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UV SPECTROSCOPIC QUANTIFICATION OF QUERCETIN IN ANNONA SQUAMOSA

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ABSTRACT

Quercetin is polyphenol flavonoid and shows anti-inflammatory, anti-hepatotoxic antihepatotoxic, antiulcer, antiallergic, antidiabetic, antiviral and antioxidant activity. Quercetin is present in herbal plants such as in Annona squamosa. The present research work is to develop a simple, accurate, precise and economic UV spectroscopic method for the estimation of quercetin in Annona squamosal leaves. The estimation of quercetin by spectroscophy is with maximum absorption at λmax 256nm using solvent ethanol. Beer-Lambert's law is obeyed in the concentration range of 10-50µg/ml and the regression equation y = 0.012x with a regression coefficient (r2) = 0.998 (n=5). For Quercetin, the value of molar absorptivity and Sandell's sensitivity are 3385.04L/mol/cm and 0.0833µg/cm2. The percentage recovery of quercetin was found to be 69.75%. The % RSD for quercetin showed ±0.22. The method is simple and economic. The developed method was effectively applied for the quantification of Quercetin in food nutrients and herbal plants.

KEYWORDS

Annona squamosa, Quercetin and UV Spectral characterization.

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INTRODUCTION

Flavonoids are a big class of natural polyphenol compounds of low molecular mass, commonly distributed in plant world, flavonoids have many important uses such as anti-oxidant and anti-lipidimic properties[1]. Flavonoids have been revealed to have a wide range of biological and pharmacological activities in *in vitro* studies such as Analgesic and anti-inflammatory[2], Anti-bacterial and cytotoxic activity[3], antifungal and antiviral[4-6], anti-cancer[7], anti-diarrheal activities[8], Anti-ulcer

activity[9] and anti-diabetic activity[10]. Flavonoids present in fruits, vegetables, wines, teas and cocoa. It acts as a photoprotectors in plants, so the flavonoid containing vegetables or food supplements can be used as sunscreens in the creams preparations[11]. From the literature survey a few numbers of papers were reported the detection and estimation of quercetin in plants by UV-spectrophotometric methods[12,13]. The present work gives best knowledge about spectroscopic method for the estimation of quercetin in the leaves of Annona squamosal leaves. The aim of this research is to decrease analysis time for the estimation of quercetin in the leaves of Annona squamosa by UV spectroscopy.

MATERIAL AND METHODS

Chemicals

Iodine crystals, potassium iodide, picric acid, copper acetate, α-naphthol, copper sulphate, sodium hydroxide, sodium potassium tartarate, ninhydrin, ferric chloride, gelatin, hydrochloric acid, sulphuric acid, acetic anhydride, petroleum ether, diethyl ether, acetone, acetic acid and silica gel R254 were purchased from Merck (India).

Collection of plant leaves

The leaves of Annona squamosa were collected from local farms of sulur, Coimbatore, Tamilnadu (India). The plant was identified by local farmers and authenticated (BSI/SRC/5/23/2021/Tech /42) by Dr. M.U. Sharief, Scientist 'E' and Head of office, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India. The Annona squamosa leaves were air dried and pulverized. They were washed, shade dried and powdered using an analytical mill. The powder was stored in air-tight container until further analyses.

Instrument and chemical

Instruments Absorbance measurements was made on Shimadzu 1800 double beam UV/VIS spectrophotometer provided with a pair of matched quartz cells of 1 cm width, Shimadzu digital balance used for weighing.

Quercetin (3, 5, 7, 3', 4'-pentahydroyflavone) (99%) was purchased from technico chemicals. Ethanol, petroleum ether was all analytical reagent and purchased form technico chemicals. Distilled water was used in all experiments.

Extraction of leaves

The 50gm of air shade dried leaves of Annona squamosa leaves was soaked in 500ml of ethanol left for 24 hours. Filtered the extraction, collected filtrate and dried. The chlorophyll is removed from the dried extract by treating with petroleum ether for the experiment. Selection of solvent after assessing the solubility of quercetin in different solvents.

Preparation of standard stock solution

The standard stock solutions of quercetin were prepared by dissolving 100mg of each pure quercetin in 100ml of ethanol and final volume was adjusted with same solvent in 100ml of volumetric flask to get a solution containing 1mg/ml of quercetin (Stock 1).

Selection of wavelength

In a 100ml volumetric flask, pipette out 10ml standard stock 1 solution of quercetin and dilute it up to the mark with the solvent ethanol to get a concentration of 100µg/ml (Stock 2). The stock 2 solution of quercetin and ethanolic extract of Annona squamosa leaves were scanned between 200 to 400nm and 256nm was found to be maximum wavelength for absorption as shown in Figure No.2 to Figure No.6 respectively. This wavelength was selected for development of UV method for estimation of quercetin in ethanolic extract of Annona squamosa leaves.

Determination of molar Absorptivity Value

The absorptivity value of quercetin solution was calculated using following formula and the results were presented in Table No.2. Molar Absorptivity = Absorbance/ (pathlenth X concentration)

Preparation of sample solution

100mg of dried extract was treated with 100ml of petroleum ether to remove chlorophyll and centrifuged, collected crude product of extract. Then this crude product is dissolved in 100ml ethanol and absorbance was measured at 256nm.

RESULTS AND DISCUSSION

Results

Linearity

From the above stock 2 solution (100µg/ml) further dilution were made and the volume was make up to 10ml using ethanol to produce 10µg/ml, 20µg/ml,30µg/ml,40µg/ml and 50µg/ml solutions. The absorbance of the spectra was measured at 256nm. The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated (Figure No.1).

Specificity

Various aliquots were prepared from the stock solution (100µg/mL) ranging from 10-50µg/mL. The solutions were scanned in UV-Visible spectrophotometer in the range 200-400nm to determine the wavelength of maximum absorbance for the drugs. The result is shown in Figure No.6.

Discussion

The UV-VIS spectra of solutions containing 100mg/L quercetin were carried out and the maximum absorbance was found at (λmax = 256nm) for all solutions as shown in Figure No.2 to Figure No.6. In this method, the overlain spectra of drugs showed the λmax of 256nm for quercetin Figure No.7. Linear regression data showed a good linear relationship over the concentration range, 10-50µg/ml for quercetin, Correlation coefficient (R2) was found to be 0.998. The sample absorbance obtained were submitted in regression equations to obtain concentration of drug present in plant leaves. The recovery was found to be 69.75% (Table No.1). The spectral parameters by UV spectroscopic method is summarized in Table No.2.

Table No.1: percentage recovery of plant extract

S.No	Sample absorbance	Percentage recovery	% RSD
1	0.837	69.75	±0.00565

Table No.2: Spectral and statistical data of quercetin

S.No	Parameters	Results
1	Beer's law limits (µg)	10 to 50 µg/ml
2	Maximum absorbance	256 nm
3	Sandell's sensitivity (µg/cm2/0.001 absorbance unit)	0.0833 µg/cm2
4	Molar absorbtivity (E max)	3385.04 L/mol/cm
5	%Relative standard deviation	±0.22.
6	Correlation coefficient	0.998
7	Regression equation (Y=b+ac)*	y = 0.012x
8	Slope (a)	0.012

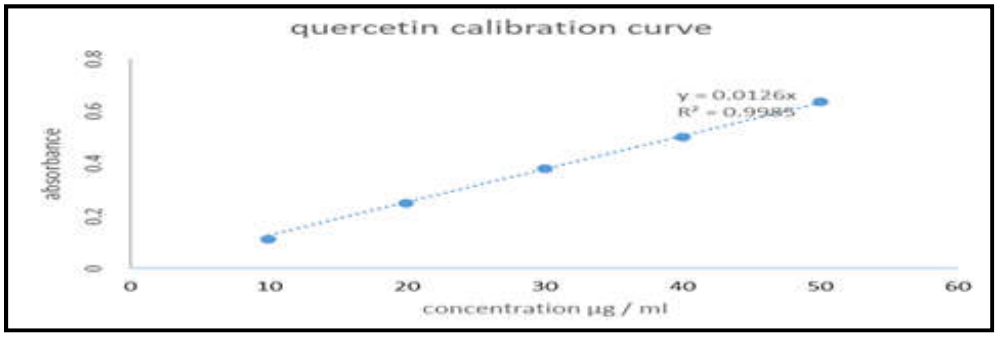

Figure No.1: Calibration curve for standard quercetin

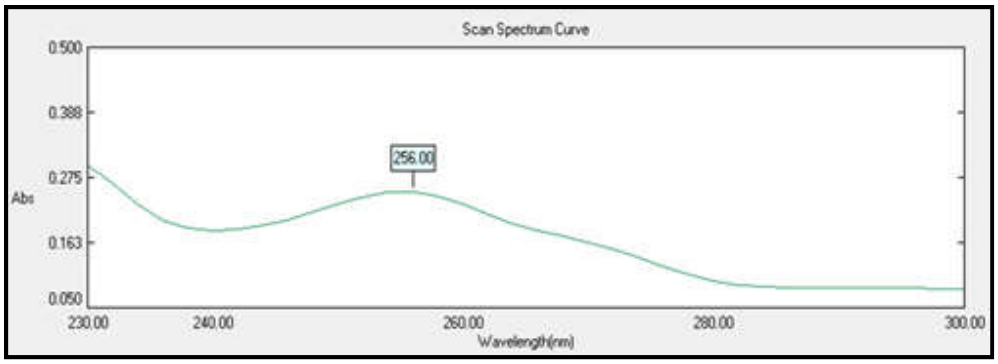

Figure No.2: Spectrum of standard quercetin 10µg/ml

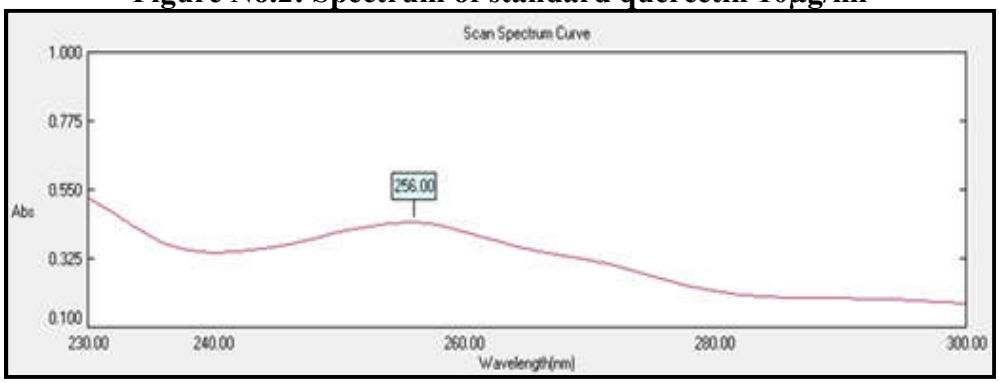

Figure No.3: Spectrum of standard quercetin 20µg/ml

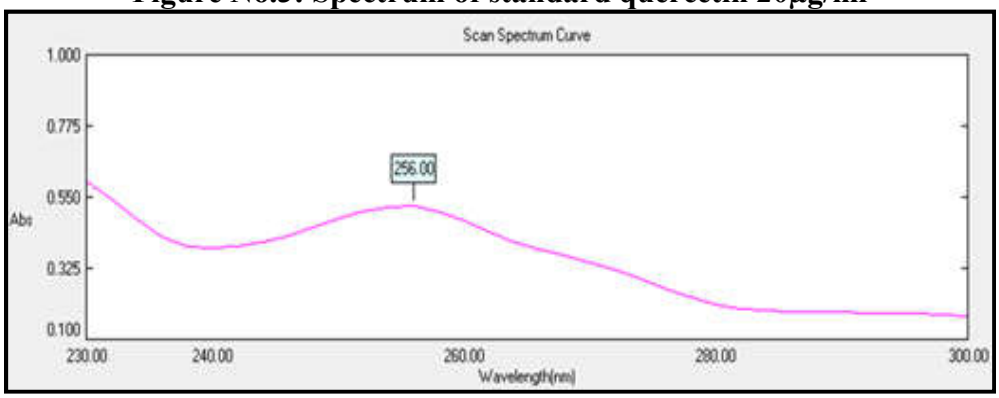

Figure No.4: Spectrum of standard quercetin 40µg/ml

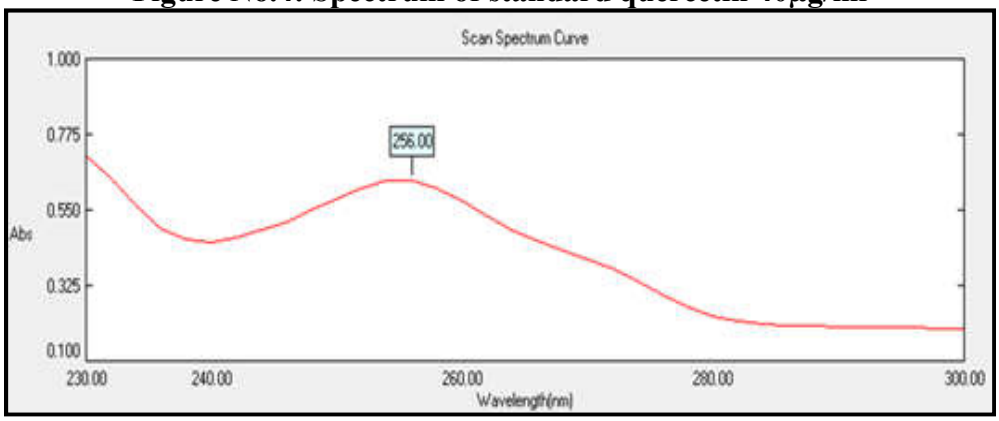

Figure No.5: Spectrum of standard quercetin 50µg/ml

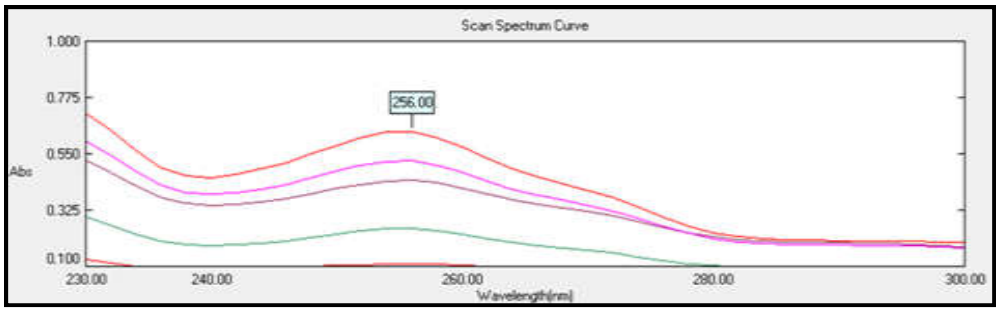

Figure No.6: Specificity and overlap spectrum of quercetin

CONCLUSION

The UV Visible Spectrophotometer analytical method developed was simple, effective, precise and reproducible. Important characteristics of this method are low cost, faster speed, adequate accuracy and good specificity to clearly test the analyte in the presence of components that might be assumed to be present. The developed UV method is simple and cost effective for the estimation of quercetin in Annona squamosa leaves.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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