Advance Research in Pharmaceuticals and Biologicals
(A peer reviewed International journal for Pharmaceutical and allied research)

Vol -2 (II)
APRIL-JUNE 2012

Received on 17/05/2012
Revised on 11/06/2012
Accepted on 21/06/2012

***Corresponding author**

Mr. Vinit Movaliya
K. B. Institute of Pharmaceutical Education & Research, Kadi Sarva Vishwavidyalaya, Sector 23, Gandhinagar, India 382023

HPTLC METHOD DEVELOPMENT AND ESTIMATION OF QUERCETIN IN THE ALCOHOLIC EXTRACT OF *AERVA JAVANICA* ROOT

***V. Movaliya and M. N. Zaveri**
K. B. Institute of Pharmaceutical Education & Research, Kadi Sarva Vishwavidyalaya, Sector 23, Gandhinagar, India 382023

ABSTRACT:

Aerva javanica belonging to family Amaranthaceae was selected for the present study. High performance thin layer liquid chromatography (HPTLC) was more popular in quality control and standardization of traditional herbs. The present study is to develop chromatography fingerprinting of *Aerva javanica*. A new, economic and rapid high performance thin-layer chromatographic method was developed for alcoholoic extract of root of *Aerva javanica*. Chromatographic separation was achieved on a pre-coated with silica gel 60 F254 plate using a mixture of toluene: acetone: methanol: formic acid (7: 2: 0.8: 0.2 v/v) as a mobile phase at a wavelength of 254 nm and 366 nm. Derivatization was done with anisaldehyde sulphuric acid as a detecting reagent. Developed HPTLC method can be used for determination of quercetin. The minimum detectable amount of quercetin was found to be 0.02196% w/w. Hence, the present method was successfully employed for the estimation of quercetin in the alcoholoic extract of root of *Aerva javanica*.

Keywords: *Aerva javanica,* HPTLC, quercetin, estimation.

INTRODUCTION

Ancient literature has prescribed various herbs for the cure of kidney disease. The term "Pasanabheda" has been cited in the literature to identify the group of plants which have been extensively used in the indigenous system of medicine to dissolve urinary calculi and stones like *Coleus aromaticus, Aerva lanata, Aerva javanica, Rotula aquatica, Kalanchoe pinnata, Ocimum basilicum.* The plant *Aerva javanica* belonging to the family *Amaranthaceae* is a tall and under shrub found plentiful in rainy season. This plant is used as Pasanabheda means one which breaks the kidney stone.[1] and therefore, in Gujarati it is commonly known as Patharphod. Roots and flowers are reported to possess medicinal properties against rheumatism and kidney troubles. [2] The selected plant is also reported as anthelmintic, diuretic, demulcent. [3] It is used for the treatment of headache. [4] The decoction of the plant is administered to remove swellings. [5-6], applied to acne like conditions of the face. [7] It contains kaempferol, sterol, triterpenes, bioflavonoids, ß-sitosterol, α-amyrin, palmitic acid, stearic acid, linoleic acid, myristic, oleic acid, palmitoleic acid, aervanone, alkaloids and ciliated iso-rhamnetin glycoside as phytoconstituents. [8-9] here, an attempt has been made to develop HPTLC method for quantification of bioflavonoid, quercetin in alcoholic extract of the root of *Aerva javanica.* Therefore, we describe in this paper a simple, sensitive HPTLC method for the determination of quercetin in alcoholic extract of the root of *Aerva javanica.* The developed method can be applied successfully for quality control and for other analytical purposes.

MATERIALS AND METHODS

Chemicals and Reagents

Pure sample of quercetin was purchased from Sigma-Aldrich Chemical Co Spectrochem. All different organic solvents and chemicals used for extraction under study were analytical grade (A.R. grade) obtained from S.D. Chem. Pvt. Ltd., Mumbai, India. Folin-Ciocalteu Reagent and anisaldehyde sulphuric acid was also obtained from S.D. Chem. Pvt. Ltd., Mumbai, India. Mobile phase was filtered using 0.45 µm cellulose acetate filters made by Millipore (USA) whereas, Whatmann filter papers No. 41 (purchased from the local market) was used in the preparation of sample solution.

Procurement of plant material and extraction procedure

Fresh roots of *A. javanica* were collected from Bhavnagar District, Gujarat, India. The authentification of the plant was

established and voucher specimen (202) deposited in the Department of Pharmacognosy and Phytochemistry, KBIPER, Gandhinagar, Gujarat, India. Identification of this plant was done by taxonomist Dr. A.S. Reddy, department of bioscience, S.P. University, V.V. Nagar, Gujarat, India. It was shade dried and reduced into coarse powder and stored in air tight container which was used for the present work. The powder of the root extracted with alcohol (95% ethanol). The dried alcoholic extract was then stored in airtight container until usage.

Phytochemical test

Phytochemical analysis of the extract was performed using standard methods. Specifically, the extract was analyzed for the presence of alkaloids, bioflavonoids, saponins, tannins, anthraquinone, and carbohydrates. Thin layer chromatography was employed to check for the presence of a quercetin. Therefore, tests for phytoconstituents were carried out and confirmed by quantitative analysis and also by thin-layer chromatography (TLC).

Estimation of total phenolic content

The total phenolic content of the extract was estimated accordingly to the method described by Singleton and Rossi. From the stock solution (1 mg/ml) of the extract, suitable quantity was taken into a 25 ml volumetric flask and mixed with 10 ml of water and 1.5 ml of Folin Ciocalteu's reagent. After 5 minutes 4 ml of 20 % w/v sodium carbonate solution was added and volume was made up to 25 ml with double distilled water. The Absorbance was recorded at 765 nm after 30 minutes. % of total phenolic was calculated from calibration curve of Gallic acid (50-250 µg) plotted by using the same procedure and total phenolics were expressed as % Gallic acid.

Estimation of total flavonoid content

The total flavonoids content of the extract was estimated. To the 10 ml volumetric flask added 4 ml of water and 1 ml of plant extract (1 mg/ml). After 5 minutes add 3 ml of 5 % sodium nitrite and 0.3 ml of 10 % aluminum chloride. After 6 minutes add 2 ml of 1 M Sodium hydroxide and make up the volume 10 ml with water. Measure the absorbance[10].

Development of HPTLC technique

To determine the phytochemical profile of *A. javanica* and quercetin by using modern chromatographic technique like High performance thin layer chromatography (HPTLC). *TLC* co-chromatography was performed on alcoholic extract and standard quercetin 3 µl of sample solution was spotted on the *TLC* plate along with a standard solution of quercetin. Stationary phase consisted of *TLC* Aluminum sheets pre-coated with silica gel 60 F254,

thickness 0.2mm, (20×20 cm) (E Merck, Germany), mobile phase consisted of toluene: acetone :methanol: formic acid (7: 2: 0.8: 0.2 v/v). Derivatization was done with anisaldehyde sulphuric acid. On the basis of *TLC* study, an *HPTLC* method was developed for the quantification of phytoconstituent quercetin in the alcoholic extract of *A. javanica* root and development of fingerprints of the same. As no method is available to date for the quantification of quercetin an attempt has been made to develop HPTLC method for quantification of this bioflavonoid in alcoholic extract of root of *A. javanica*[11].

Determination of phytoconstituents by HPTLC

An accurate and sensitive HPTLC method was developed for estimation of quercetin present in alcoholic extract. With the help of micro liter syringe, the standard or sample solutions of appropriate volume were applied on TLC plate under nitrogen stream using semiautomatic spotter (Camag Linomat V). The plates were allowed to dry in air and developed up to 80 mm at constant temperature using toluene : acetone : methanol : formic acid (7: 2: 0.8: 0.2 v/v) as mobile phase in Camag Twin trough chamber previously saturated with mobile phase for 25 minutes. The plate was removed; dried and photometric measurement was performed at 254 nm and 366 nm in absorbance/reflection mode with Camag TLC Scanner-3 with WINCATS software with distance run was 80 mm, slit dimensions : 5×0.45 mm, temperature : 25^0C ± 0.5, spotting parameter band width-6mm.

Sample preparation

10 mg/ml of test solution of alcoholic extract was prepared and 20.0 µg/ml of quercetin. Spot number 1-3 represent alcoholic extract at the concentration of 10µl while spot 4-7 and 8-12 are of standard quercetin at the concentration of 3 µl and 6 µl respectively [12].

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

In the preliminary phytochemical screening the root of *A. javanica* showed presence of alkaloids, bioflavonoids, phenolics, as shown in Table 1.

Total phenolic and flavonoid content

Total phenolic content of alcoholic extract of root of *Aerva javanica* was found to be 0.52 mg/ml. Total flavanoid content of alcoholic extract of root of *Aerva javanica* was found to be 0.12 mg/ml.

Estimation of quercetin in root of *Aerva javanica* by HPTLC method

The plate was developed at 254 nm as shown in figure 1 and 366 nm as shown in figure 2. The % content of quercetin in the root of *Aerva javanica* was found to be

0.02196 % w/w. The R_f value of standard quercetin was found to be 0.45 in all tracks. Chromatogram of standard quercetin was shown in Figure 4. Figure 5 depicts the chromatogram for the test sample and Figure 3 depicts the spectral comparison of test sample track along with standard tracks.

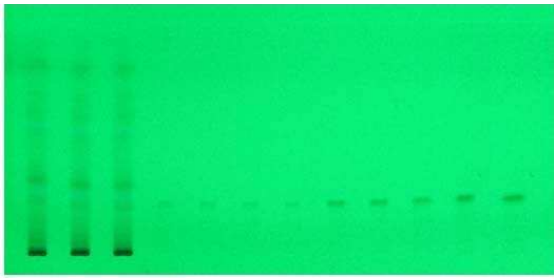

Fig.1. TLC profile of alcoholic extract of *Areva javanica* roots at 254 nm

Fig.2. TLC profile of alcoholic extract of *Areva javanica* roots at 366 nm

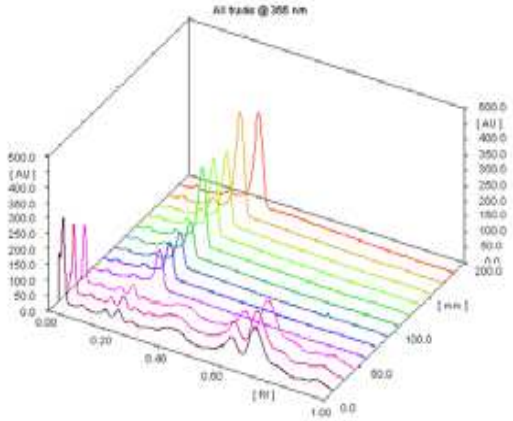

Figure 3. Spectral comparison of Track 1-3 (test sample) with track 4-12 (standard quercetin**)**

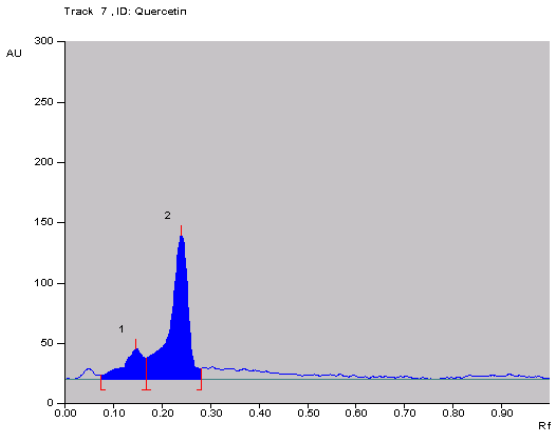

Fig.4. HPTLC chromatogram of standard quercetin

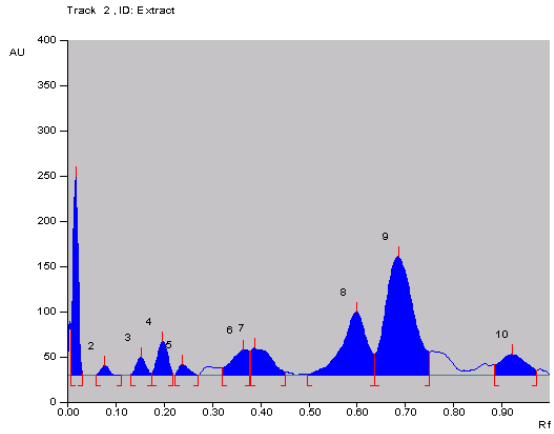

Fig.5. HPTLC Chromatogram of test sample

Recent years have seen an exponential raise in research for antioxidant properties of medicinal plants. Polyphenols like quercetin attribute to the antioxidant property and has potential therapeutic uses in the prevention of Cardio vascular diseases (CVD), cancer, nephrotoxicity, cataract etc. High performance thin layer chromatography (HPTLC) is an invaluable quality assessment tool for the evaluation and quantification of botanical materials. It allows for the analysis of a broad number

of compounds both efficiently and cost effectively.

CONCLUSION

It can be concluded that the approach given for standardization of *Aerva javanica* by developing an analytical method pure reference standard of reported active ingredient may have be procured. Using quercetin as a reference standard, it will be possible to quantitatively determine the active ingredient in the *Aerva javanica*. The present investigation is suggested as future line up for research in this study. A simple and accurate HPTLC method has been developed for the determination of quercetin in alcoholic extract of root of *Aerva javanica*.

Acknowledgement

The authors acknowledge the grant received from All India Council of Technical Education (AICTE) for this project under Research Promotion Scheme.

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