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Research Article

SIMULTANEOUS DETERMINATION OF PHENOLIC COMPOUNDS IN *BRASSICA OLERACEA L.VAR CAPITATA.* BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

MOHAMMED FAZIL AHMED[1,*], A. SRINIVASA RAO[2]

[1]Nizam Institute of Pharmacy & Research Center, Deshmukhi, Pochampally (M), Near Ramoji Film City, Nalgonda, A. P. 508284, [2]Bhaskar Pharmacy College, Yeknapally, Moinabad(Mandal), R.R(Dist), Hyderabad-500075, India. Email: mohdfazil_pharma@yahoo.co.in

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ABSTRACT

Objective: The present study was carried out to estimate the phenolics compound, Rutin, Quercetin and Kaempferol in *Brassica oleracea L.var capitata.* by high-performance liquid chromatography (HPLC).

Methods: For the estimation of Rutin, Quercetin and Kaempferol by HPLC method, the mobile phase used are, Acetonitrile and Phosphate buffer (pH=5.8) in ratio of 55: 45.Quantification of Rutin, Quercetin and Kaempferol was carried by Athena C18 column and absorbance was measured at 254 nm with flow rate of 1 ml/min.

Results: In HPLC analysis the retention time(Rt) of standards, Rutin, Quercetin and Kaempferol were found to be 2.357, 6.093 and 9.373 respectively, while the Retention times of Rutin and Kaempferol in *Brassica oleracea L.var capitata* are 2.387, 6.060 and 9.113 which are found to be matching with standards retention time values respectively. Conclusion: Thus this HPLC method was found to be simple and rapid for quantitative determination of Rutin, Quercetin and Kaempferol in *Brassica oleracea L.var capitata.*

Keywords: *Brassica oleracea L.var capitata*, HPLC, Phenolic compounds, Rutin, Quercetin and Kaempferol.

INTRODUCTION

In the last decades, special attention was paid towards the edible plants, especially those plants are rich in secondary metabolites (phytochemicals) and nowadays, there is an increasing interest in the antioxidant activity of such phytochemicals present in diet. Cruciferous vegetables act as a good source of natural antioxidants due to the high levels of carotenoids, tocopherols and ascorbic acid. In addition to carotenoids, tocopherols, and ascorbic acid, most of the antioxidative effect related to plant food intake is mainly due to the presence of phenolic compounds of fruits and vegetables. Cruciferous vegetables such as cabbage are among the most important dietary vegetables consumed in Europe owing to their availability in local markets, cheapness and consumer preference.[1] Brassica foods are very nutritive, providing nutrients and health-promoting phytochemicals such as vitamins, carotenoids, fiber, soluble sugars, minerals, glucosinolates and phenolic compounds.[2,3] Among phytochemicals possessing antioxidant capacity, phenolic compounds are one of the most important groups.[3] "Phenolic compounds" is a generic term that refers to a large number of compounds (more than 8,000) widely dispersed throughout the plant kingdom and characterized by having at least one aromatic ring with one or more hydroxyl groups attached. Phenolics are produced in plants as secondary metabolites via the shikimic acid pathway. Phenolics range from simple, low molecular-weight, single aromatic-ringed compounds to large and complex tannins and derived polyphenols.[4,5] Flavonoids are polyphenolic compounds comprising fifteen carbons with two aromatic rings connected by a three-carbon bridge. Quercetin, kaempferol and isorhamnetin, the main flavonols in Brassica crops, are most commonly found as O-glycosides.

The current knowledge indicates that the aging processes may result from oxidative stress leading to a variety of alterations within the human organism caused by reactive oxygen species (ROS). Oxidative stress occurs when the generation of ROS in a system exceeds the system's ability to neutralize and eliminate them. If not controlled properly, the excess of ROS can lead to damage of cellular lipids, proteins or DNA, impairing their normal function. There are increasing evidences that increased consumption of fruits and vegetables and intake of certain non-nutrients that are present in foods reduce the risk of various pathological events such as cancer [6,7] and cardio- and cerebro-vascular diseases.[8]Plant foods with apparent anticancer [9,10] and cardioprotective properties include varieties of Brassica oleracea [11], which have exhibited genotoxic properties[12]and high antioxidant and antimicrobial activities [13,14] in earlier studies. The vegetables are rich sources of many nutrients and antioxidant vitamins. *Brassica oleracea var. capitata (Cabbage)* (Family Brassicaceae) is an excellent source of vitamin C. It also contains significant amounts of glutamine, an amino acid that has anti-inflammatory properties. Cabbage can also be included in dieting programs, as it is a low calorie food.The present study was directed to investigate the phenolics compound, Rutin and Kaempferol in *Brassica oleracea L.var capitata.* by high-performance liquid chromatography (HPLC) method.

MATERIALS AND METHOD

Reagents and Materials

All chemicals and solvents used were of analytical grade. The standard Rutin and Quercetin were purchased from Yucca Enterprises,MumbI (purity >97%).The standard Kaempferol MP Biomedicals, Mumb(purity >97%). Phosphate buffer (pH=5.8) and solvent Acetonitrile used as mobile phase were obtained from S.D.Fine Chem Limited, Mumbai. The column type was, Athena C18 250X 4.6 (CNW Technology).

Plant material

The basic plant material of *Brassica oleracea var. capitata (Cabbage)* was obtained from local market, Hyderabad. The plant was identified and authenticated by Department of Botany and Research office (Botanist) Anwar-ul-loom college of Pharmacy, Hyderabad.

Extraction of plant material for HPTLC analysis

Brassica oleracea var. capitata (Cabbage) were dried under shade and powdered in a mechanical grinder. *Brassica oleracea var. capitata (Cabbage)* powder weight about (250 g) were individually packed in the thimble of soxhlet apparatus and extracted with ethanol at 55°C for 18 h. The extract was concentrated to get dry residue and stored in the dessicator and it was used for subsequent experiments. Preliminary photochemical screening revealed the presence of Polyphenols, flavanoids and glycosides.

Preparation of standard and sample solutions

Phosphate buffer (pH=5.8) and solvent Acetonitrile used as mobile phase.10 mg of Standard Rutin and Quercetin were dissolved in 25ml of mobile phase, while 15mg of kaempferol were dissolved in 25ml of mobile phase and 10 mg of Sample solution of extract of

Catharanthus roseus were dissolved in 25ml of mobile phase as above as standard preparation.

Chromatographic conditions

Flow rate : 1 ml/min

Detection : 254 nm

Injection quantity: 0.02ml

Column used : Athena C18 250X 4.6

Column temperature: 35°C

Mobile phase ration : 55: 45 % v/v

Mobile phase: Phosphate buffer (pH=5.8) and Acetonitrile

The operating temperature was maintained at room temperature. Identification of the compounds was achieved by comparison with retention times of standards with the samples.

Assay formula

$$\frac{\text{Sample Area}}{\text{Standard Area}} \times \frac{\text{Standard weight}}{\text{Standard dilution}} \times \frac{\text{Sample dilution}}{\text{Sample weight}} \times \text{Standard Purity}$$

RESULTS AND DISCUSSION

Quantification of Rutin and Kaempferol in *Brassica oleracea var. capitata* (B1)

The retention time (Rt) of standards Rutin, Quercetin and Kaempferol were found to be 2.357, 6.093 and 9.373 with 100% area (Fig 1-3 and Table 1). The retention time (Rt) of Rutin and Kaempferol in ***Brassica oleracea var. capitata*** **(B1)** extract, was found to be 2.387, 6.060 and 9.113 respectively (Fig 4 and Table 2), which are matching with standards Rt values respectively. The amount of rutin, quercetin and Kaempferol in ***Brassica oleracea var. capitata*** **(B1)** was found to be 2.83 %,0.056 % and 0.019 % w/v respectively. The mobile phase include Acetonitrile and Phosphate buffer (pH=5.8) were tested and the results showed the good resolution and good peaks shape.

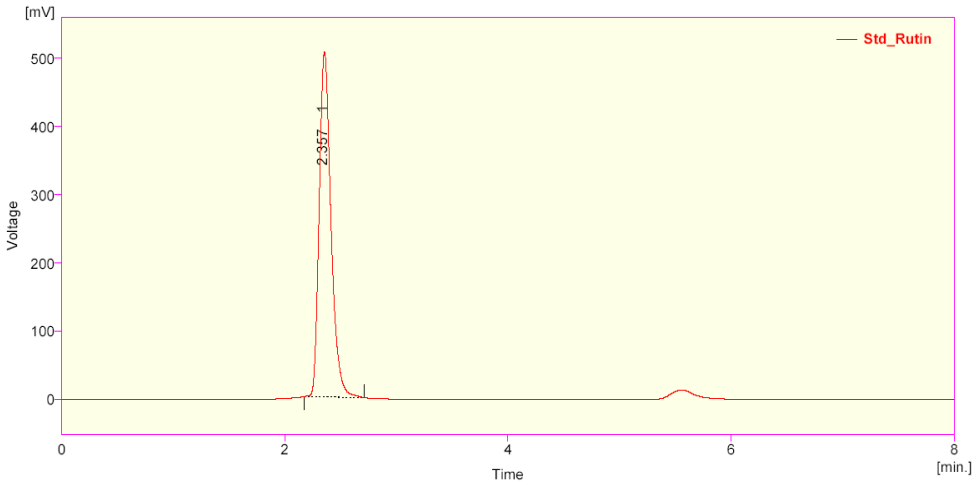

Fig. 1: HPLC Chromatogram of standard Rutin

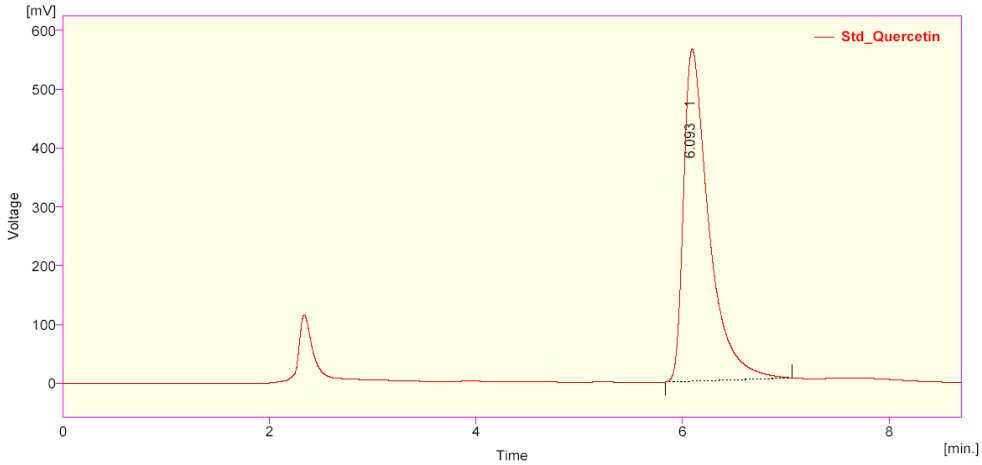

Fig. 2: HPLC Chromatogram of standard Quercetin

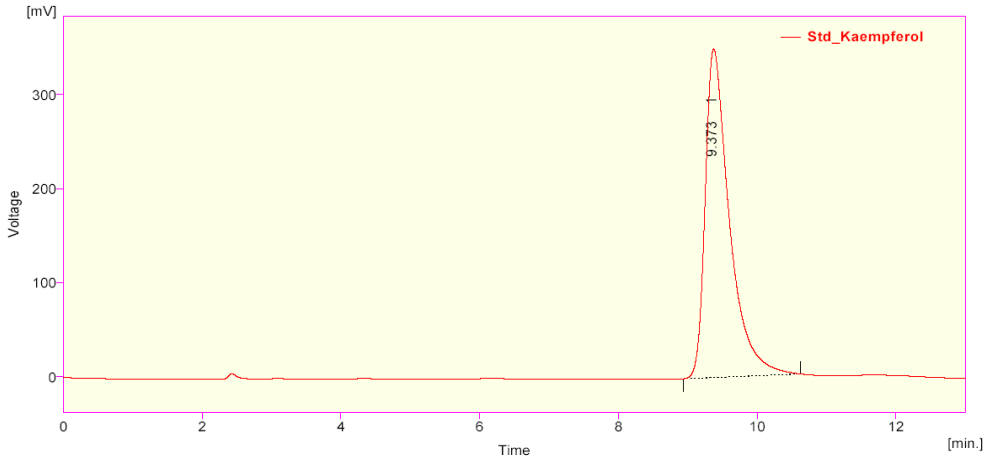

Fig. 3: HPLC Chromatogram of standard Kaempferol

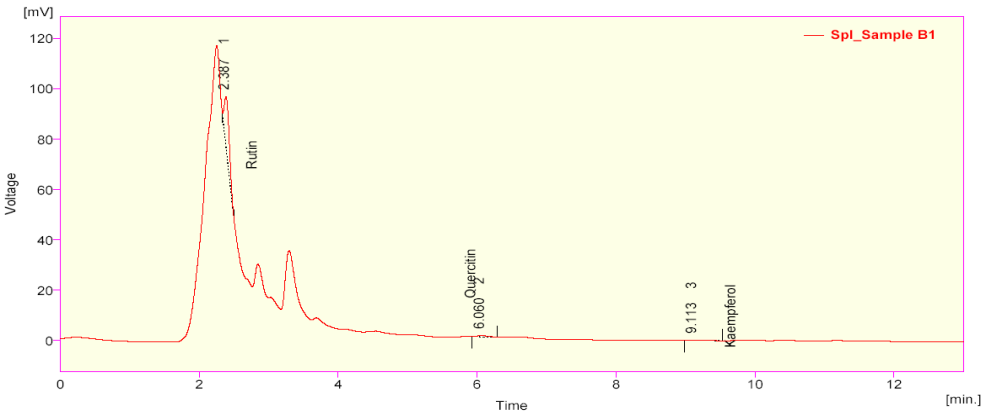

Fig. 4: HPLC Chromatogram of extract *Brassica oleracea var. capitata* (B1)

Table 1: Retention time, Height and % Area of Standards Rutin, Quercetin and Kaempferol

Standards	Retention time(min)	Area(mV.s)	Height(mV)	Area (%)
Rutin	2.357	3700.301	505.494	100
Quercetin	6.093	9594.659	564.435	100
Kaempferol	9.373	8468.410	348.791	100

Table 2: Retention time, Height and % Area of Rutin, Quercetin and Kaempferol in extract of *Brassica oleracea var. capitata* (B1):

Standards	Retention time(min)	Area(mV.s)	Height(mV)	Area (%)
Rutin,	2.387	108.820	20.147	94.36
Quercetin	6..060	5.356	0.433	4.64
Kaempferol	9.113	1.155	0.060	1.00
Total		115.330	20.640	100

CONCLUSION

The developed in-house HPLC analytical method was found to be excellent technique for simultaneous determination of Rutin, Quercetin and Kaempferol in ethanolic leaves extract of ***Brassica oleracea var. capitata***. The cost and Running time per analysis are found to be low relatively in comparison with other methods. Hence this method can be applied for the Quantitative analysis of rutin, Quercetin and Kaempferol. Furthermore, the method can be used as quality control for phenolic compounds (rutin, Quercetin and Kaempferol) and was found to be rapid, efficient and simple.

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