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ESTIMATION OF QUERCETIN FROM PEEL OF UNRIPE FRUIT OF *TRICHOSANTHES ANGUINA*, LINN. BY REVERSE-PHASE HIGH PERFORMANCE CHROMATOGRAPHY

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ABSTRACT

Several polyphenols have been tested in *Trichosanthes anguina*, Linn. The flavonoid content is important because of the pharmacological properties of these compounds, whereas quercetin has been proved to be an anti-oxidant, anti-inflammatory and hepatoprotective compound. A reversed-phase HPLC method has been developed and applied to determine quercetin content in hydroalcoholic extract of dried flowers of *Trichosanthes anguina*, Linn. in a single analysis. The quercetin was analysed with a HiQ Sil C-18 column by isocratic elution using 0.01% (v/v) phosphoric acid–acetonitrile as the mobile phase. The flow rate was 1.2 ml min−1, and detection was set at 265 nm. The recovery of the method was in the range of 98.50–99.40%, and all the compounds showed good linearity (r > 0.98332) in a relatively wide concentration range.

Keywords: *Trichosanthes anguina* Linn.; Quercetin; RP- HPLC.

INTRODUCTION

Trichosanthes anguina Linn. (Nymphaeaceae), a medicinal plant has been reported traditionally for the treatment of liver disorders in Ayurveda, an ancient system of medicine. The leaves, roots and flowers have a wide range of pharmacological activities and are used for diabetes, eruptive fevers and as cardiotonic, emollient, diuretic, narcotic and aphrodisiac[1,2]. The plant also has antihepatotoxic[3], antidiabetic[4] and antihyperlipidaemic[5] activities. The flowers of plant contain[6] flavanoids, quercetin, astragallin, quercetin and kaempferol. Structurally they have phenolic groups which serve as a source of readily available hydrogen atoms such that the subsequent radicals produced can be delocalized over

the phenolic structure (Figure 1).[7,8] The interest in these compounds is due to their pharmacological activity as radical scavengers.[9,10] They have been proved to have potential preventive and therapeutic effects in many diseases, where the oxidative stress has been implicated, including cardiovascular diseases, cancer, neurodegenerative disorders and in aging.[11-15] The phenolics are also of interest in food, cosmetic and pharmaceutical industries, as substitutes for synthetic antioxidants. These phenolic compounds widely distribute in the plant kingdom.[16-18] Several high-performance liquid chromatographic (HPLC) methods and capillary electrophoretic methods have been developed for the determination of these constituents in herbs and Chinese medicinal preparations[19-31], but none on estimation of quercetin in flowers of *N. stellata* by HPLC method. In this study, HPLC with UV detector was developed for hydroalcoholic extract of dried flowers of *Trichosanthes anguina*, Linn.. The assay was validated to determine linearity, precision, recovery, selectivity and stability.

EXPERIMENTAL

Materials

The herbarium of Nymphea stellata Willd. was identified and authenticated from Prof. B. D. Patil, Department of Botany, Sant Gadage Maharaj College of Science, Tal. Karad, Dist. Satara, M. S. India, having voucher no. SGM/BDP/03/2009.

Quercetin, acetonitrile were purchased from Loba chemie Pvt. Ltd., India. The deionized water was prepared from Millipore water purification system (Bharti Vidhypeeth, Pune, M.S., India) and was filtered with 0.45 µm membrane.

Plant Material

The fresh fruits were washed under running tap water to remove adhered dirt, followed by rinsing with distilled water, the peels of unripe fruits were scraped, shade dried and pulverized in a mechanical grinder to obtain coarse powder.

About 300 gm of the powder was taken in a Soxhlet extractor, defatted with petroleum ether and then extracted with hydroalcohol (70% v/v). The solvent was recovered by glass distillation. The residue was concentrated, dried and stored in the dessicator for subsequent use of experiments.

HPLC system

An Isocratic HPLC PU 2080 Plus (JASCO) with UV- 2075 Plus detector and RP-C18 column was used. A Rheodyne injector with a 20 µl loop was used for the injection of sample. The HPLC system was equipped with Borwin software for data processing. Separations were carried out with a HiQ Sil C-18 reversed-phase column (250mm × 4.6 mm) (KYA TECH Corporation, Japan). The mobile phase was isocratic of aqueous 0.01% (v/v) phosphoric acid–acetonitrile (60:40% v/v). The flow rate was 1.2 ml min−1. The detection was set at UV 380 nm, and absorption spectra of compounds were recorded between 200 nm and 400 nm. The column temperature was 25^0 C, and the sample injection volume was 20 µl. The quercetin was identified from hydroalcoholic extract by comparing retention time value (5.15 min.) with those of the standard. All solvents were of HPLC- grade and were filtered and degassed before their use.

Calibration curve

Marker compound, quercetin was accurately weighed and dissolved in 50% methanol to give serial concentrations within the range of 0.5-5 µg ml−1. The calibration curve was obtained from peak areas of the standard solutions over the concentrations. Concentration of quercetin in sample was calculated from this regression analysis.

Sample preparation

The Hydroalcoholic residue was dissolved with 50% methanol into a volumetric flask. The final volume of the extracting solution was set to 50 ml. For determination of quercetin, the solutions were filtered through a membrane (0.45 µm) and then injected into HPLC directly.

Recovery study

An appropriate amount of extract was taken as control and spiked with 50%, 100% and 150% of standard quercetin with that of sample extract. Samples were filtered through a 0.45 µm membrane filter and assayed by HPLC to calculate recoveries.

RESULTS AND DISCUSSION

Optimization of separation conditions

Absorption maxima of quercetin was observed to be in the range of 200–400 nm on the UV spectra with dimensional chromatograms and a monitoring wavelength for quantitative determination at 380 nm was altered to obtain the baseline separation of marker compound. Isocratic elution was carried out to successfully separate the compounds in hydroalcoholic

extract of *Trichosanthes anguina*, Linn. using 0.01% (v/v) phosphoric acid–acetonitrile (60:40, v/v) as mobile phase in less than 10 min. There were no interfered peaks within the time frame in which quercetin in the extract was detected.

Regression equation

Linear regression analysis for quercetin was performed by the external standard method. The calculated results were given in Table 1, where *a*, *b* and *r* were the coefficients of the regression equation $y = ax + b$, *x* referred to the concentration of the marker compound (µg ml−1), *y* the peak area, and *r* the correlation coefficient of the equation. The marker substance showed good linearity ($r = 0.98805$) in a relatively wide concentration range. The limits of detection (LOD) for quercetin were 10.35 ng ml−1, detected at 380 nm.

Precision and accuracy

The within-day and the day-to-day accuracy data for marker substance are listed in Table 2, relative standard deviations (R.S.D.) of the within-day and day-to-day were 0.45– 1.03% and 0.021– 0.19%, respectively.

Recovery

The average recovery of standard spiked into extract was 98.92 % for quercetin, as shown in Table 3.

Determination of quercetin in hydroalcoholic extract

Fig. 2 shows the chromatogram obtained from RP-HPLC separation of standard quercetin, calibration chromatogram of standard quercetin, typical chromatogram of hydroalcoholic extract and comparative chromatogram of quercetin with hydroalcoholic extract, respectively. The retention time of the standard quercetin was 5.15 min. The content of quercetin was calculated from the corresponding calibration curve. Its content in hydroalcoholic extract of *Trichosanthes anguina*, Linn. was 2.75 mg/g.

Table 1 Linear relation between peak area and concentration (*n*=6)

Marker compounds	Regression equation	r	Linear range (µg ml−1)	LOD (ng ml−1)
Quercetin	y= 30390.02 x + 482.5209.	0.98805	0.5-2.5	10.35

y = peak area, *x* = concentration (µg ml−1). Triplicate assay about the different concentration (*n* = 6).

Table 2 Within-day and day-to-day relative standard deviation (R.S.D.) for the RP-HPLC method for the determination of quercetin

Conc. (µg ml−1)	Observed concentration (µg ml−1) ±S.D.		R.S.D. (%)		Accuracy (%)	
	Within-day[a]	Day-to-day[b]	Within-day[a]	Day-to-day[b]	Within-day[a]	Day-to-day[b]
2.5	2.48 ± 1.04	2.47 ± 0.17	1.03	0.17	99.20	98.80
2	2.03 ± 0.58	1.98 ± 0.021	0.57	0.021	101.50	99.02
1.5	1.52 ± 0.46	1.49 ± 0.19	0.45	0.19	101.33	99.33

[a] Within-day precision test at six times in 1 day.

[b] Day-to-day precision on four different days.

Table 3 Recovery of quercetin (*n*=3)

Initial amount (µg ml−1)	Added amount (µg ml−1)	Detected amount (µg ml−1)	Recovery (%)	R.S.D. (%)
1	0.5	1.48	98.66	0.034
1	1	1.97	98.50	0.089
1	1.5	2.49	99.60	0.023

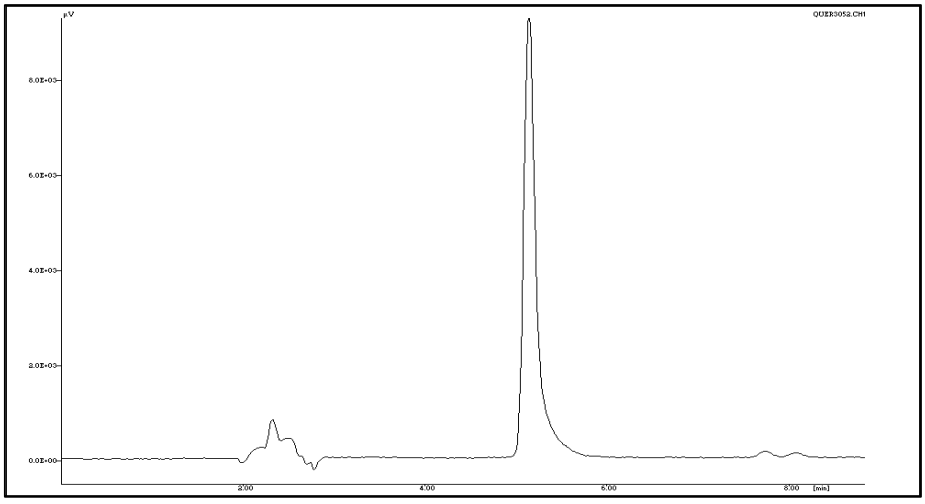


Fig.2. (a) Typical chromatogram of quercetin, 3 µg ml−1.

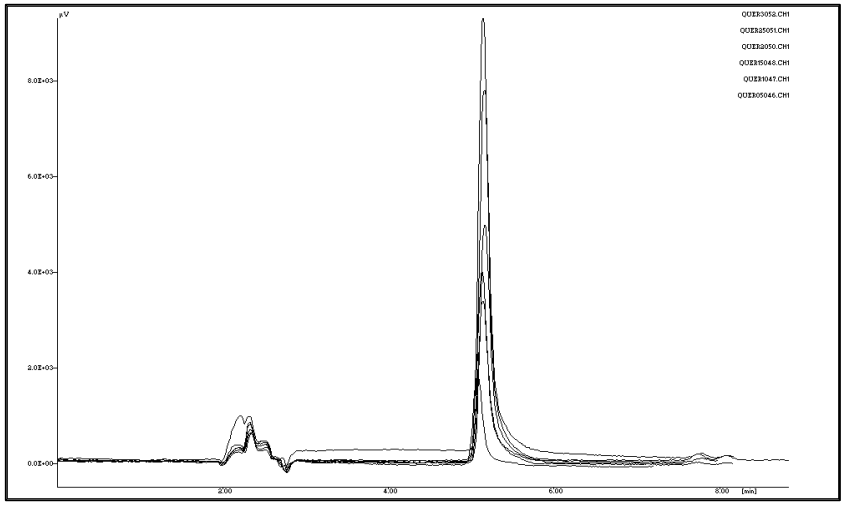
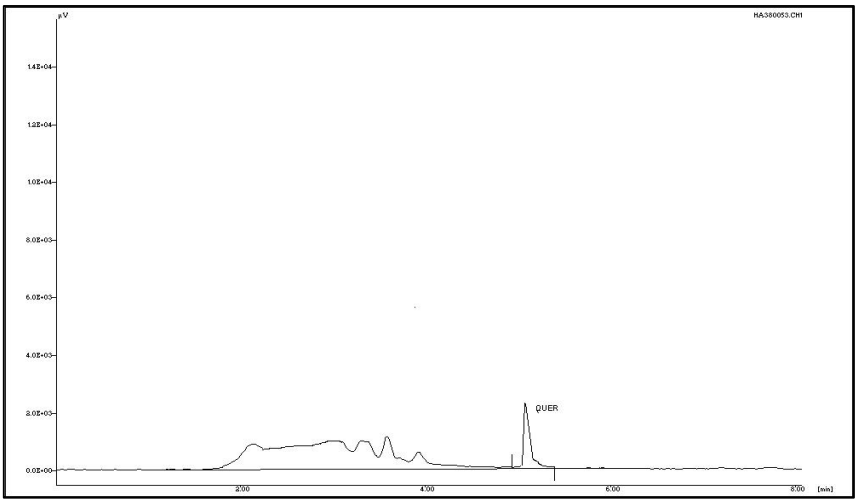


Fig.2.(b) Calibration chromatogram of quercetin.



(c) Typical chromatogram of Hydroalcoholic extract of *Trichosanthes anguina*, Linn. 200 µg ml−1.

CONCLUSION

This was the first report of identification and quantification of the quercetin in hydroalcoholic extract of *Trichosanthes anguina*, Linn. with a short analysis time. A simple, rapid and accurate assay approach was presented. The experimental results indicated that hydroalcoholic extract of dried flowers of *Trichosanthes anguina*, Linn. contained high concentration of quercetin. Since the phenolic compounds have been of interest of health benefits, the present analytical study could be a potential application to identify and quantify the phenolic compound in other flower extracts.

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