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Research Article

Comparative HPTLC Analysis of Leaves of *Allium cepa* L., *Ficus carica* L. and *Ziziphus mauritiana* L. with Standard Quercetin

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ABSTRACT
Flavonols aglycones are well established in plant extracts with omnipresence of common flavonols like kaempferol, quercetin and myricetin. Quercetin has been reported for various pharmacological activities with major ones in the field of inflammation, cancer, arthritis, diabetes and its complications. Leaves of plants selected for this study viz. *Allium cepa* L., *Ficus carica* L. and *Ziziphus mauritiana* L. reported to have antioxidant, anticancer, hypoglycemic activities. It is hypothetised thisactivities are due to flavonoids present in these plants. Hence the ethylacetate fraction of methanolic extract of these leaves was obtained as it provides flavanoid rich fraction and the samples were prepared and HPTLC analysis was performed against standard quercetin and their quantitative analysis was performed, the solvent system toluene: ethyl acetate: formic acid (9: 1: 0.5) was selected. It was observed that these plants shows presence of quercetin where *A. cepa* has highest concentration with *F. carica* and *Z. mauritiana* having quercetin in declining order. The calibration curve was linear in the range of 0.1 to 0.7 µg for quercetin. Further a correlation coefficient of 0.999 indicates good linearity between concentration and area. Thus, it was proved that the plants used in the study has presence of quercetin which is possibly the reason having the pharmacological uses.

Keywords: Flavonoids, *Allium cepa* L., *Ficus carica* L., *Ziziphus mauritiana* L., Quercetin, HPTLC.

INTRODUCTION

Flavonoids are the most common and widely distributed group of plant phenolic compounds, which occur virtually in all plant parts, particularly the photosynthesising plant cells[1]. A large number of studies have emphasized the potential health-promoting and disease preventing effects of fruits and vegetables in the diet.

Fruits and vegetables contain a multitude of flavonoids and related phenolic compounds that also act as natural antioxidants[2]. They have enormous biological and pharmacological activities conferring many health benefits to the human. They are the group of compounds which received considerable attention from the researchers as depicted from the scientific literature[3]. *Allium cepa* L., *Ficus carica* L. and *Ziziphus mauritiana* L. are important source of phytoconstituents and food flavor.

They possess high level of antioxidant activity attributal to flavonoids quercetin and pigments such as anthocynins are found in *A. cepa*[4]. Herbs contain different biologically active phytoconstituents which exhibit therapeutic effects. Quercetin constitutes phenolic compounds which are structurally derived from the parent substance flavones. Quercetin is reported for its antiallergic, antitumor, immunomodulatory activities.

Phytochemical evaluation is one of the tools for the quality assessment which includes preliminary phytochemical screening, chemo profiling and marker compound analysis using modern analytical techniques. High performance thin layer chromatography method is suitable method for estimation of chemical constituents present in plant materials[5].

A comparative HPTLC analysis of *A. cepa*, *F. carica*, and *Z. mauritiana* was performed to determine the presence of quercetin in ethyl acetate fraction of methanolic extract of leaves of these three plants. In accordance with the aim of study first method was developed and solvent system was determined using TLC.

Traditional methods of solvents for flavonoids analysis were compared and TLC was obtained followed by quantification using HPTLC.

Experimental

Reagents and Materials

The leaves of *A. cepa*, *F. carica* and *Z. mauritiana* were collected from local area of Aurangabad region (MS, India). Shade dried and coarsely powdered. Standard Quercetin was purchased from M/s Deepa chemicals. Aluminium plates precoated with silica gel 60F254 of 0.2 mm thickness (E.merck, Darmstadt, Germany) were used without pretreatment. All chemicals and solvents used were of analytical grade. Derivatizing reagent used was Anisaldehyde sulphuric acid.

Preparation of extract

The powdered material was extracted in Petroleum ether (60 – 80°C) to remove lipids and chlorophyll, filtered and filtrate was discarded, residue extracted with methanol in

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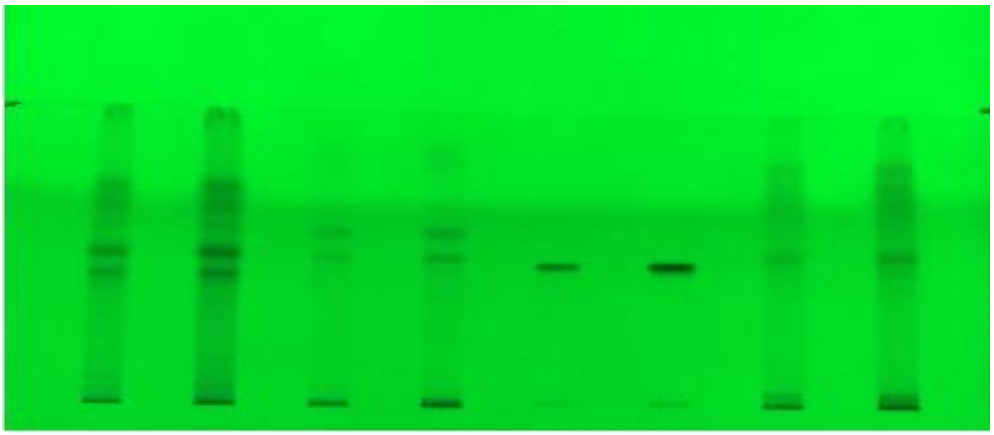

Figure 1: TLC fingerprint profile of ethyl acetate fraction of methanolic extract of leaves of Quercetin, *A. cepa*, *F. carica* and *Z. mauritiana* under 254 nm.

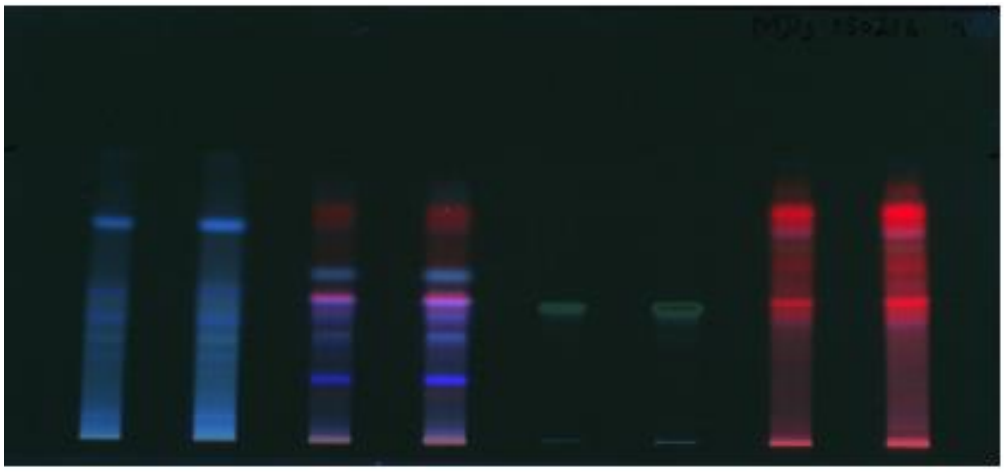

Figure 2: TLC fingerprint profile of ethyl acetate fraction of methanolic extract of leaves of Quercetin, *A. cepa*, *F. carica* and *Z. mauritiana* under 366 nm.

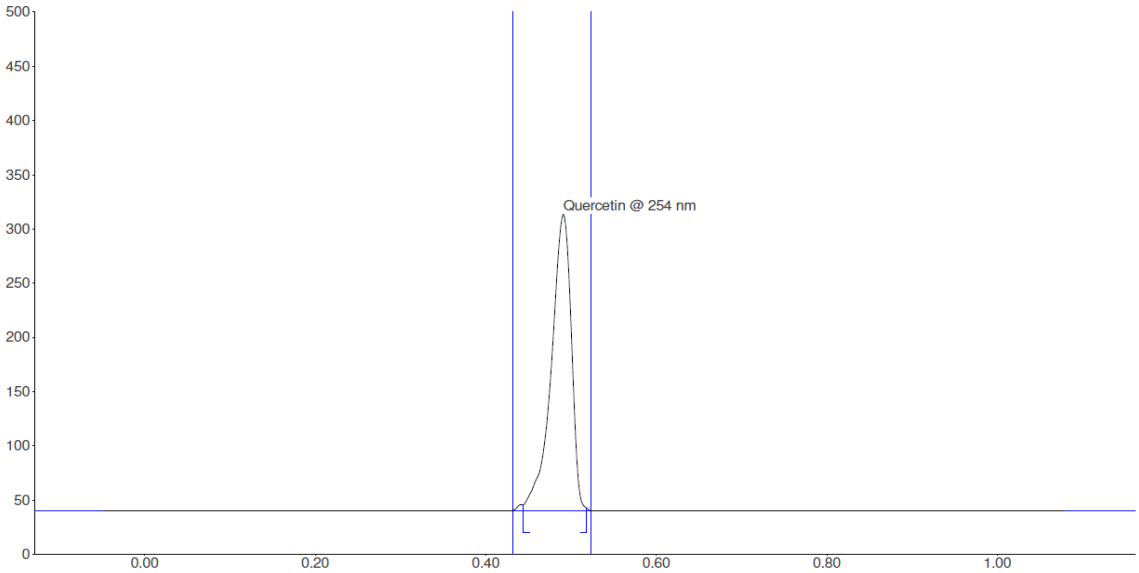

Figure 3: HPTLC chromatogram of Quercetin.

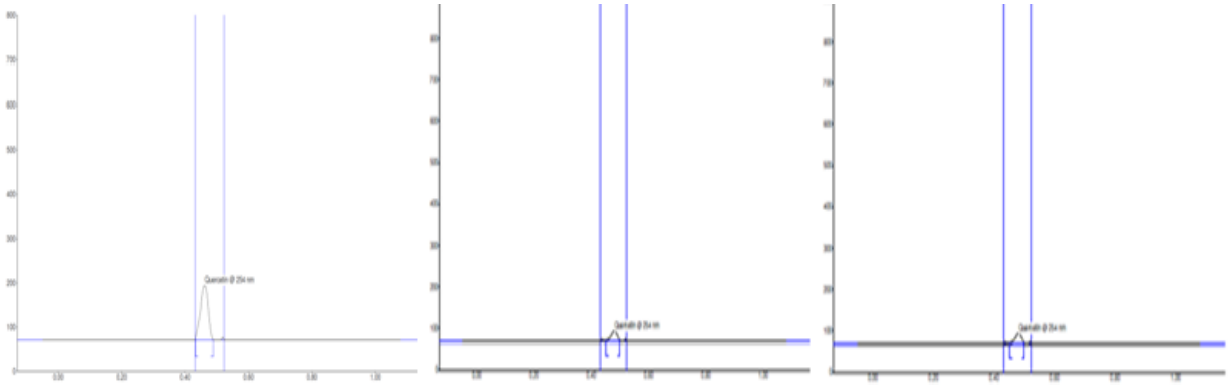

Figure 4: HPTLC chromatogram of *A. cepa*, *F. carica* and Z. mauritiana.

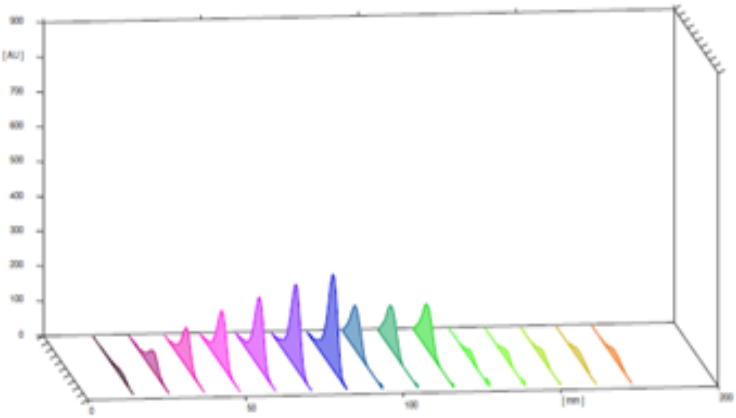

Figure 5: UV spectra of Calibration curve for *A. cepa* with Quercetin as marker.

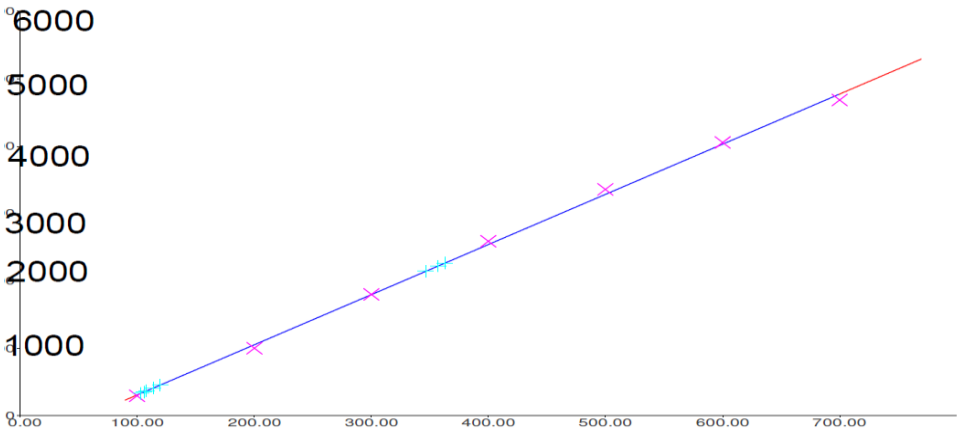

Figure 6: Calibration plot obtained by chromatography of marker compound Quercetin. Regression via area, Regression mode- Linear.

soxhlet apparatus for 72hr. After extraction the solvent was filtered and evaporated in a vacuum, whatever residue may be obtain was dissolved in distilled water and extracted with Ethyl Acetate using separating funnel. The filtrate obtained was evaporated on water bath to obtain dry mass[6].

Preparation of standard and stock solution

Standard solution of quercetin

A stock solution of quercetin was prepared by dissolving 10 mg of accurately weighed quercetin in methanol and making up the volume to 10 ml with methanol to get final concentration of 1000 µg/ml. It was then sonicated for 10 minutes.

Preparation of sample solution

Sample solution was prepared by dissolving 100 mg of fraction in ethyl acetate and making up the volume to 10 ml to get the concentration of 10 mg/ml. It was then sonicated for 10 minutes.

Development of TLC technique

Table 1: Details of TLC and HPTLC Chromatogram of Quercetin.

Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
0.45	5.8	0.49	273.9	100.00	0.52	3.1	4673.9	100.00	Quercetin

Table 2: Details of TLC and HPTLC Chromatogram of ethyl acetate fraction of methanolic extract of leaves of *A.cepa.*

Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
0.44	23.7	0.46	127.2	100.00	0.50	0.7	2253.4	100.00	Quercetin

Table 3: Details of TLC and HPTLC Chromatogram of ethyl acetate fraction of methanolic extract of leaves of *F. carica.*

Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
0.45	0.2	0.48	24.5	100.00	0.5	1.1	357.97	100.00	Quercetin

Table 4: Details of TLC and HPTLC Chromatogram of ethyl acetate fraction of methanolic extract of leaves of *Z. mauritiana.*

Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
0.45	2.8	0.48	22.7	100.00	0.51	0.1	444.30	100.00	Quercetin

Thin Layer Chromatography was performed using standard methods. Small quantity of sample (2 mg/ml) was dissolved in Ethyl acetate. Quercetin (1 mg) standard was dissolved in methanol. Different mobile phases with varying concentrations were employed in the screening programme and selected the one in which separation of flavonoid was clear: toluene: ethyl acetate: formic acid (5:4:1)[7,8]. All plates were visualized directly after drying and with the help of UV at 254 nm and 366 nm in UV TLC visualizer (CMAG). Later it was derivatized using Anisaldehyde-sulphuric acid reagent [7]. The Rf value of the different spots that were observed was calculated.

Development of HPTLC technique

The samples were spotted in the form of bands with CAMAG microlitre syringe on a precoated silica gel GF254 plates (20 cm×10 cm with 0.2 mm thickness, E.Merck) using CAMAG linomat 5. Automatic sample spotter of band width 8 mm. The plates were developed in a solvent system in CAMAG glass twin trough chamber previously saturated with the solvent for 30 min. The distance was 7 cm subsequent to the scanning, TLC plates were air dried and scanning was performed on a CAMAG TLC scanner in absorbance at 254 nm operated by Wincats software 4.03 version.

Calibration curve for Quercetin

The standard solution of quercetin (1000 µg/ml) in different volumes were located on the different TLC plate for preparation of calibration curve (1-2 µl of quercetin) checked for reproducibility. The calibration curve was prepared by plotting the concentration of standard *vs.* average peak area after scanning at 254 nm.

Quantification of Quercetin

Stationary phase: Silica gel GF254 plates
Mobile phase: Toluene: Ethyl acetate: Formic acid (5:4:1)
Standard: Quercetin (1000 µg/ml)
Sample: Ethyl acetate fraction of Methanolic extract of *A. cepa, F. carica* and *Z. mauritiana* leaves (10 mg/ml)
Migration distance: 70 mm
Scanning wavelength: 254 nm
Mode of scanning: Absorption (deuterium).

RESULT AND DISCUSSION

Optimization of sample preparation

Ethyl acetate fraction of methanolic extract leaves of *A.cepa, F. carica* and *Z. mauritiana* were subjected to preliminary phytochemical investigation that revealed the presence of flavonoids. Hence the extract was selected for further HPTLC analysis[9].

TLC and HPTLC fingerprint and chromatograph

TLC and HPTLC analysis so far has helped in variety of research work to get confirm results about active phytoconstituents present in medicinal traditaional herbs. In this study we have targeted to achieve presence of flavonoids and quantify the amount of active metabolite present by considering Quercetin as the marker molecule. Different solvent systems were tried to achieve a good resolution [7, 10, 11]. Finally, the solvent system toluene: ethyl acetate: formic acid (9: 1: 0.5) was selected. Fingerprint of TLC was studied at both 254nm and 366nm (Fig I and II). The band at Rf 0.44 was observed for Quercetin and that for the herbal fraction was 0.45 after spray with anisaldehyde-sulphuric acid reagent. The HPTLC chromatogram of standard quercetin, *A. cepa, F. carica* and *Z. mauritiana* are shown in fig III and IV respectively. The calibration curve (fig VI) was linear in the range of 0.1 to 0.7 µg for quercetin. Further a correlation coefficient of 0.999 indicates good linearity between concentration and area. The method allows reliable quantification of quercetin and provides good resolution and separation of quercetin in the three plants. The peak purity of quercetin was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot (fig III, fig IV, fig V). In system suitability, the relative standard deviations of the quercetin peak areas and retention factors were 100%and 2.47, respectively. Peak area comparison and concentrations of standard and sample indicates Ethyl

acetate fraction of methanolic extract of *A. cepa* leaves has highest 4.822% Quercetin. The results obtained by adoption of methodologies outlined, clearly support that these may help in quali-quantitative analyses of quercetin from any unknown plant source. Within the range of the designed standardized curve for Quercetin (Table I, II, III and IV). Methods that are so far in use elute several other compounds along with the flavonoids while the currently investigated technique is found to be optimum for identifying and separating flavonoids. The linear relationship between the peak areas and the concentrations prove the dependability of the present method. Applying the result that Ethyl acetate fraction of methanolic extract of these plants' leaves has Quercetin in them proves that these fractions are valuable in the fields of medicine and therapeutics. However, as will be required, these fractions needs to be further proceed towards pre-clinical and clinical studies for its benefits in treatment of many oxidative stress diseases.

CONCLUSION

A rapid and simple HPTLC method for quantitative estimation of quercetin present in the ethyl acetate fraction of methanolic extract of leaves of *A. cepa*, *F. carica* and *Z. mauritiana* has been developed. The method used in this work resulted in good peak shape and enabled good resolution of quercetin from other constituents of the plant material. Because recovery was 100%, there was no interference with the quercetin peak from other constituents present in the plant. As it was found that Ethyl acetate fraction of methanolic extract of these plants contains Quercetin, these fractions justifies its use for many medicinal studies.

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