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Isolation of a Flavonol Quercetin from Onion

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Abstract

High Performance Liquid Chromatography (HPLC) is a chromatographic method used to separate component parts, to identify each component and to measure each component where the standing phase consists of packing of small particles (1.8 - 15 μm) contained in a column with a small drill (2 - 4.6 mm), one end connected to liquid eluent (cellular category). Quercetin analysis was performed on an Agilent 1100 HPLC (Chemstation software) system equipped with universal injector, 10 μl injection volume and DAD (Diode-Array Detector) - UV-VIS detector. The HPLC method was designed to quantify the amount of Quercetin extracted from alcoholic onions, with octadecylsilane (250 x 4.6 mm, C18 Inertsil DDS-3V) as a standing phase and the moving phase of Quercetin using 0.05% orthophosphoric acid and methanol. Our HPLC volume analysis revealed that the onion sample contained 995 μg in 1.17 μg of quercetin.

Keywords: HPLC, DAD, Onion, Quercetin.

INTRODUCTION

Flavonoids exhibit many different biochemical and pharmacological effects including anti-oxidation, anti-inflammatory, anti-platelet, anti-thrombotic action and immune effects. It can inhibit enzymes such as prostaglandin synthase, lipoxygenase and cyclooxygenase, which are closely related to tumorigenesis. Flavonoids also known as 'Vitamin-P' and citrin are second Phase Metabolites. Flavonoids are a group of about 4000 naturally occurring polyphenolic compounds, which are found worldwide in plant foods. These are considered primarily pigments that cause leaf colors, especially in autumn. Quercetin is one of the most abundant flavonoids found in apples and onions and is a powerful antioxidant. Several in vitro and in vivo studies have shown that quercetin can prevent malignant growth and metastasis in a variety of cancer cells by demonstrating a strong ability to induce apoptosis, which may make it an additional cancer treatment option.

The main objective of this study was to identify and extract quercetin from onions as there was no report on the distribution and distribution of quercetin in onions.

MATERIALS AND METHODS:

Sample adjustment

The soluble systems used for extraction were ethanol. The flask removal process was changed to be removed. A 25-gram sample of powdered stem is immersed in a solvent-containing flask, wrapped in aluminum foil and placed in a shaker for 48 hours at 120-130 rpm. After 48h, the extract was filtered using Whatman filter paper No 1. The solvent extract was concentrated in an air-conditioned oven at 54°C until completely dry. The dry parts were stored at 4°C for further analysis.

Column chromatography and sub-chromatography (TLC)

In order to separate the lead molecule from ethanol extracted from onions, crude extract is included in the chromatography column. Column chromatography is a type of adsorption chromatography technique. Here segmentation depends on the level of advertising of the standing category.

Silica gel (standing phase) was properly moistened with ethyl acetate (cell phase) and packed sufficiently in the column with a cotton pad below. Sodium sulfate was added to the top of the column to hold water molecules and a solid gel was added to protect the column. A collection tube was placed under the column near the end to collect the elutes. The empty column was removed with hexane for 10 minutes and subsequent clarification was performed in the form of a gradient. The discharge items were placed on top of a full standing section. Gradient elution were as follows hexane, hexane: ethyl acetate (5:1), hexane: ethyl acetate (3:1), hexane: ethyl acetate (1:1), ethyl acetate, ethyl acetate: alcohol (5:1), ethyl acetate: alcohol (3:1), ethyl acetate: alcohol (1:1) and alcohol. The volume of the collected fraction was 2.0ml and 21 fractions were collected. The collected particles were subjected to TLC and the solvent system used for TLC was toluene: ethyl acetate: methanol (4: 0.5: 0.5). 14, 15 and 16 are mixed together and included in the chromatography column again due to the similarity of the fractions. Column chromatography was performed in the same way as in previous tests except for cell phase and analysis method. Here the standing phase was prepared with silica gel (Merk) and ethyl acetate and a blank column was removed with ethyl acetate for 10 minutes.

The gradient elution were as follows: ethyl acetate, ethyl acetate: alcohol (6:4), ethyl acetate: alcohol (4:6), ethyl acetate: alcohol (2:8), alcohol, alcohol : methanol (6:4), alcohol. : methanol (4:6), alcohol: methanol (2:8) and methanol.

The volume of the collected fraction was 2.0ml and 21 fractions were collected. Collected fractions were subjected to TLC and the solvent system used for TLC was toluene: ethyl acetate: methanol (4:0.5:0.5). 14, 15 and 16 fractions were stitched together and the column column was repeated and here again 21 fractions were collected. 7, 8, 9, 11, 12 and 13 components were blended together and subjected to double TLC to compare the lead compound with standard quercetin (Sigma Aldrich). TLC silica gel 60 F254 plates (Merck, 10x06 cm) was used to separate quercetin from onion extract. The plates are then placed in a room filled with iodine vapor to determine the color of the paint (blue). The enhanced plate was sprayed with a 5% solution of ethanolic ferric chloride to determine the color of the stains in both the sample and normal quercetin. Rf values calculated from the sample are divided and compared to the corresponding level. Mixed pieces were concentrated and stored for further analysis.

Analysis of Flavonoids

Determination of the existing flavonoid in the concentrated plant sample was performed by adding 1 ml of dilute sodium hydroxide to 1 ml of the concentrated plant sample. The appearance of yellow indicates the presence of flavonoids.

Detection of quercetin by HPLC

The concentrated plant quercetin was subjected to High Performance Liquid Chromatography. Quercetin purchased from Ultra lab products, Bangalore (Q4951, HPLC grade, Sigma Aldrich) and methanol (HPLC grade, Merck) chromatography, Merck specialty Private Limited, Mumbai). Orthophosphoric acid (Spectrochem Private Limited, Mumbai), HPLC water, other chemicals and reagents are purchased from authorized suppliers. Quercetin is soluble in water, methanol, ethanol based on the solubility we selected in the cellular phase and performed analysis by regression phase and gradient elution.

Phase A mobile fix:

Pipe 1.0 ml of orthophosphoric acid in 2000 ml of volumetric flask and three times the pipette rinse with water and make the volume increase with water.

Adjustment of Normal Quercetin

Measured and transferred 10 mg of standard quercetin (Sigma Aldrich) to 100 ml of volume flux and added about 50 ml of diluent. The solution is sonicated 5min and cooled to room temperature (21° -C) and makes it volume per diluent.

It also transfers 5.0 ml of the above solution to 50.0 ml volume flask and diluted in volume with a diluent. 2.4.2 Sample preparation Properly measured 5 mg of the sample per 100 ml of volume flux and added about 50 ml of diluent. The solution is sonicated for 5 min and lowered to room temperature and make it volume with diluent. Empty diluent, standard solution and sample test solution were injected into each column. Record chromatogram at 273 nm and report results

RESULTS AND DISCUSSION

Thin layer chromatography When TLC plates are developed they are full of iodine vapor, showing areas corresponding to those of quercetin and ethanol extract of onions. Plates formed under UV light showed bright spots on onion ethanol extraction and produced the results of a thin layer of chromatogram of the concentrated test sample.

Most phytochemical studies on natural sources of quercetin reveal that onions, apples, berries, green and black tea and other citrus plants are the origin of the main plant. Studies of TLC, UV and IR spectral have given new dimensions to flavonoid chemistry so that their presence is taxonomically significant (9). Many authors report that there are flavonoids from several plant species (*Lycium barbarum*), *Passiflora plamer*, *Cassia angustifolia*, *Jatropha curcas*. Quercetin has been reported for many plant species such as *Cicer arietinum* and *Acacia catechu*. Quercetin glycosides are present in the combined 'apple leaves' and *Azadirachta Indica*.

CONCLUSION

In our work, quercetin has been identified in the extraction of onion ethanol by thin layer chromatography. The identification of a major component of flavonoid such as quercetin was tested by direct comparison of its storage time, UV structure and 3-D at the level normally obtained. Our HPLC volume analysis revealed that the onion sample contains about 90% pure quercetin.

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