Research Paper

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Estimation of rutin in ethanolic extract of *Brassica oleracea L.* var *capitata*. leaves by HPTLC method

Pharma

KEYWORDS : *Brassica oleracea* L. var capitata. Linn , HPTLC, Flavonoidal compounds and Rutin.

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ABSTRACT

The aim of the study is to estimation biologically active flavonoidal compound, rutin, in ethanolic leaves extract of Brassica oleracea L. var capitata. Linn leaves by using high-performance thin-layer chromatography (HPTLC). Pre coated silica gel 60 F254 used as stationary phase and Toluene: Ethyl Acetate: methanol in ratio of 5: 3: 2 are used as mobile phase. Densitometric determination and quantification of this compound was carried out at 254 nm.The standard Rf values of rutin is found to be 0.17 . The total peak areas of the standards rutin were compared and the corresponding peak areas of extract were estimated to be 99.36 respectively. This HPTLC method was found to be simple and convenient for rapid screening of active compounds and quantification of the investigated flavonoid in Brassica oleracea L. var capitata. Linn.

INTRODUCTION

Herbal medicines have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects.Flavonoids are a group of polyphenolic compounds, which are widely distributed through out the plant kingdom. To date about 300 varieties of flavonoids are known.[1] Flavonoids belong to a group of polyphenolic compounds, which are classified as flavonols, flavonones, flavones, flavanols, flavan-3-ols and isoflavones according to the positions of the substitutes present on the parent molecule.Rutin, 5,7,3', 4',tetrahydroxy flavonol -3- rhamnoglucoside and quercetin 5,7,3', 4',- tetrahydroxy flavonol exhibit anti-inflammatory, antihepatotoxic[2], antiulcer[3], antiallergic and antiviral actions and some of themprovides protection against cardiovascular mortality.[4,5] Both possess antioxidant activity-and antidiabetic reduce low density lipoproteins [LDL] oxidation[6]. Quercetin in combination with other flavonoids, inhibits a number of enzymes like bradykinin[7], tyrosine kinase[8], and 5'- nucleotidase activity.[9] High performance thin layer chromatography [HPLC] method is the suitable method for estimation of chemical constituents present in plant materials.

Cabbage (Brassica oleraceavar. capitata) is economically one of the most important varieties of Brassicagenus. Cabbagecontains the high amounts of vitamins C, K, A and folic acid, fiber, flavonoids, proteins and minerals. 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 [10, 11], and cardio- and cerebro-vascular diseases.[12] The vegetables are rich sources of many nutrients and antioxidant vitamins.Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species (ROS), which are the harmful by products generated during normal cell aerobic respiration.[13] The importance of antioxidants in health and disease is now recognized by every branch of medicine and biological science.[14]

In *Brassica oleracea L. var capitata*. Linn rutin and quercetin are important active constituents and is estimated by HPLC method. Phytochemical evaluation is one of the tool for the quality assessment, which includes preliminary phytochemical screening, chemoprofiling and marker compound analysis using modern analytical techniques. In the last two decades high performance thin layer chromatography [HPTLC] method has emerged as an important toll for the qualitative and quantitative phytochemical analysis of herbal drugs and formulations. This includes TLC fingerprint profiles and estimation of chemical markers and biomarkers. [15] The major advantage of HPTLC is that several samples can be analysed simultaneously using a small quantity of mobile phase. Rutin is the important active constituents of *Brassica oleracea L. var capitata* were estimated by HPTLC method.

MATERIALS AND METHODS

Reagents and Materials:

All chemicals and solvents used were of analytical grade and obtained from Desaga Sarstedt Gruppe (Germany). The standerd Rutin were purchased from Lobo Chemie, Mumbai, India (purity >97%). Stock solutions (1 mg/ml) of the standards were prepared daily in methanol immediately before use. TLC aluminum plates pre-coated with silica gel 60 F_{254} (100x 100 mm, 0.2 mm thick) used were obtained from E. Merck Ltd (Mumbai, India).

Plant material:

The basic plant material of *Brassica oleracea L. var capitata* leaves was obtain from local market, Hyderabad. The plant ware 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:

The leaves of *Brassica oleracea L. var capitata* were dried under shade and powdered in a mechanical grinder. The leaves powders of *Brassica oleracea L. var capitata,* 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

Standard stock solutions of rutin were prepared by dissolving 10mg of rutin in 10ml of methanol. From this 10 µl each of these solutions was applied using sample applicator. 100mg of ethanolic extract of *Brassica oleracea L. var capitata* was dissolved in 10ml of methanol and filtered. The filtrate (10mg/ml) was used for the HPTLC chemoprofiling.

Chromatographic conditions:

Chromatography was performed on pre-activated (at 110°C) silica gel 60 F_{254} HPTLC plates. Sample(10µl) and standard (10µl each) compounds were applied to the layer as 10 mm wide bands, positioned 10 mm from the bottom of the plate, using an automated TLC applicator Desaga Sarstedt Gruppe (Germany),with nitrogen flow providing delivery from the syring

Detection and quantification of compounds:

TLC was performed with Toluene: Ethyl Acetate : methanol (5: 3: 2, v/v) as mobile phase. Chromatograms were developed at room temperature (24 ± 1°C) in glass twin-trough chambers (20 mm × 20 mm, with metal lids) previously saturated with mobile phase vapor for 30 min. The development distance was 86 mm. Ascending mode was used for development of thin layer chromatography.

Following the development, the TLC plate was dried in a current of air with the help of an air dryer at 110°C for 10min, and immediately scanned at λ = 254 nm and the densitograms were obtained with Desaga Sarstedt Gruppe (Germany),having proquant 1.6 version in absorption reflection scan mode.

The presence or absence of the investigated compounds was determined according to their Rf values with the corresponding pot of Standards.Calculations for percentage were done considering standard and sample Rf , AUC and dilution factor. For validation of the method, calibration curve was obtained by plotting peak area Vs concentration of rutin. Spectra of samples and standard rutin were matched. [16]

RESULTS AND DISCUSSION

The use of HPTLC has expanded considerably due to the development of forced flow (FF) and gradient TLC methods, improved stationary and mobile phase selection, as well as new methods of quantitation methods.[17] Recent reviews show that the HPTLC techniques can be used to solve many qualitative and quantitative analytical problems in a wide range of fields, including medicine, pharmaceuticals, chemistry, biochemistry, food analysis, toxicology and environmental analysis.[18] The selected mobile phase, Toluene: Ethyl Acetate : methanol (5: 3: 2, v/v) showed good resolution. Well defined spot were obtained after chamber was saturated for 20 min at room temperature. The identity of rutin were confirmed by comparing chromatogram of standard rutin with that of extract and by comparing retention factor of reference with standard.

The ethanolic extract of *Brassica oleracea L. var capitata* was able to resolve 2 compounds in the developing solvent system. The identity of the band of rutin in the ethanolic extract of *Brassica oleracea L. var capitata* was confirmed by comparing the UV-Vis absorption spectra with those of standards. (Fig. 1-3).

The *Rf* value and peak area of standards rutin were found to be 0.17 and 3187.87 (Fig.2 and Table.1). The ethonolic extract of *Brassica oleracea L. var capitata* showed two peaks, the second peak Rf value [0.18] was coinciding with standard rutin Rf value and its peak area was 99.36 (Table.2). The amount of Rutin was found to be0.311µg in the extract.

Figure 1: TLC chromatogram of Brassica oleracea L. var capitata (B1) at UV 254nm in Visible range

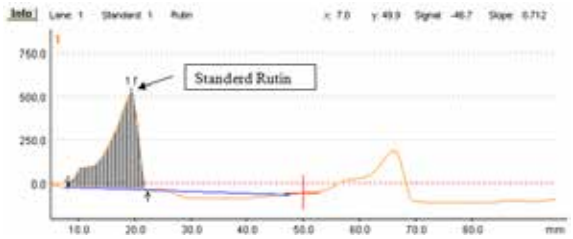

Figure 2: HPTLC chromatogram of rutin, densitogram showing the separation of peaks in rutin

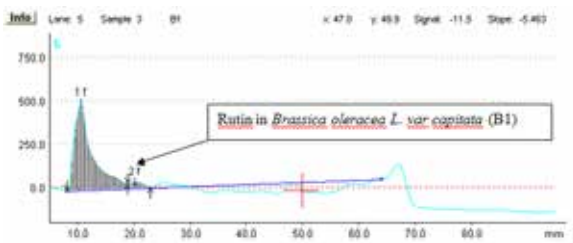

Figure 3: HPTLC chromatogram of *Brassica oleracea L. var capitata* (B1) leaves extract.

Table 1: Peak list & densitogram of Standerd Rutin at UV 254nm with Rf values of the spots.

Peak no	Y-Pos	Area	Area (%)	Height	Rf values
1	19.4	3187.87	100.0	556.53	0.17

Table 2: Peak list & densitogram of *Brassica oleracea L. var capitata* (B1)at UV 254nm with Rf values of the spots.

Peak no	Y-Pos	Area	Area (%)	Height	Rf values
1	10.6	1875.67	95.0	503.64	0.03
2	20.2	99.36	5.0	36.51	0.18

CONCLUSION

In conclusion densitometric HPTLC method can be used for the quantitative determination of rutin in *Brassica oleracea L. var capitata* leaves; mainly because of its simplicity, accuracy, and selectivity. HPTLC method is also the most suitable method for estimation of chemical constituents present in plant materials. The results of the present study also support that the presence of rutin in ethonolic leaves extract of *Brassica oleracea L. var capitata* could be a potential source of natural anti-oxidantand.

REFERENCE

1. Anonymous, Indian Pharmacopoeia, Vol II. Controllerof Publications, New Delhi, 1996, 53. | 2. Cesarone M R, Laurora G, Ricci A, Belcaco G and Pomante P, J Vas Disease., 1992, 21: 76-80. | 3. Clack W, Heller W, Michel C and Saran M, J Allergy., 1950, 21,133-147. | 4. Colergie Smith P O, Thomas P, Scurr J H and Dormandy J A, Br Med J., 1980, 296, 1726-176. | 5. Hertog M G L, Hollman P C H, Katan M B and Klohout M, Nutr Cancer., 1993, 20, 21-29. | 6. De-whalley C, Rankin S M, Houct J R S, Jessup W, and Leake D S, Biochem Pharmacol.,1990,39, 1743-1750. | 7. Bamard D L, Smee D F, Huffman J H, Meyerson C R andSidwell R W, Chemotherapy.,1993, 39, 203-211. | 8. Hur C Q, Chen K, Shi Q, Kikushkie RE, Cheng YC and Lee KH, J Nat Pro, 1994; 57, 42-50. | 9. Beladi, I. Musci, R. Pusztai, M. Bakay, I. Rosztoczy, M. Gabor. 1987, 57: 42-50. | 10. Goodwin JS, Brodwick M. J Am Diet Assoc 1996; 96:1027-1039. | 11. Rimm EB, Ascherio A, Giovannucci E, Spiegelman D, Stampfer MJ, Willett WC. JAMA 1996; 275:447-451. | 12. Trease GE, Evans WC, A Text book of Pharmacognosy, 11thedition, Bailliere Tidall, London, 1978, 530. | 13. Salah,N., Miller,N.J., Paganga,G., Tijburg,L., Bolwell,GP and Rice-Evans,C (1995) Polyphenolic flavanols as scavengersof aqueous phase radicals and as chain-breaking antioxidants. Archives of Biochemistry and Biophysics,322:339-346. | 14. Luis, A (1999) Oxygen, Free Radicals and Oxidative Stress in Plants. Free Radical Research, supplement,31:1-256. | 15. Ravishankar M N, Shrivastava N, Jayathirthaa M G, Padh H and Rajani M, J. Chromatography., 2000; 744: 257-262. | 16. P.D. Sethi. HPTLC, CBS Publishers and distributors, 1st edition New Delhi,1996. | 17. Poole CF, Poole SK. Instrumental Thin Layer chromatography. Anal Chem., 1994; 66(1): 27A-37A. | 18. Weins C, Hauck HE. Advances and developments in thin layer chromatography. LC-GC Int., 1996; 4(6): 455-71.