

IDENTIFICATION OF QUERCETIN IN *Echinops tenuisectus* Family Compositae

By

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الخلاصة:

أكدت هذه الدراسة على التحري عن الكيورستين وهي مادة فلافينودية تدرس لأول مرة في نبات بري عراقي مسمى (*Echinops tenuisectus*) نظرا للاهمية الطبية لمادة الكيورستين من جهة, وعدم وجود اي منشورات علمية تتناول المكونات الكيميائية لصنف *tenuisectus* لجنس *Echinops* أخذت هذه الدراسة أهميتها. تم الكشف عن مادة الكيورستين في كل من مستخلص البذور ومستخلص النبات للاجزاء العلوية, بواسطة طريقتين من طرق الكروماتوغرافية, الأولى هي تقنية كروماتوغرافية الطبقة الرقيقة TLC باستخدام رقائق TLC ذات النوعية Gf254 وكاشف الأشعة فوق البنفسجية U.V بالطول الموجي 254 واثان من المحاليل الناقلة المختلفة, حيث أن قيمة Rf لمادة الكيورستين القياسية طابقت قيمة Rf لمادة الكيورستين في المستخلص النباتي. طريقة HPLC هي الطريقة الكروماتوغرافية الأخرى التي أكدت وجود مادة الكيورستين في المستخلص النباتي بتطابق retention times لكل من الكيورستين القياسي و الكيورستين في المستخلص النباتي. كما وأكدت النتائج أن نسبة وجود مادة الكيورستين في مستخلص البذور هي أعلى من نسبة وجود هذه المادة في مستخلص النبات للاجزاء العلوية للنبات.

Abstract

This study is emphasized on the detection and identification of Quercetin Flavonoid in a newly studied, wild Iraqi plant, named *Echinops tenuisectus* of Compositae family. The medicinal importance of Quercetin on one hand, and the absence of any phytochemical investigation on *tenuisectus* specie of *Echinops* genus on the other hand, acquired this s importance. study it

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Quercetin was identified in the plant extract of both, the aerial part's and the seed's extracts, by two chromatographic methods, first Thin Layer Chromatography (TLC) using TLC ready made Gf254 plates, UV detector at 254 nm, and two different solvent systems in which the Rf value of the standard quercetin matched with the Rf value of the plant extract quercetin. HPLC was the other chromatographic method that proved the presence of quercetin in the plant extract by identical retention times. The result indicated that the quercetin content in the seed extract was higher than that in the plant extract of the aerial parts.

Introduction

The studied plant is *Echinops tenuisectus* of the family Compositae (Fig-1). It is a wild, Iraqi plant first studied in Iraq. The *Echinops* genus consists of 100 spp (1). The *Echinops tenuisectus* is a perennial, 40-100 cm high. Stems simple or branching from the base, sparsely cobwebby-cane scent. Leaves lanceolate or oblong-lanceolate, the lower ones 10-15 cm long, 4-6cm wide, with triangular-lanceolate, prickly lobes, greenish, shiny, sub glabrous above, densely whitish- tomentose below; stem –leaves gradually smaller, subpinnatisect, prickly, the uppermost ones narrow linear – lanceolate, diminute. Heads 5-7 cm in diameter, Penicil about 1/3as long as the involucre the bristles scabrous. Involucral bracts 12-14, the outer bracts as long as the penicil, narrow spatulate – deltoid, the intermediate ones subulate- attenuate, up to 2.5-3.5 cm long, produced into a long slender prickly horn, twice to twice and a half times as long as the outer ones, the innermost ones of about equal length, acute, fimbriate, connate to the middle. Pales of pappus barbellate, connate at base into a contiguous corona.

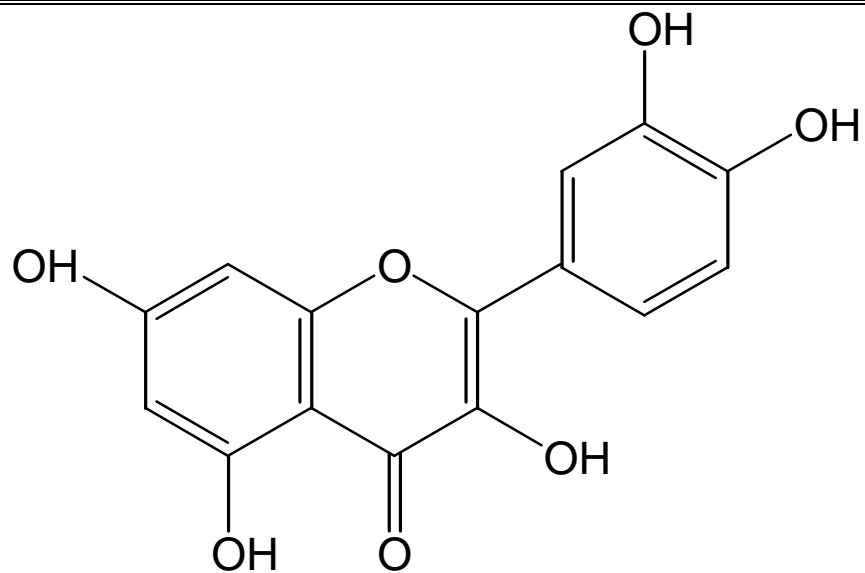
The distribution of this plant is in Sharaban, Diyalah, Badrah, - Upper Tigris Plain (2).



Figure –1 Photography of *Echinops tenuisectus*

Quercetin is a member of the class of flavonoids called flavonols. It is widely distributed in the plant kingdom, it is known chemically as:

2- (3,4- dihydroxyphenyl)- 3,5,7- trihydroxy- 4H-1- benzopyran-4- one and 3,3',4', 5,7- pentahydroxy flavone⁽³⁾



Quercetin

Figure-2 Structure of Quercetin

Quercetin is consistently the most active of the flavonoids in experimental studies, and many medicinal plants owe much of their activity to their high Quercetin content. It is best known as an anti-inflammatory/ anti-allergy agent. Because it stabilizes mast cell membranes and prevents the release of histamine and other inflammatory agents, it is often for food and inhalant allergies, asthma, eczema, psoriasis, gout and ulcerative colitis ⁽⁴⁾. Due to its antioxidant effect, Quercetin can inhibit inflammatory processes mediated by “leukotrienes” (inflammatory agents a thousand times more powerful than histamines), hyaluronidase (collagen-destroying enzymes), and lysosomal enzymes (other promoters of localized inflammation) ⁽⁵⁻⁸⁾.

Many flavonoids inhibit tumor formation, but again Quercetin has consistently demonstrated significant anti-tumor activity against a wide range of cancers including cancers of prostate, breast, ovaries, colon, rectum, and squamous cell carcinoma, leukemia ⁽⁹⁻¹³⁾. Preliminary studies indicate that Quercetin may inhibit the proliferation of androgen independent human prostatic tumor cells. In rats Quercetin, in combination with finasteride, reduces prostate weight. ⁽⁹⁾

Quercetin can significantly decrease the accumulation of “sorbitol” in the lens of diabetic animals, effectively delaying the onset of cataracts. ⁽¹⁴⁾

It is also indicated in diabetes for its ability to enhance insulin secretion ⁽¹⁵⁾, protects the pancreatic beta cells from the damaging effects of free radicals ⁽¹³⁾, and inhibits platelet aggregation ⁽¹⁶⁾. Very recent study indicate that Quercetin inhibits the proliferation and migration of aortic smooth muscle cells, along with inhibition of mitogen activated protein kinas phosphoryation, these finding provide new insights and a rationale for the potential use of Quercetin in the prevention of cardiovascular diseases ⁽⁸⁾.

Quercetin reduces damage to kidneys in rats given a toxin or a chemotherapy drug such as cisplatin ⁽¹⁷⁾.

Quercetin has potential for the treatment of neuroleptic-induced extra pyramidal side effect, such as from haloperidol. Quercetin also is a powerful antioxidant that may protect brain cells from damage ⁽¹⁸⁾.

Quercetin appears to be extremely safe to use, carcinogenic and teratogenic studies in rats and rabbits have shown that it is without apparent side effects even when consumed in very large quantities (2000 mg. Per Kg of body weight) for long periods of time (up to 2 years) Unlike the citrus bioflavonoids, Quercetin has no interaction with any drug. It can be used even during pregnancy ⁽¹⁹⁾.

This study indicates that Echinops tenuisectus of Compositae family serve as another important source of that important medicinal compound.

Materials and Methods

The plant material was collected during July 2005 From Sharaban/ Iraq. The plant was identified by the Department of Pharmacognosy, college of Pharmacy/University of Baghdad; and authenticated by the Herbarium of Baghdad University (Prof. Dr. Ali- Al-Mussawi) /Iraq.

Fifty grams of the powdered plant material (aerial part) were first defatted by reflux with 100 ml of petroleum ether (60°-80° C) for one hour and filtered. The defatted dried plant material was then extracted by reflux using 100 ml of 70% ethanol for three hours. This step was repeated for four times, then the combined filtrates were evaporated under reduced pressure using Buchi rotatory evaporator attached to vacuum pump at 40° C, to a thick residue of ethanol extract (F1). This residue was then hydrolyzed with 2NHCl in aqueous methanol (1:1) under reflux for three hours; the resultant solution was then partitioned with 100 ml of ethyl acetate (F2). This fraction was evaporated under reduced pressure to dryness, as shown in the following diagram (Fig-3). Then the same extraction method was repeated exactly on 50gm of the seed part of the same plant.

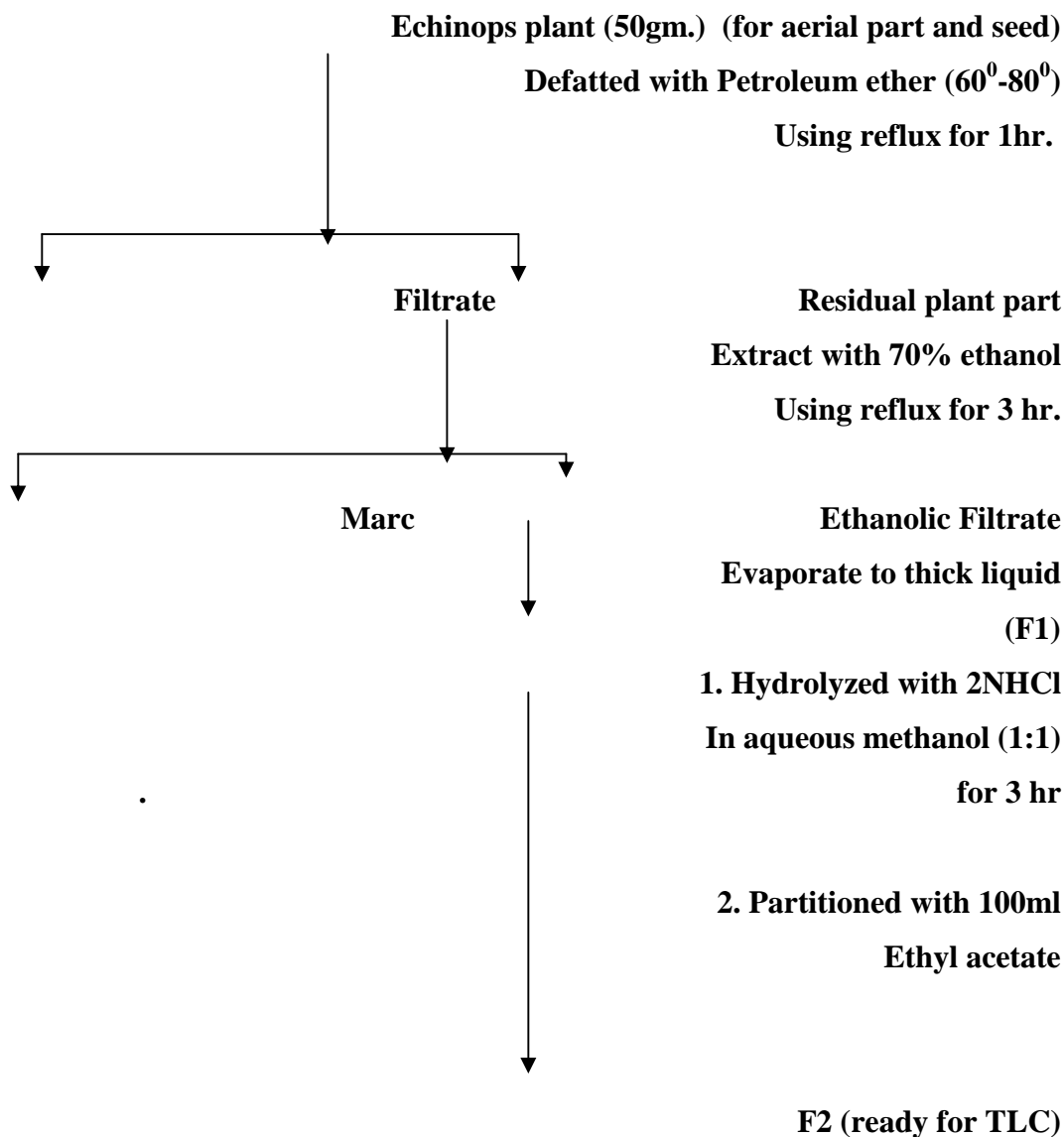


Figure-3 [Schematic representation of Quercetin extraction from *Echinops tenuisectus*]

Identification of Quercetin in the plant extract.

The Identification of Quercetin in the aerial plant extract and the seed extract, was performed first by TLC, using TLC ready made Gf254 plates, UV detector at 254 nm, Standard Quercetin and two different solvent systems that were⁽²⁰⁾:

Solvent (1): chloroform: acetone: formic acid (75:16.5:8.5)

Solvent (2): n.butanol: glacial acetic acid: water (40: 10:50)

Then this identification was authenticated by HPLC with the standard Quercetin

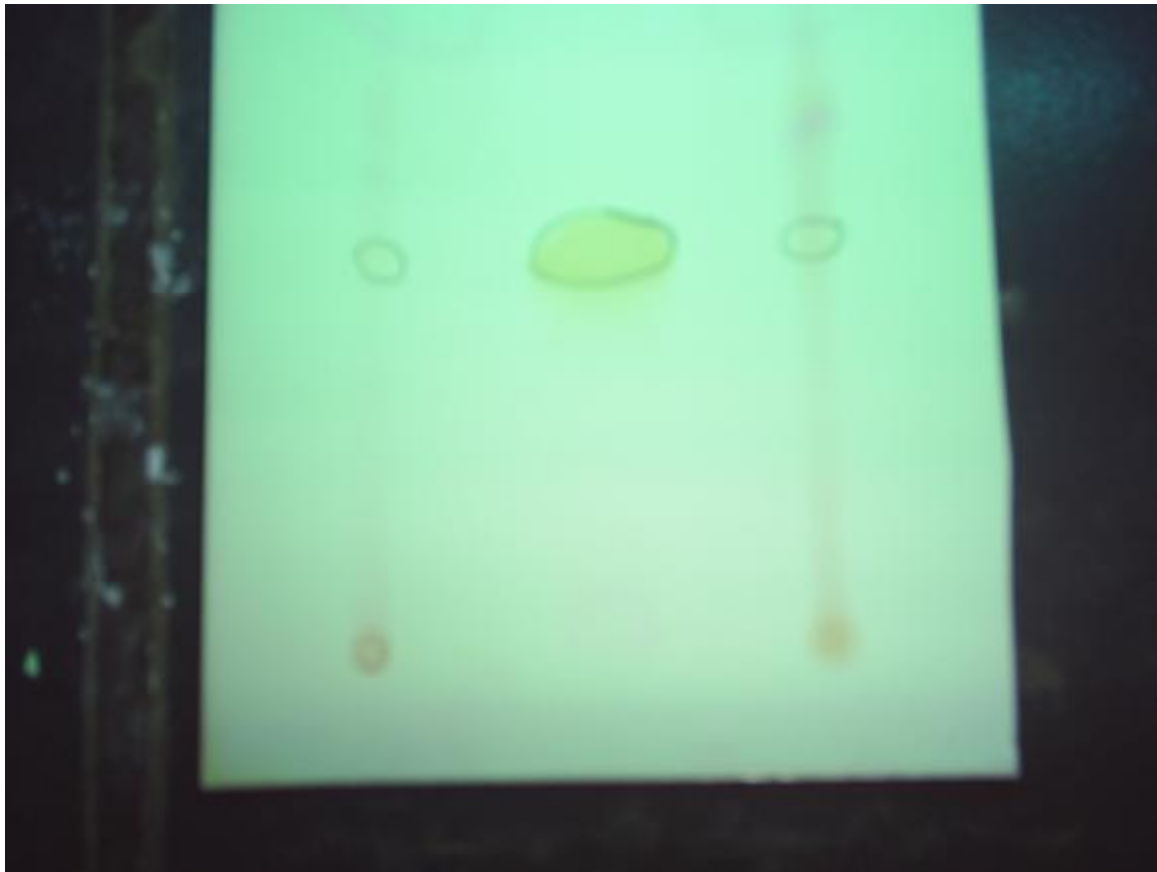
Results and Discussion

Most of the phytochemical studies on the natural sources of quercetin revealed that the onion, apples, berries, green and black tea and some of citrus plant is the main plant origin, but none, at all, was reported on Echinops tenuisectus). Preliminary investigation of this plant indicates the presence of a number of flavonoid compounds, among them is Quercetin compound. Since most of the flavonoids occur in plants in the form of glycosides, we used 70% ethanol to extract good quantities of Quercetin. Then for further fractionation, the glycoside residue hydrolyzed for 3 hours, under reflux with 2N hydrochloric acid in aqueous methanol (1:1). Then the aglycone was taken with organic solvent (ethyl acetate)

Identification of Quercetin by TLC.

The detection of quercetin was maintained for both the aerial plant extract and the seed extract, by TLC. Using two different solvent systems, in the presence of standard Quercetin, and U.V detector (wave length 254nm) as demonstrated by the following TLC-plates.

(Fig.-4, 5).



C

B

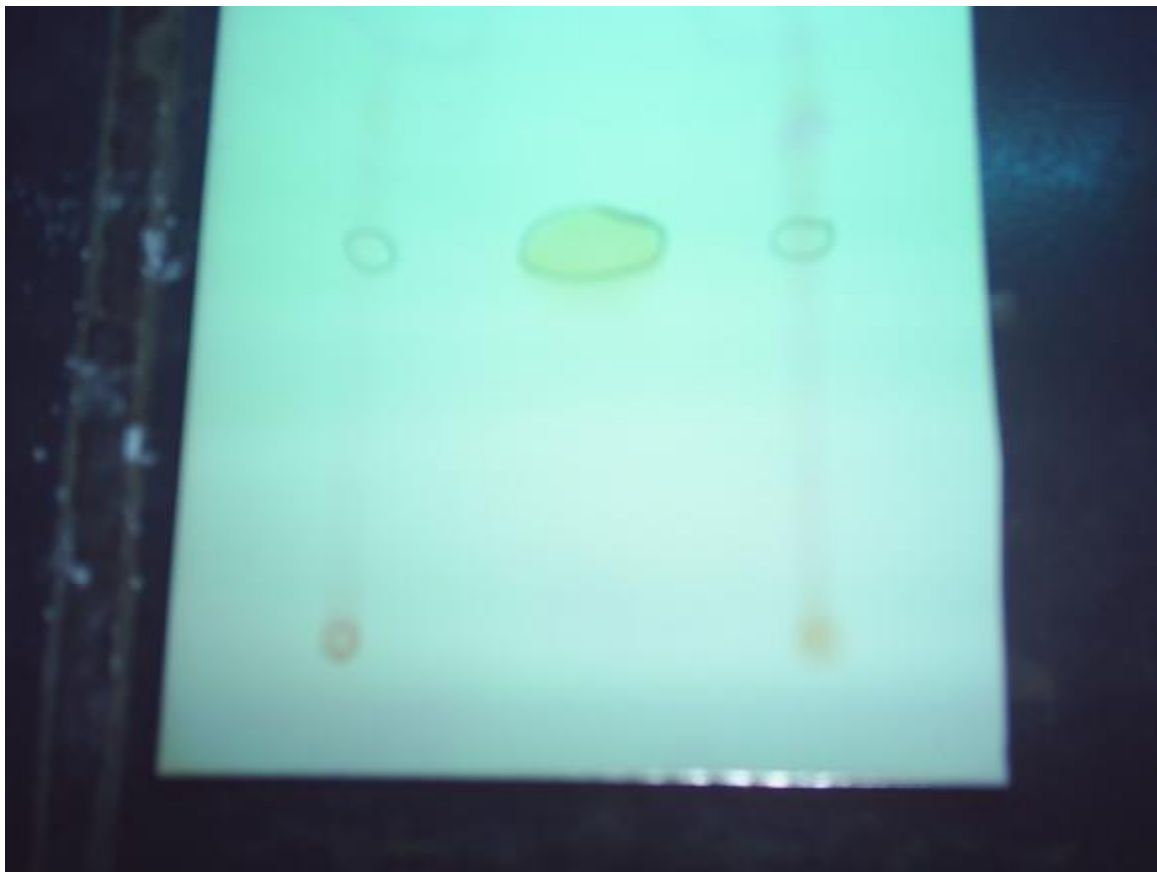
A

Figure (4) [TLC Gf254 plate of the aerial plant extract, seed extract, and standard using S1 mobile phase)

A= Seed extract

B= Standard quercetin

C= Aerial plant part extract



C B A

Figure (5) [TLC Gf254 plate of the aerial plant extract, seed extract, and standard using S2 mobile phase)

A=Seed extract

B=Standard quercetin

C=Aerial plant part extract

The Rf values of the standard quercetin, the aerial plant extracts and the seed extract are tabled below. (Table-1)

Solvent system	Rf standard	RF seed extract	Rf aerial pant extract
S1	0.496	0.5	0.5
S2	0.79	0.78	0.8

Table 1-(table of Rf values)

Identification of Quercetin by HPLC.

Quercetin was authenticated by HPLC .The HPLC conditions are listed in the following table, and the following charts were obtained (Fig 6-8)

(Table-2) HPLC conditions

HPLC Conditions	
Mobile phase	Acetonitril : Methanol : Acetic acid 70 : 30 : 0.1
Column	C18 25cm
Flow rate	0.5ml/min
Detector	288 nm

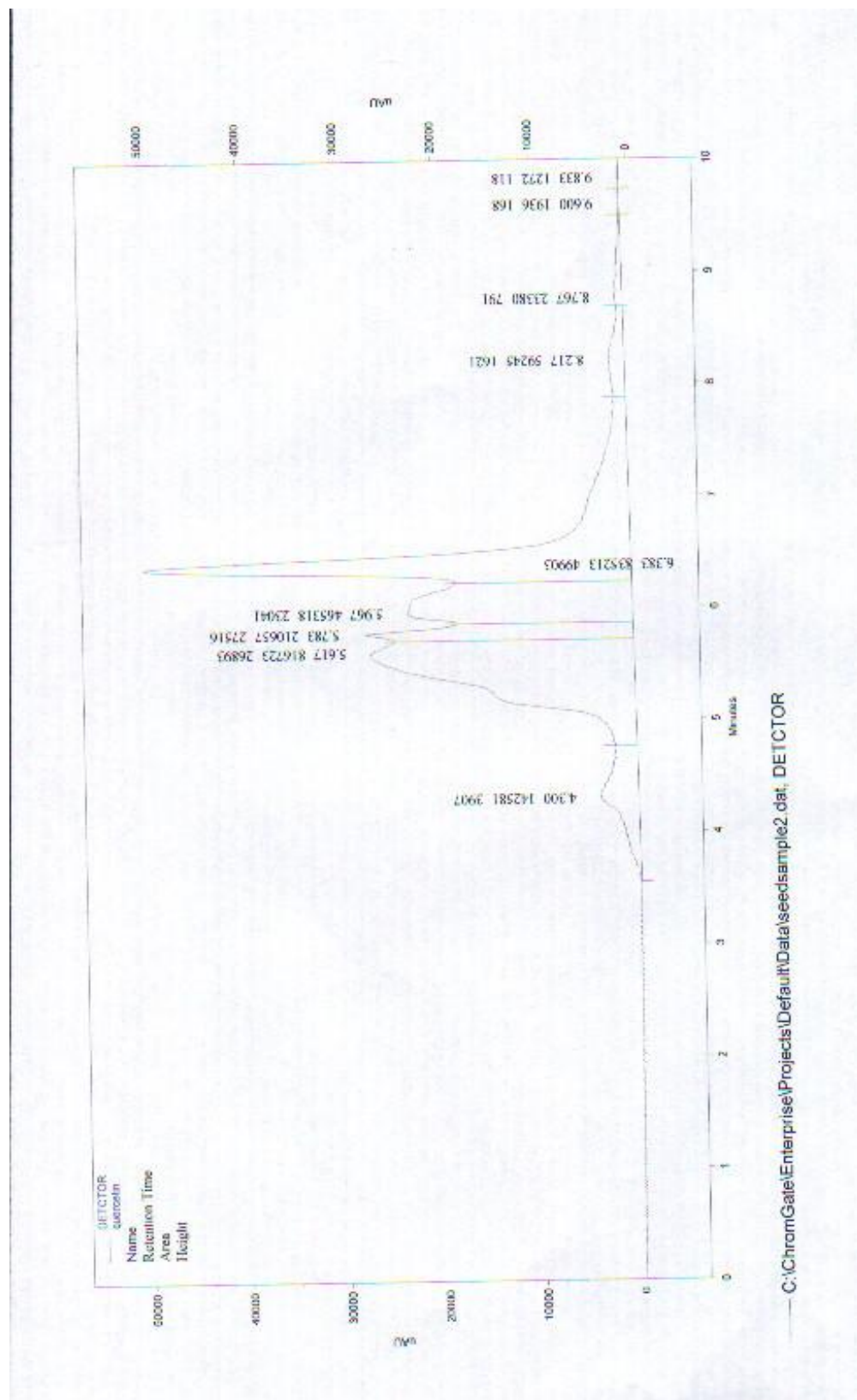


Fig. 6- HPLC of the seed extract

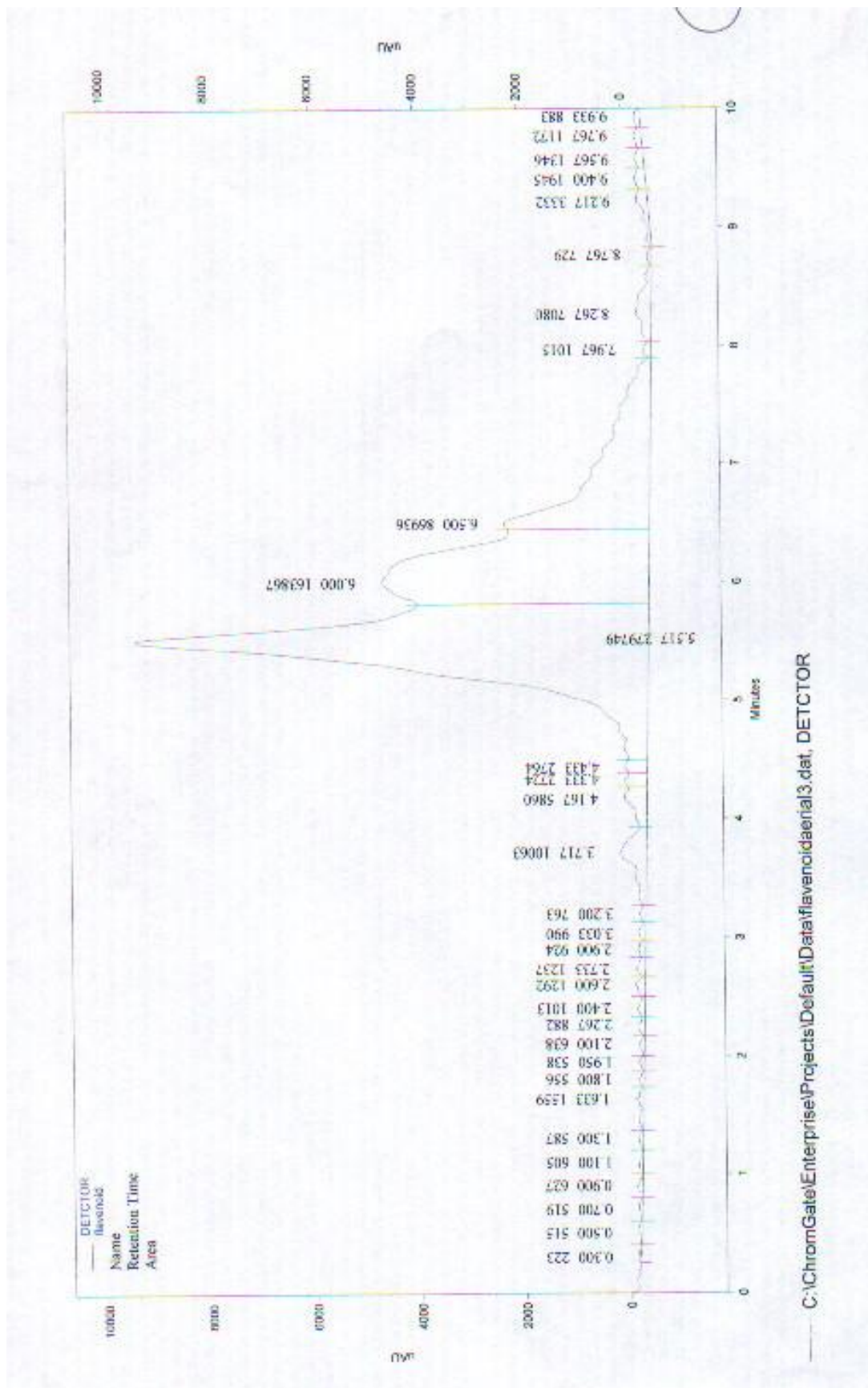


Fig. 7- HPL C of aeri a l plant extra ct

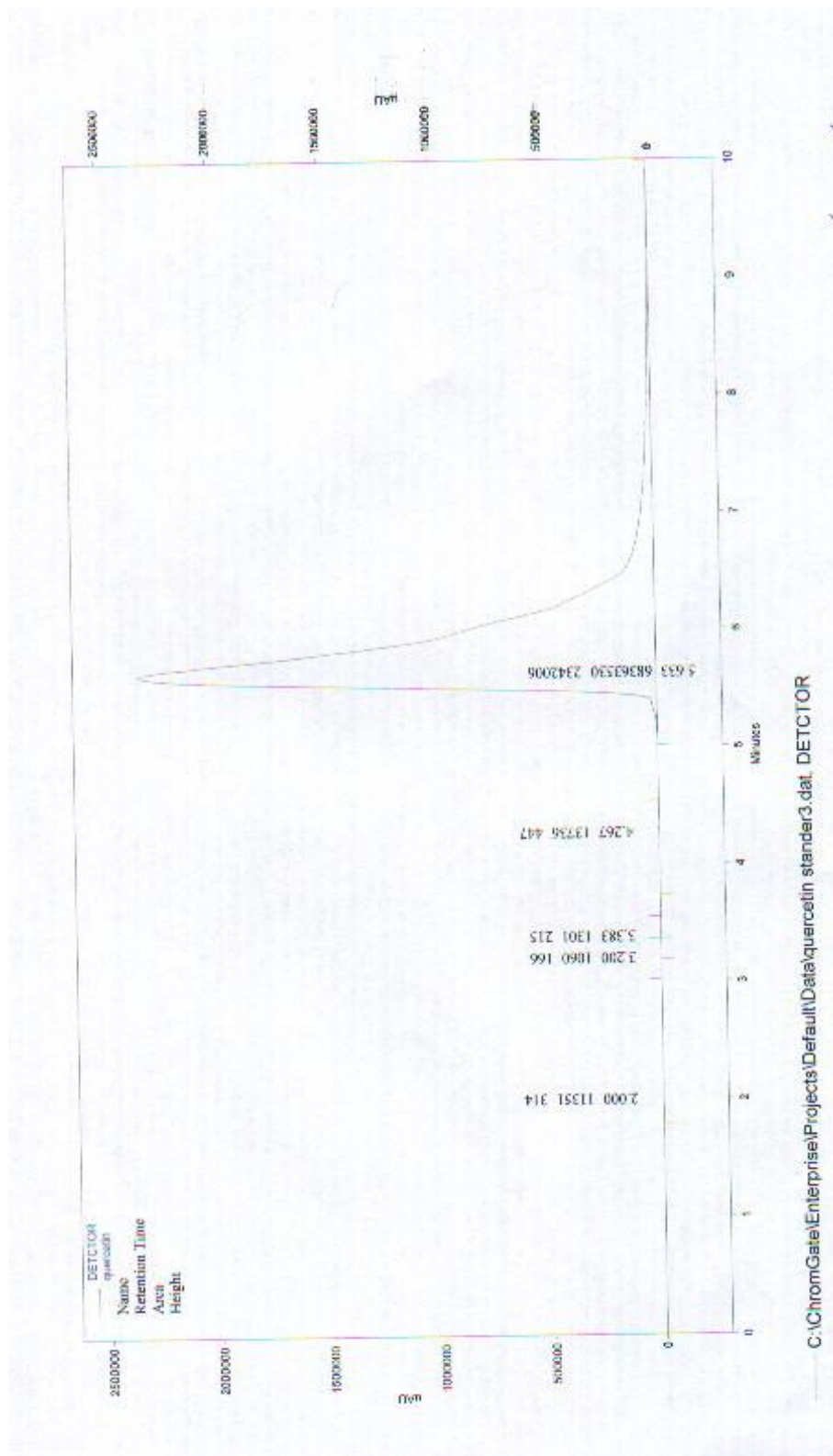


Fig.8- HPLC of standard quercetin

It is obvious from the previous charts that both the aerial plant extract and the seed extract contain quercetin Flavonoid glycoside.

Calculations:-

The quantitative analysis of quercetin in the extracts from the HPLC chart represented as follow: -

1. Percent yield in the aerial plant part extract: -

$[\text{AUC of the sample}/\text{AUC of the standard}] * \text{conc. of the standard.}$

The conc. of standard quercetin use in HPLC= 10mg/ml

The AUC of the aerial plant part extract quercetin in the HPLC chart= 279749

The AUC of the standard quercetin in the HPLC chart = 68363530

Therefore the equation will be:-

$[279749/68363530] * 10 \text{ mg/ml} = 0.04\text{mg/ml}$

$0.04\text{mg/ml} * \text{dilution factor (20)} = 0.8\text{mg/ml} \rightarrow 0.0008\text{g/ml}$

The % of the quercetin content in the aerial plant part extract = $[0.0008/50]$

$*100=0.0016\%$.

2. Percent yield in the seed extract:-

The conc. of standard quercetin use in HPLC= 10mg/ml

The AUC of the seed extract quercetin in the HPLC chart = 816723

The AUC of the standard quercetin in the HPLC chart = 68363530

Therefore the equation will be:-

$[816723/68363530] * 10 \text{ mg/ml} = 0.12\text{mg/ml}$

$0.12\text{mg/ml} * \text{dilution factor (20)} = 2.4\text{mg/ml} \rightarrow 0.0024 \text{ g/ml}$

The % of the quercetin content in the seed extract = $[0.0024/50] * 100=0.0048\%$.

Conclusion

Quercetin compound is considered as one of the important class of natural compounds, which is widely used to treat many diseases such as cancers of prostate, breast, ovaries, colon, rectum and kidney damage. So this study indicate that Echinops tenuisectus) serves as another source of quercetin production, and also both the aerial plant parts and the seed part contain quercetin Flavonoid glycoside, and by calculate the percentage of Quercetin in both aerial plant and seed extracts, we found that the percentage of Quercetin content in the seed extract is higher than that in the aerial plant part.

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