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Research Article

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Comparative Analysis of Total Quercetin Content in Aqueous and Ethanolic Extract of *Artocarpus heterophyllus* by Liquid Chromatography

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Abstract: The ethanolic and aqueous extract of *Artocarpus heterophyllus* was evaluated by the well-known HPLC method was developed using a Thermo C-18 column with 250 x 4.6 mm i.d. and 5 µm particle size column. Methanol and Acetonitrile was taken in the same proportion as same proportion and detected at 256 nm. Flow rate employed was 1mL min^{-1}. The aqueous and ethanolic extract of *Artocarpus heterophyllus* was analysed at optimised condition and the content of quercetin of in crude aqueous extract was found as 7.11 mg per 100 mg while in crude ethanolic extract it was found 18.48 mg per 100 mg. Quercetin is a naturally occurring flavonoid which is a polar auxin transport inhibitor and also induce insulin secretion by activation of L-type calcium channels in the pancreatic β-cells. Hence the presence of Bio flavonoid quercetin gives promising advice for antidiabetic potential of the plant.

Keywords: *Artocarpus heterophyllus*, Bioflavanoids**,** Liquid Chromatography, Quercetin.

INTRODUCTION

Recently, synthetic products are being restricted in the industries, because of harmful effects observed such as human toxicity and environmental pollution[1].Additionally, the new demands by the consumers which pressure on the industries particularly for safer products[2, 3]. From this point of view,

an increasing tendency towards the use of natural products instead of synthetic products has been observed in a high demand for food, cosmetics and pharmaceutical manufacturers. For thousand years the plant natural products have been used in the medicine, cosmetics, nutrition and flavoring without or less harmful effects. Thus, plant extracts appear to be a feasible alternative for this problem and the industries have put the attention in the bioactive phytochemicals present in the plants[3].

Bio-flavonoids are the group of phenolic secondary plant metabolites known for their multi-directional biological activities including anti-diabetic efficacy. It has also been demonstrated that flavonoids can act as insulin secretagogues or insulin mimetics, probably by influencing the pleiotropic mechanisms. As a result, bio-flavonoids are now-a-days regarded as promising and significantly attractive natural substances to enrich the current therapy options against diabetes[4]. Among flavonoids, quercetin is a naturally occurring polar auxin transport inhibitor. Quercetin may also induce insulin secretion by activation of L-type calcium channels in the pancreatic β-cells[5]. Thus one can know antidiabetic efficacy of a plant or plant part by estimating flavonoid content or say quercetin (**Figure 1**).

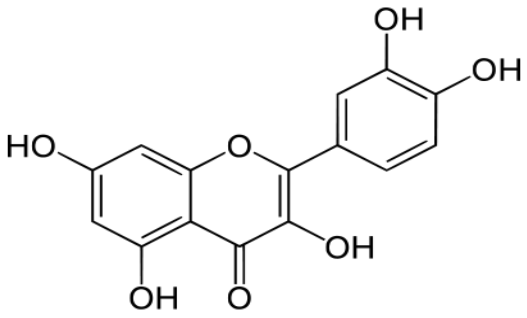

Figure 1: Chemical structure of quercetin

Traditionally *Artocarpus heterophyllus seed* have been used for the treatment of diabetic mellitus for years together but there is no scientific evidence present to prove antidiabetic potential of seeds of the plant[6]. The present investigation deals with the quantitative estimation of quercetin in aqueous and alcoholic extract of *Artocarpus heterophyllus* seeds to prove antidiabetic potential of seeds of the plant.

MATERIAL AND METHODS

Reagents and chemicals: Quercetin was kindly provided by Scan Research Laboratories, Bhopal (India). Methanol and acetonitrile were of HPLC grade and purchased from Merck Ltd, New Delhi, India. Water used was of HPLC grade water from Merck Ltd, New Delhi, India.

Plant material: The seeds of plant *Artocarpus heterophyllus* were collected from local area of Bhopal (M.P.) in the month of June, 2014. The plant specimen was authenticated by Dr. Zia Ul Hasan, HOD, Dept. of Botany, Saifia Science College, Bhopal (M.P). The accession No. for the specimen is 498/Bot/Safia/14. A voucher specimen has been preserved in the laboratory for future reference. The aqueous and ethanolic extraction process was carried out in the Pharmacognosy Department of Scan Laboratory, Bhopal.

Preparation of the plant extract: The seeds *Artocarpus heterophyllus* was collected and shade dried. The shade dried seed material was coarsely powdered mechanically, using manual grinder and subjected to extraction with petroleum ether (60-80°C) in a Soxhlet apparatus. The extraction was continued till the defatting of the material had taken place. The defatted powdered seeds were shade dried nicely and extracted with ethanol separately using Soxhlet's apparatus. The resulting extract was

rapidly filtered through Whatman No 1 filter paper and later with cotton wool to obtain a homogenous filtrate. These filtrates were then concentrated *in vacuum* at low temperature (37-40°C) to about one tenth the original volume using a rotary evaporator. The concentrates of seeds were allowed open in a water bath (40°C) for complete dryness. The extract was refrigerated at 2-8°C until use.

Instrumentation: A thermo spectronic model of Labindia 3000 + UV/VIS Spectrophotometer with 1cm. matched quartz cells was used for determination of λmax. The HPLC system (Waters) consisted of a pump, a UV Visible detector, a Thermo C18 (250 X 4.6 mm, 5µm) column, a Data Ace software.

Determination of wavelength maxima: Stock solution of drug was prepared in methanol and UV spectrum of 10 µg mL^{-1} solution of *Artocarpus hetrophyllus* seed was taken, it showed maximum absorbance at 256 nm (**Figure 2**).

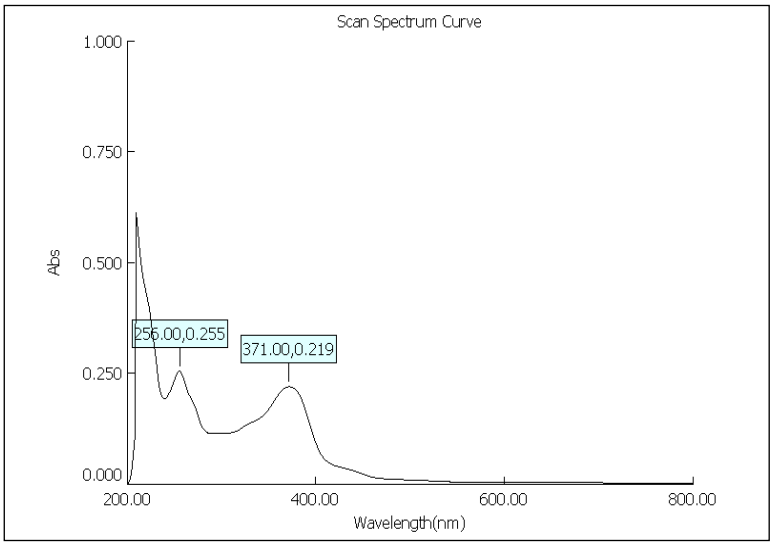

Figure 2: *In situ* spectrum of *Artocarpus hetrophyllus* seed

Chromatographic conditions: A small sample volume of 20 µL of extracted drug was used for each sample run, injected into the HPLC system. Initially methanol and water in different ratios were tried. It was found that with variation in water concentration in the mobile phase, the asymmetry and plate count changes drastically yet not found up to the mark. Hence water was replaced by acetonitrile with no change in flow rate and there was improvement in resolution. It was found that acetonitrile: methanol in the ratio 50:50 v/v isocratically eluted at flow rate 1mL min^{-1} gives acceptable retention time, asymmetry, plates and good resolution of drug. The chromatogram was monitored with UV detection at a wavelength of 256 nm.

RP-HPLC method: HPLC method was developed using a Thermo C-18 column with 250 x 4.6 mm id and 5 µm particle size column. Mobile phase selected for this method contained Methanol and Acetonitrile in the ratio of 50:50 and filtered through 0.2 µ membrane filter. Flow rate was employed 1 mL min^{-1} and detection of eluent was carried out at 256 nm.

Preparation of standard stock solution: 10 mg of quercetin was weighed accurately and transferred to a 10 mL volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000 ppm.

Preparation of working standard solution: From stock solutions of quercetin 1 mL was taken and diluted up to 10 mL from this solution 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mL solutions were transferred to

10mL volumetric flasks and make up the volume up to 10 mL with mobile phase, gives standard drug solution of 5, 10, 15, 20, 25 µg/ mL concentration respectively.

Preparation of the calibration curve of the quercetin: Each of the standard drug solutions were injected 3 times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using calibration curve.

Sample preparation: 10 mg of Aqueous and ethanolic extract was taken in 10 ml of volumetric flask separately and diluted upto the mark with Methanol to obtain concentration of 1000 µg/ml. The resulting solution was filtered and then sonicate for 10 min.

A reverse phase C-18 column pre equilibrated with mobile phase methanol: acetonitrile (50:50, v/v) was used. Mobile phase flow rate was maintained at 1mL $min^{-1}$and effluents were monitored at 256 nm. The sample was injected using a 20 µl fixed loop, and the total run time was 10 min. The sample solution was chromatographed and a concentration of quercetin in Extracts samples was found out using regression equation.

RESULTS

HPLC Condition: At optimized condition with C-18 column with 250 x 4.6 mm i.d. and 5-µm particle size column. HPLC Chromatogram data for extracted drug was shown in **Figure 3** and **Table 1**.

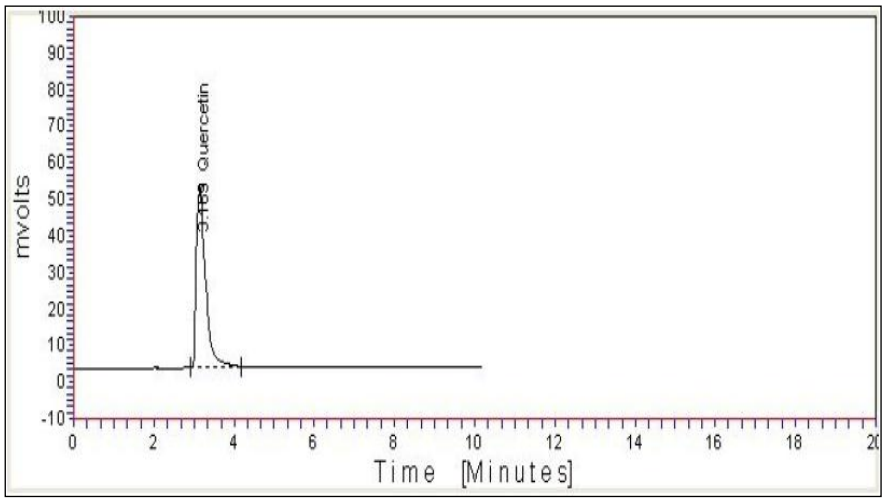

Figure 3: Chromatogram of quercetin standard 10 µg mL^{-1}

Table 1: Data of system suitability parameter for quercetin standard

Chromatogram	Retention time (min)	Asymmetry	Plates
Quercetin 10 µg mL^{-1}	3.189	1.14	7390

Calibration curve of the quercetin: After injecting each concentration 3 times, the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation and calibration curve was found as follows (**Figure-4** and **Table-2, 3**).

Table 2: Data of calibration curve of quercetin

S. No.	Conc.	Mean AUC
1	0	0
2	5	160.29
3	10	334.812
4	15	488.56
5	20	735.45
6	25	930.986

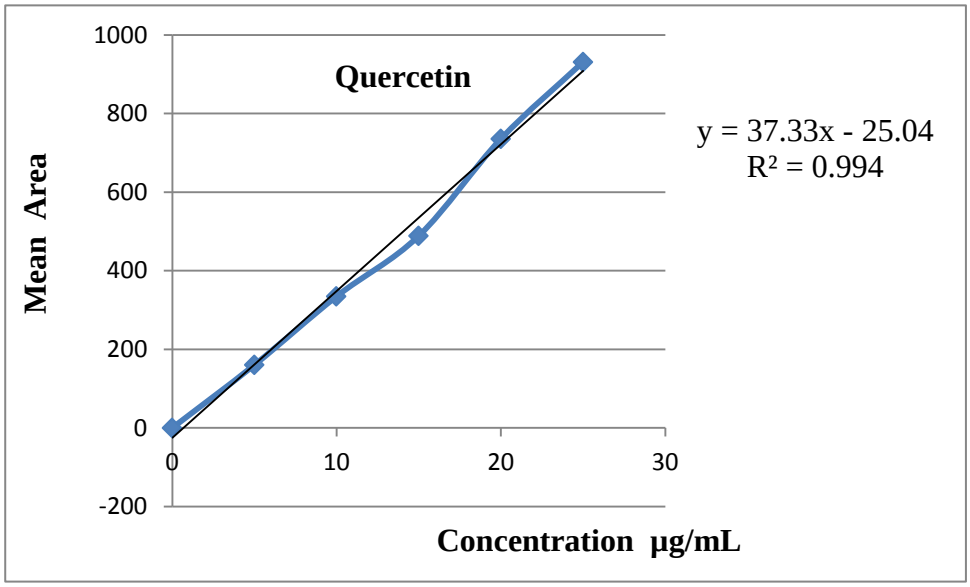

Figure 4: Calibration graph of quercetin

Table 3: Characteristics of the analytical method derived from the standard calibration curve

HPLC				
Compound	Linearity range µg/ml	Correlation co-efficient (r^2)	slope	intercept
Quercetin	5-25	0.994	37.33	-25.04

Analysis of extracts: The aqueous and ethanolic extract of *Atrocarpus heterophyllus* was analysed at optimised condition of methanol: acetonitrile (50:50, v/v) at flow rate of 1 mL min^{-1} and λmax of 256 nm respectively and the chromatogram and a concentration of quercetin in Extract samples was showed as follows (**Figure 5** and **6, Table 4** and **5**). The content of quercetin of in crude aqueous extract was found as 7.11 mg/100 mg while in crude ethanolic extract it was found 18.48 mg/ 100 mg.

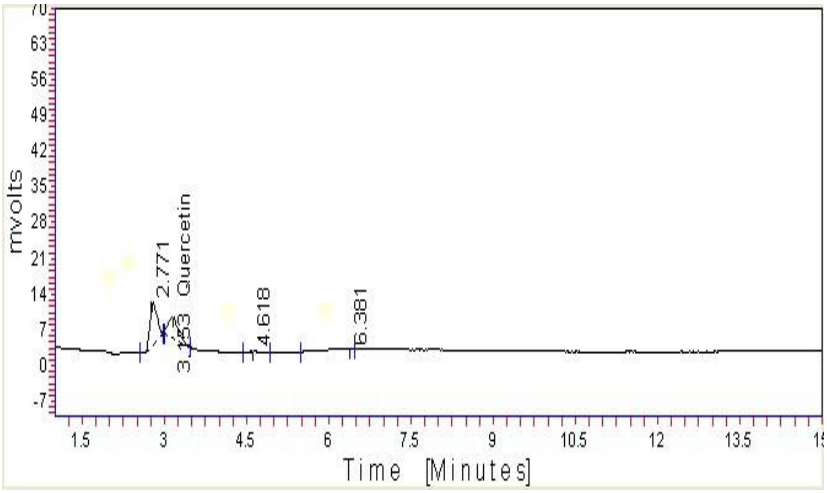

Figure 5: Chromatogram of aqueous extract *Artocarpus hetrophyllus*

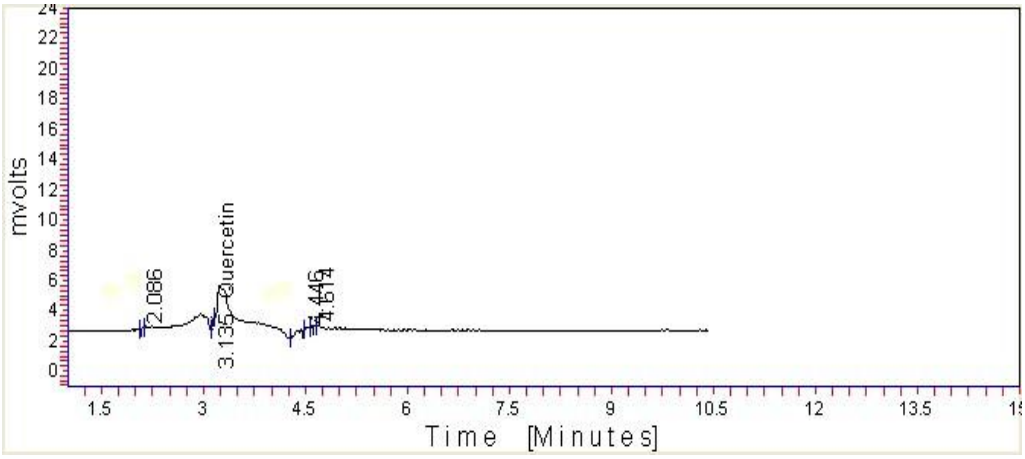

Figure 6: Chromatogram of ethanolic extract of *Artocarpus hetrophyllus*

Table 4: Percentage estimation in aqueous extract of A. Heterophyllus

S. No.	Type of Extract	Retention time (min)	% Estimation Found
1	Aqueous Extract	3.153	7.928

Table 5: Percentage estimation in ethanolic extract of A. Heterophyllus

S. No.	Type of Extract	Retention time (min)	% Estimation Found
1	Ehanolic Extract	3.135	18.48

DISCUSSION

Result of estimation of total content in aqueous and ethanolic extract of *Artocarpus heterophyllus* showed the significant amount of quercetin. The presence of quercetin in herbal plant is supposed to use as antidiabetic activity that may be the reason of high medicinal potential of plant. Optimization of the chromatographic conditions were carried out with various combinations of Methanol and acetonitrile and by observing the peak parameters, the run time of the method was set at 15 min, quercetin appeared on the typical chromatogram at 3.18 min, which indicates a good base line. When the same drug solution was injected 3 times, the retention time of the drug was same. Linearity range was observed in the concentration range of 5-25 µg/ml. The regression equation of quercetin concentration over its peak area ratio was found to be Y = 37.33X – 25.04 (r = 0.994) where Y is the peak area ratio and X is the concentration of quercetin. The result of Estimation of quercetin in Aqueous and Ethanolic Extract were found to be 7.92 % and 18.48% respectively.

Bio-flavonoids are now-a-days regarded as promising and significantly attractive natural substances to enrich the current therapy options against diabetes. Quercetin is a naturally occurring flavonoid which is a polar auxin transport inhibitor and also induces insulin secretion by activation of L-type calcium channels in the pancreatic β-cells. Hence the presence of Bio flavonoid as quercetin gives promising advice for antidiabetic potential of the plant.

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