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DEVELOPMENT OF A SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF RUTIN AND QUERCETIN FROM THE FLOWER BUDS OF SOPHORA JAPONICA L.

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Abstract

A simple, rapid, precise, and economical spectrophotometric method for the simultaneous determination of rutin and quercetin isolated from the flower buds of Sophora Japonica L. has been developed. Since rutin and quercetin showed maximum absorbance at 258 and 374 nm, respectively, absorbance was measured at these wavelengths for the determination of rutin and quercetin, respectively. Rutin and quercetin obeyed Beer-Lambert's law in the concentration range of 1-10 µg/ml. The method can be adopted for the routine simultaneous determination of rutin and quercetin.

Key words

Sophora Japonica L., Rutin, Quercetin, UV- and IR- spectroscopy, Calibration curve, Validation.

Introduction

Sophora japonica L. is an arboreal, ornamental perennial plant. The main components of its flower buds include flavonoids, tetraglycosides, isoflavonoids, isoflavone tetraglycosides, triterpene glycosides, phospholipids, alkaloids, amino acids, and polysaccharides. Additionally, Sophora japonica contains five primary flavonoids: rutin, quercetin, isorhamnetin, genistein, and kaempferol [1].

Flavonoids are widely distributed compounds in the plant world and exhibit antioxidant, anti-inflammatory, anticancer, and neuroprotective activities [2-4]. Rutin and quercetin, among the most common flavonoids, are widely used in the food and pharmaceutical industries due to their numerous biological effects.

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Currently, rutin is extracted from the aerial parts of the plant and from the flower buds of Japanese sophora (Sophora japonica L.) [5]. Quercetin is obtained by hydrolyzing the extracted rutin with mineral acids [6, 7].

A literature review indicates that TLC, UV, and HPLC methods have been proposed for the qualitative and quantitative determination of rutin and quercetin [8-11]. However, an inexpensive, rapid, and selective method for the simultaneous quantitative determination of rutin and quercetin has not been developed. Therefore, finding new sources of rutin and quercetin and developing rapid methods for their simultaneous determination is an important task.

Materials and Methods

Quercetin (purity ≥ 97.0%; molecular weight 302.24 g/mol) and rutin (purity ≥ 98.0%; molecular weight 610.52 g/mol) were purchased from Sigma-Aldrich®, USA.

UV spectra were obtained using a UV-visible spectrophotometer (Shimadzu, UV-1900i, Japan) equipped with a 2 nm spectral bandwidth, 0.5 nm wavelength accuracy, and a pair of 1 cm quartz cuvettes, using UV Probe 2.0 computer software.

Preparation of Extracts. Rutin was isolated from the flower buds of Japanese Sophora by aqueous extraction followed by recrystallization in ethyl alcohol. The obtained rutin was hydrolyzed using mineral acids to obtain quercetin [10].

Calibration of the Analytical Method. The analytical method was calibrated by preparing a standard solution using a standard sample and constructing a calibration curve. Standard solutions of rutin and quercetin were prepared. For this, 10 mg of each compound was dissolved in ethanol and then brought to 100 ml in a 100 ml volumetric flask with the same solvent. As a result, a 100 μg/ml concentration solution was obtained for each drug. A 10 μg/ml concentration working standard solution was prepared and the maximum absorption wavelength (λmax) was determined in the wavelength range 600-200 nm. Calibration curves were plotted to study the concentration-absorption relationships for rutin and quercetin and to establish regression equations according to Beer-Lambert's law.

Results and discussion

Infrared spectroscopy was employed to authenticate the purity of rutin and quercetin samples procured from the Japanese Embassy. The acquired IR spectra are depicted in Figure 1 and tabulated in Table 1.

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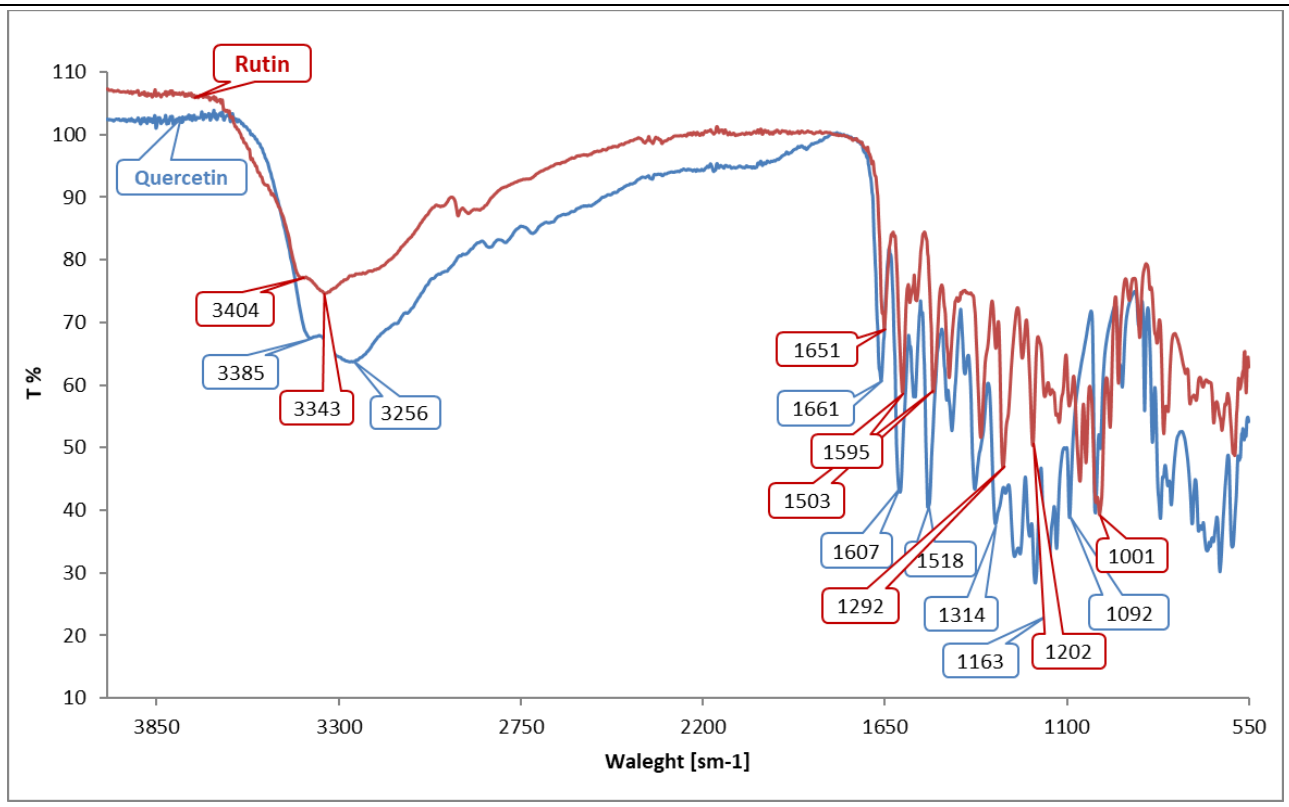

Figure 1: IR spectra of Rutin and Quercetin

According to the obtained IR spectral data, the valence and deformation vibrations corresponding to the functional groups of rutin and quercetin were found to be in full compliance with the standards.

Table 1

IR spectra of Rutin and Quercetin (cm^{-1})

	v(C = O)	v (C = C)	v (O-H)	v (C-O-H)	v (C-O-C)
Rutin	1654	1600, 1504	3473-3244	1362, 1294	1064, 1010
Quercetin	1661	1611	3406-3323	1319	1262

The linearity range for rutin and quercetin at the appropriately selected wavelengths is 1-10 µg/mL. The correlation coefficient for rutin at 257 nm and for quercetin at 372 nm is 0.9967 and 0.9955, respectively. Both compounds exhibit good regression values at the corresponding wavelengths, and the recovery results indicate that any small change in the concentration of the substance in the solution can be accurately determined by the proposed methods.

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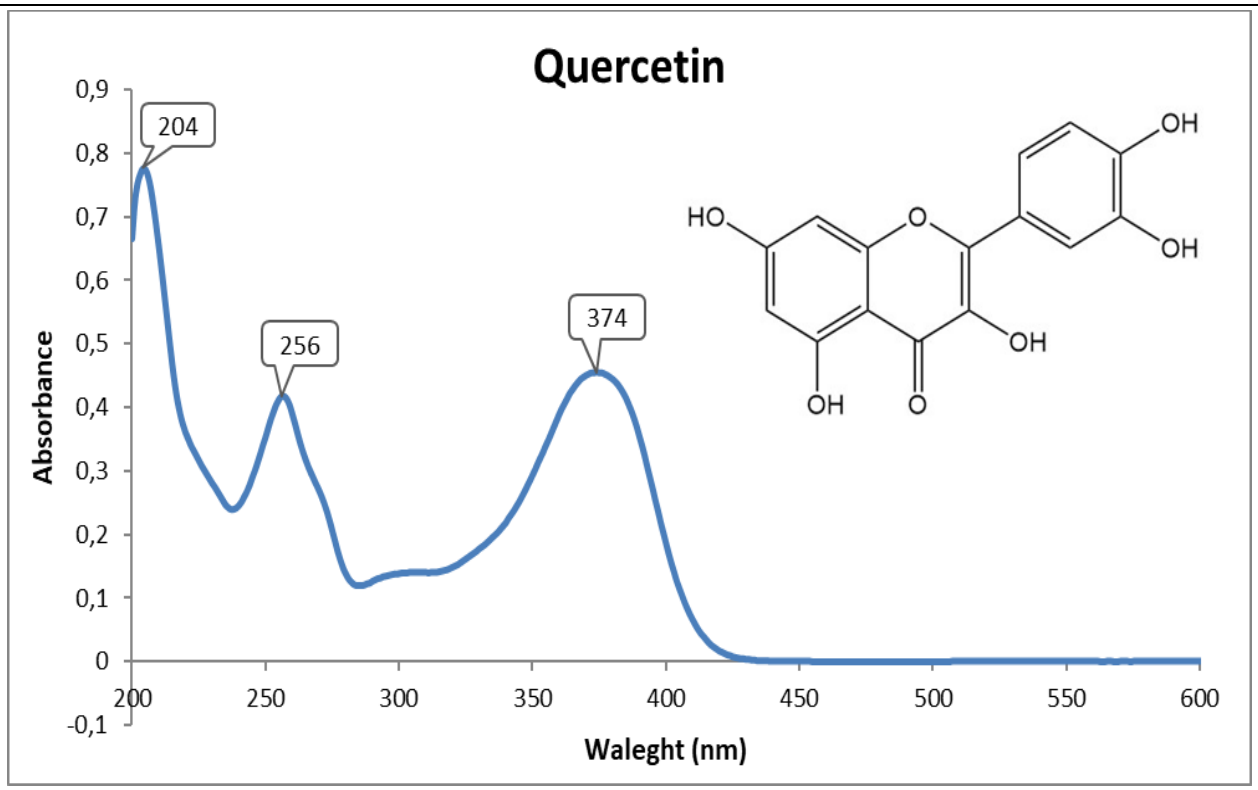

Figure 2: UV-Vis spectrum of quercetin in ethanol.

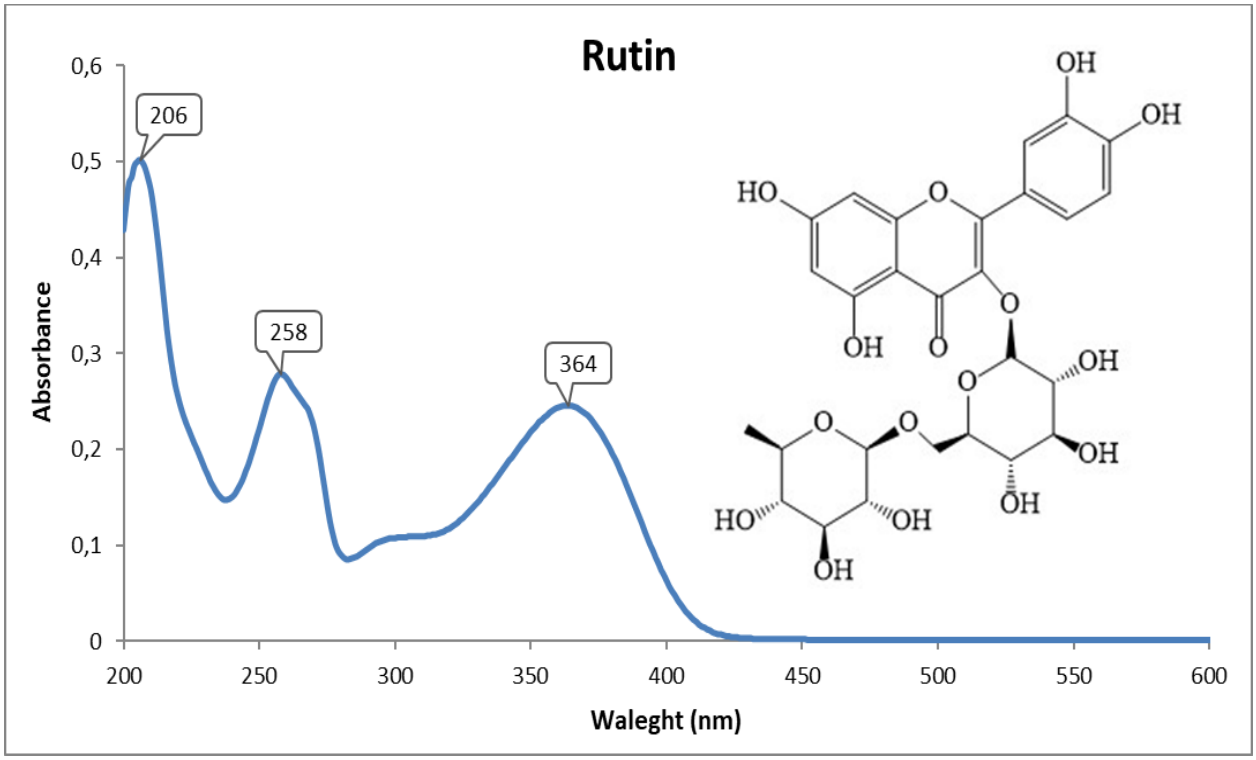

Figure 3: UV-Vis spectrum of rutin in ethanol.

To determine the accuracy of the method, the optical values of six different concentrations (1, 2, 4, 6, 8, 10 µg/ml) of rutin and quercetin were measured three

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times. Based on the obtained results, a calibration curve and equation were constructed for both rutin and quercetin (Figure 3).

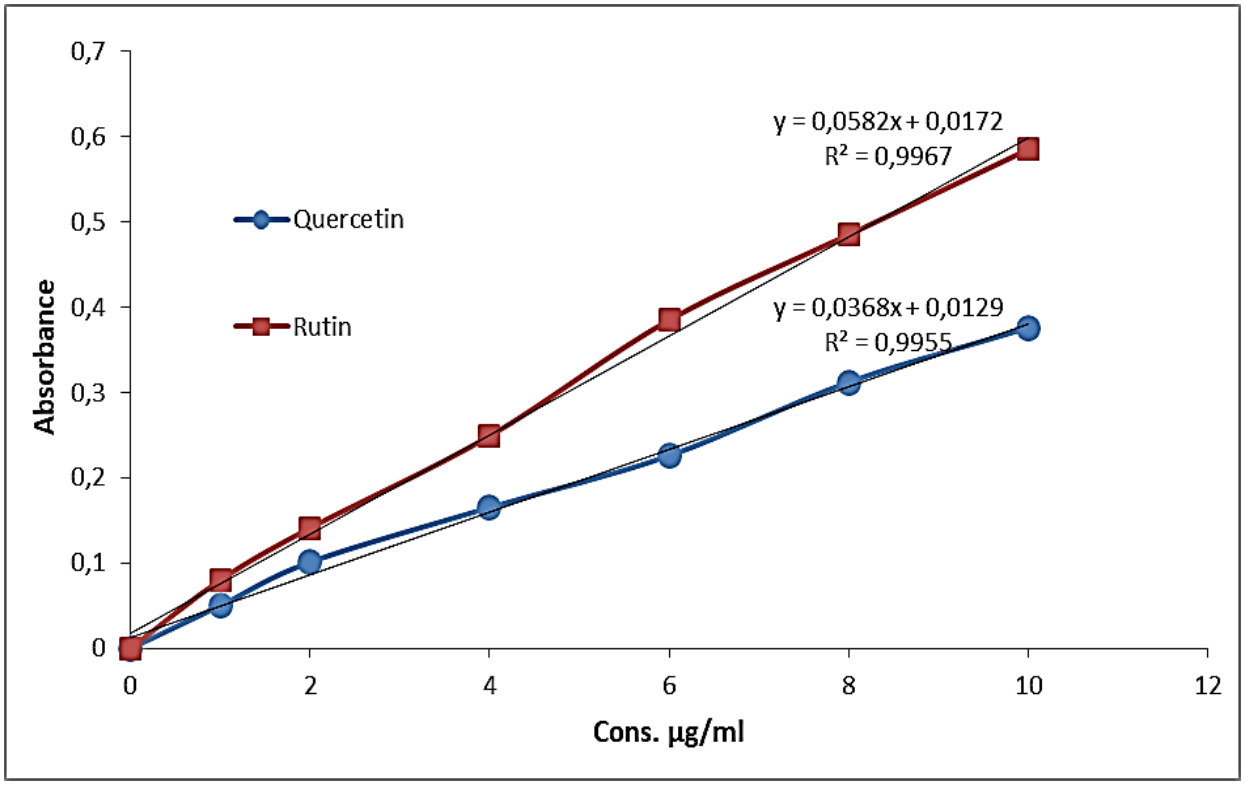

Figure 4: Calibration curve Rutin and Quercetin

Standard solutions of each substance were prepared according to established procedures and analyzed. The Beer-Lambert concentration range for rutin and quercetin was determined to be between 1 and 10 µg/mL. Linearity data for the method are presented in Table 2.

Table 2

Result of validation parameter

Parameter	**Rutin**	**Quercetin**
Wavelenght (nm)	258	374
Linearity range (µg/ml)	1-10 µg/ml	1-10 µg/ml
Linear equation	y = 0,0582x + 0,0172	y = 0,0368x + 0,0129
Correlation (R^2)	0,9967	0,9955
Slope (b)	0,0582	0,0368
Intercept (a)	0,0172	0,0129

Conclusion

The proposed spectrophotometric method is simple, rapid, accurate, and economical, and has been validated in terms of linearity, precision, specificity, and

repeatability. This method can be successfully applied for the simultaneous determination of Rutin and Quercetin.

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