Nelumbo nucifera leaves, a simple, precise and accurate method was established in this research. Mobile phase n-Butanol: Glacial acetic acid: Water: 0.1% Formic acid (7:1:1:0.25v/v/v/v) with a dosage speed of 20µL/sec and aluminium back-coated silica gel with a stationary phase of 60F 254 were used as optimal conditions for HPTLC. Satisfactory outcomes according to ICH guidelines were obtained under the optimized conditions. The High Performance Thin Layer Chromatography technique was simple, economical compared to other techniques such as HPLC and GC, and the estimation of constituents in plant extract is done with high efficiency in a simple way. The value of the retardation factor for Rutin was 0.68. For qualitative analysis such as the identification of constituents and quantitative analysis like quantification of constituents present in the plant, the optimized method was used and therefore this methodology can be employed for routine analysis.

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