Identification Of Silymarin In *Echinopus tenuisectus* Family Compositae

التحري عن السليمارين في مكونات نبات *Echinopus tenuisectus*

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Abstract.

This study emphasized on the detection and identification of silymarin (Silybinin) Flavonoid in a newly studied, wild Iraqi plant, named *Echinopus tenuisectus* of Compositae family. The medicinal importance of silymarin on the one hand, and the absence of any phytochemical investigation on tenuisectus specie of *Echinopus* genus on the other hand, acquired this study its importance. Silymarin 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 three different solvent systems in which the Rf value of the standard silymarin matched with the Rf value of the plant extract silymarin. HPLC was the other chromatographic method that proved the presence of silymarin in the plant extract by identical retention times. The result indicated that the silymarin content in the seed extract was higher than that in the plant extract of the aerial parts.

المستخلص

أكدت هذة الدراسة على التحري عن السيليمارين وهي مادة الفلافينودية تدرس لاول مرة في نبات بري عراقي مسمى *Echinopus tenuisectus* نظرا للاهمية الطبية لمادة السيليمارين من جهة وعدم وجود اية منشورات علمية تتناول المكونات الكيميائية لصنف tenuisectus لجنس *Echinopus* ، أخذت هذة الدراسة أهميتها .

تم الكشف عن مادة السيليمارين في كل من مستخلص البذور ومستخلص النبات للاجزاء العلوية ، بواسطة طريقتين من طرق الكروماتوغرافية ، الأولى هي تقنية الكروماتوغرافية الطبقة الرقيقة TLC باستخدام رقائق TLC ذات النوعية Gf254 وكاشف الاشعة قوق البنفسجية U.V بالطول الموجي 254 وثلاثة محاليل ناقلة مختلفة ، حيث ان قيمة Rf لمادة السيليمارين القياسية طابقت قيمة Rf لمادة السيليمارين في المستخلص النباتي . طريقة HPL

هي الطريقة الكروماتوغرافية الاخرى التي اكدت وجود مادة السيليمارين في المستخلص النباتي بتطابق retention times لكل من السيليمارين القياسي والسيليمارين في المستخلص النباتي . كما واكدت النتائج ان نسبة وجود مادة السيليمارين في مستخلص البذور هي اعلى من نسبة وجود هذه المادة في مستخلص النبات للاجزاء العلوية للنبات .

Introduction

The studied plant, *Echinops tenuisectus*, belongs to the family Compositae (Fig-1). It is an Iraqi wild plant first studied in Iraq. The *Echinops* genus consist of 100 spp. [1], very few reports investigated this family. The *Echinopus tenuisectus* is a perennial, 40-100 cm high. Stems are simple or branching from the base, sparsely cobwebby-canescent. Leaves are lanceolate or oblong-lanceolate, the lower ones are 10-15 cm long, 4-6cm wide, with triangular-lanceolate, prickly lobes, greenish, shiny, subglabrous above, densely whitish-tomentose below; stem–leaves are gradually smaller, subpinnatisect, prickly and the uppermost ones are narrow liner – lanceolate, diminute. Heads are 5-7 cm in diameter and Penicil is about 1/3 as long as the involucres the bristles scabrous. Involucral bracts 12-14, the outer bracts as long as the penicil, narrow spathulate – 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 are 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, 250-800 m [2].

Figure 1: Photography of Echinops tenuisectus

Flavonoids belong to the family of the benzo gamma-pyrones. More than 4000 different flavonoids are currently known; they are ubiquitous not only in the plant kingdom, where they are particularly abundant in the photosynthetic cells of higher plants, but also in the animal kingdom. [1,3]. Silymarin is a flavonolignan, which has been introduced fairly recently as a hepatoprotective agent [4,5,6,7,8,9,10], is the most well known compound of the flavonoids; it is composed mainly of silibinin (or silybin), with small amounts of other silibinin stereoisomers, namely isosilybin, dihydrosilybin, silydianin and silychristin [4,11]. The structure of the constituents of silymarin (Fig-2) was clarified in the 1960s [4, 11, 12].

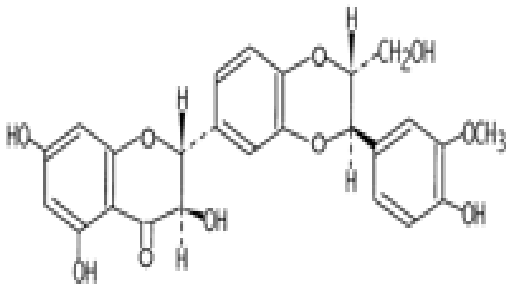


Figure-2 : Structures of Silybinin formally called silymarin(13)

Silymarin is soluble in acetone, ethyl acetate, methanol, and ethanol, sparingly soluble in chloroform and practically insoluble in water [13]. Of the isomers that constitute silymarin, Silybinin is the most active [3,14]. From a medical point of view, silymarin and Silybinin have been found to provide cytoprotection and, above all, hepatoprotection. Their mechanisms of action are still poorly understood. However, the data in the literature indicate that silymarin and Silybinin act in four different ways [3]:

1. As antioxidants, scavengers and regulators of the intracellular content of glutathione [15,16,17,18,19,20].
2. As cell membrane stabilizers and permeability regulators that prevent hepatotoxic agents from entering hepatocytes [21,22].
3. As promoters of ribosomal RNA synthesis, stimulating liver regeneration. [23,24,25].
4. As inhibitors of the transformation of stellate hepatocytes into myofibroblasts, the process responsible for the deposition of collagen fibers leading to cirrhosis. [26,27,28].

The key mechanism that ensures hepatoprotection appears to be free radical scavenging [3].

Anti-inflammatory [25,28,29] and anti-carcinogenic properties have also been

documented [30,31,32]. Silymarin is included in the pharmacopoeia of many countries under the trademark LegalonTM or HepatronTM and is often used as supportive therapy in food poisoning due to fungi and in chronic liver disorders, such as steatosis [33] and alcohol-related liver disease [34].

The ripe seeds of *Silybum marianum* of the family Compositae serve as a main source of Silymarin [14]. This study indicates that *Echinopus tenuisectus* of Compositae family serves 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 then 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 rotary 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.

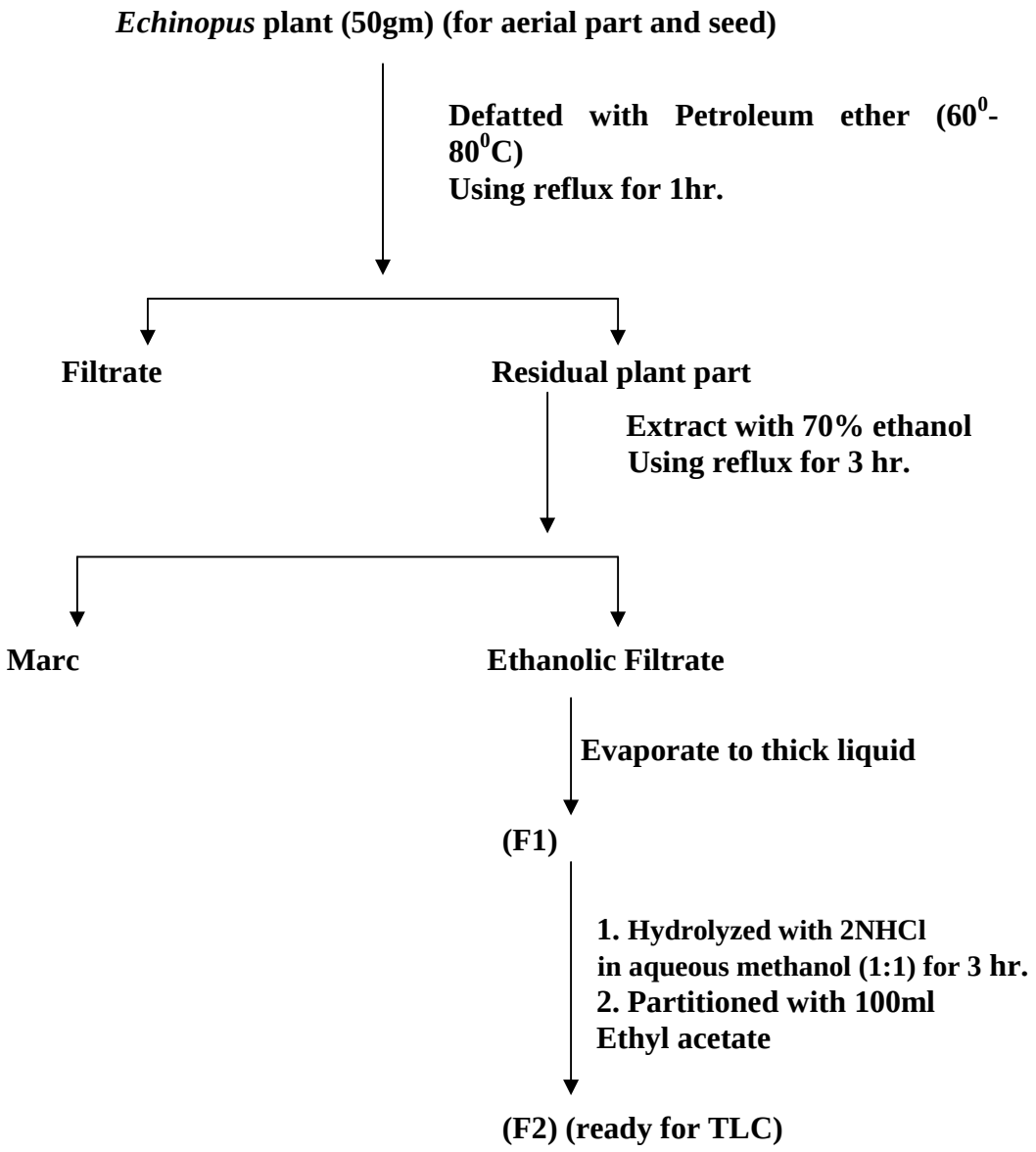


Figure-3: [Schematic representation of silymarin extraction from *Echinopus tenuisectus*]

Identification of Silymarin in the plant extract.

The Identification of Silymarin 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 Silymarin, Standard Silybinin and three different solvent systems that were [35]:

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

Solvent (2): Benzene: ethyl acetate [70:30]

Solvent (3): ethyl acetate: hexane [40:60]

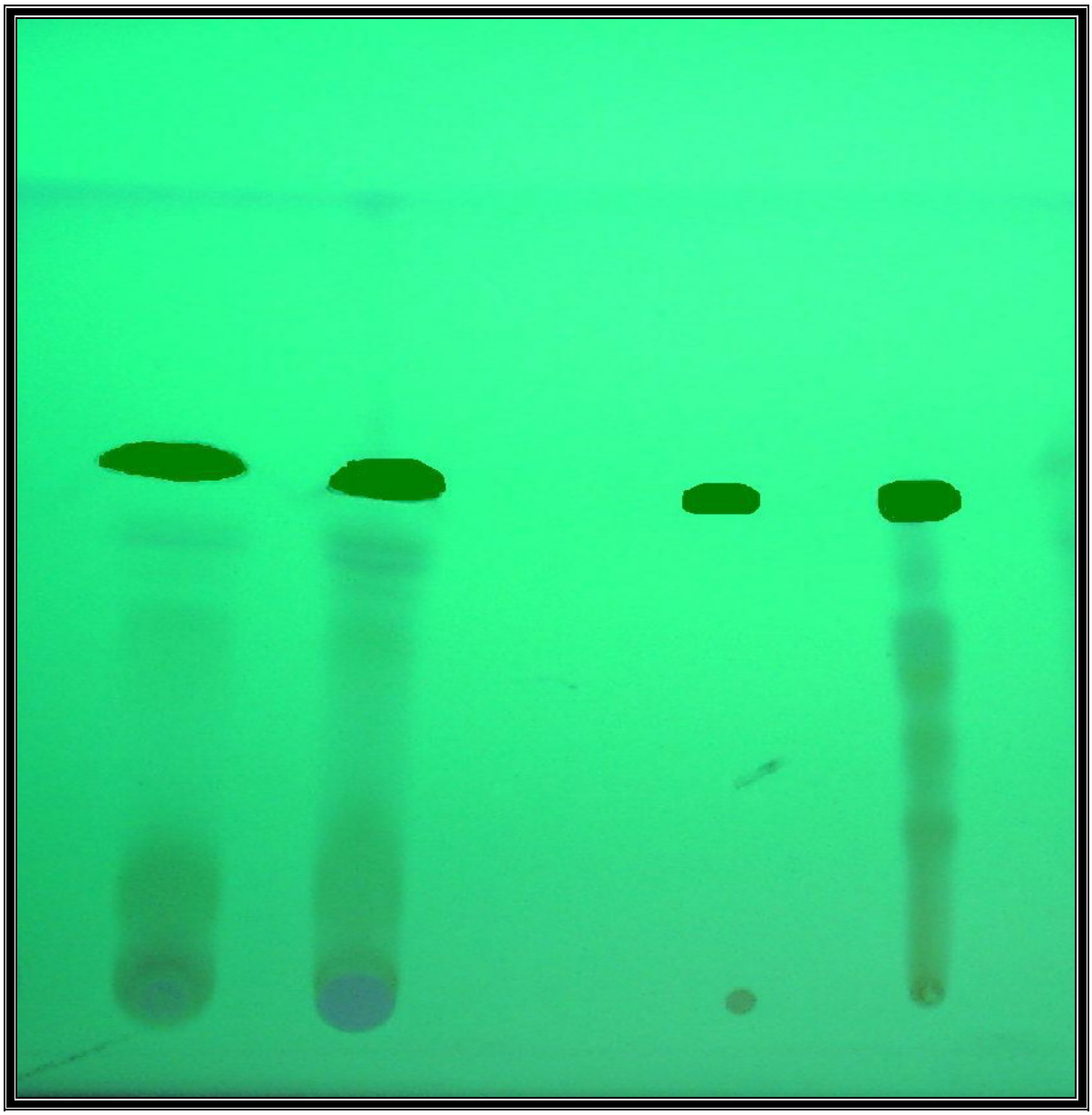
Then this identification was authenticated by HPLC with the standards silymarin and Silybinin

Results

Most of the phytochemical studies on the natural sources of silymarin revealed that the fruit of *Silybum marianum* of the family Compositae is the main plant origin, but none, at all, was reported on *Echinopus tenuisectus*). Since this plant resembles *Silybum marianum* in its shape, we found it worth to investigate its possible silymarin content.

Identification of silymarin (Silybinin) by TLC.

The detection of silymarin (Silybinin) was maintained for both the aerial plant extract and the seed extract, by TLC. Using three different solvent systems, in the presence of standard silymarin, standard Silybinin, and U.V detector (wave length 254nm) as demonstrated by the following TLC-plates. (Fig. 4, 5, 6).



B D C A

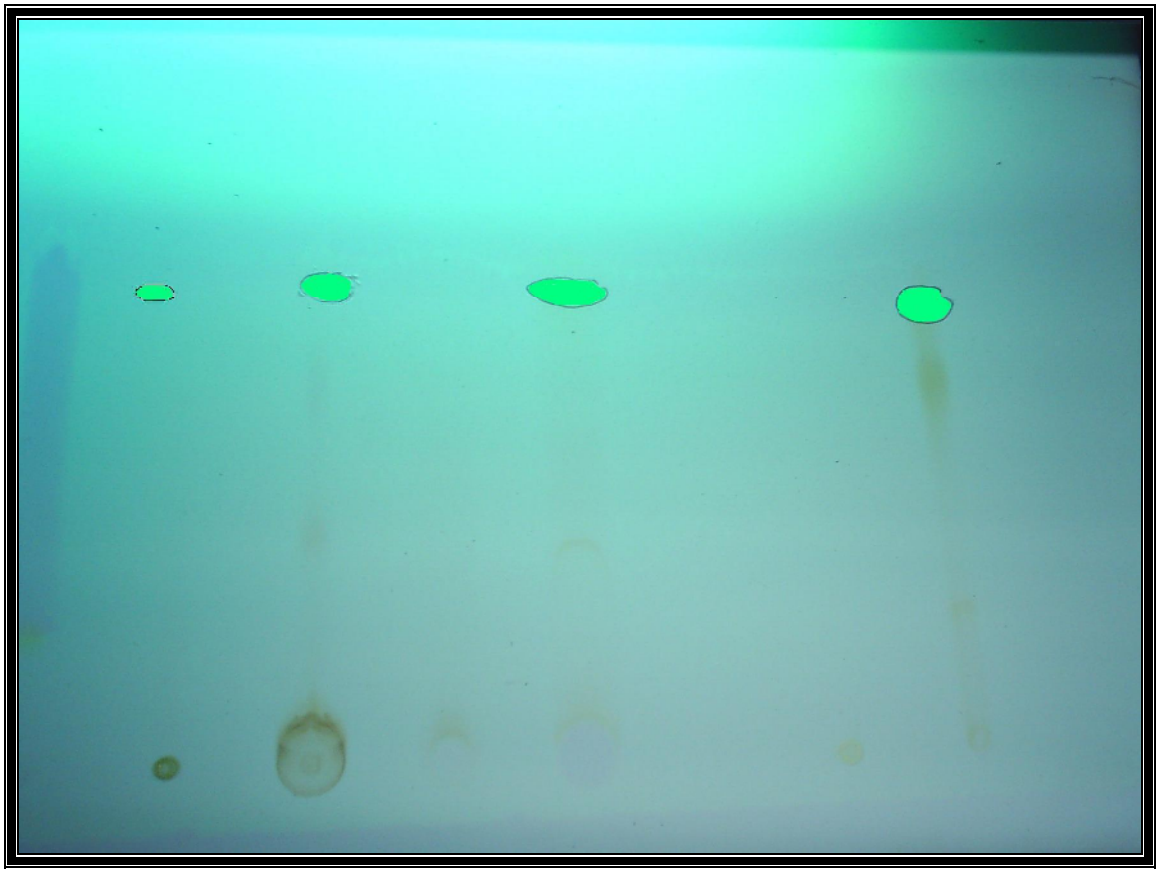
Figure (4): [TLC Gf254 plate of the aerial plant extract, seed extract, and standards using S1 mobile phase)

A=Standard silymarin

B=aerial plant extract

C=standard Silybinin

D=seed extract



C B D A

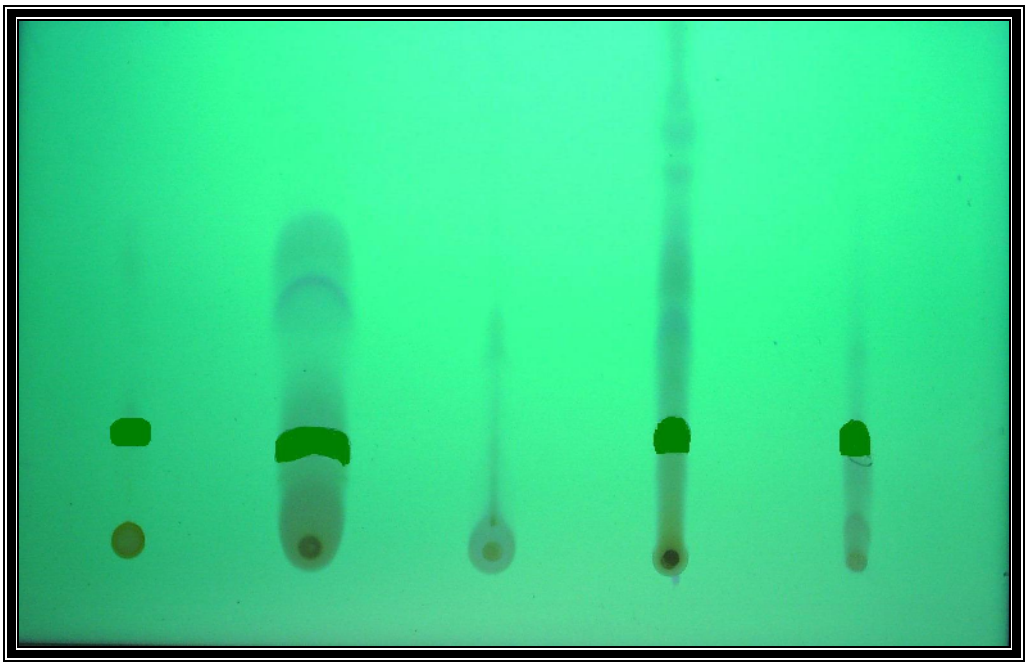
Figure (5): [TLC Gf254 plate of the aerial plant extract, Seed extract, and standards using S2 mobile phase)

A=Standard silymarin

B=aerial plant extract

C=standard Silybinin

D=seed extract



C D B A

Figure (6): [TLC Gf254 plate of the aerial plant extract, seed extract, and standards using S3 mobile phase)

A=Standard silymarin

B=aerial plant extract

C=standard Silybinin

D=seed extract

The R**f** values of the standard silymarin, standard Silybinin, the aerial plant extract and the seed extract are tabled below table (1):

Table 1: Table of Rf values

Solvent system	**Standard silymarin**	**Standard Silybinin**	**Aerial plant extract**	**Seed extract**
S1	0.63	0.63	0.64	0.63
S2	0.57	0.57	0.58	0.57
S3	0.25	0.26	0.26	0.26

Identification of silymarin (Silybinin) by HPLC.

Silymarin (Silybinin) was authenticated by HPLC .The HPLC conditions are listed in the following, table (2):

Table 2: HPLC conditions

HPLC Conditions	
Mobile phase	Methanol: water (50:50)
Column	C18 25cm
Flow rate	1ml/min
Detector	288 nm

The following charts were obtained (Fig. 7-10):

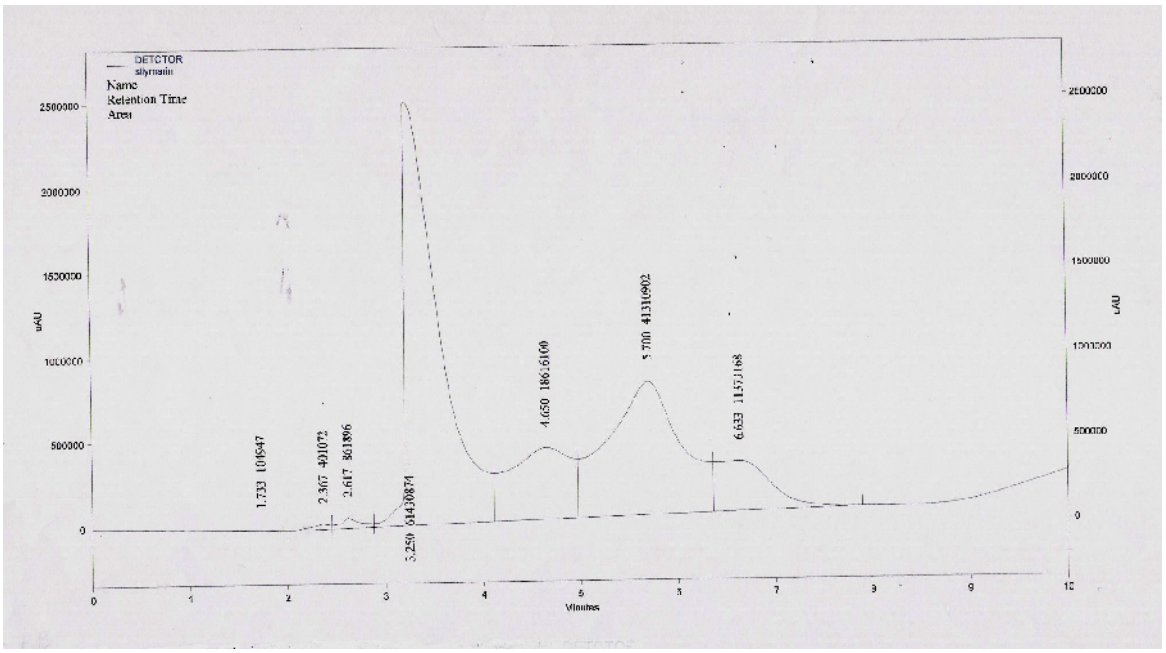


Figure (7): [HPLC of standard silymarin]

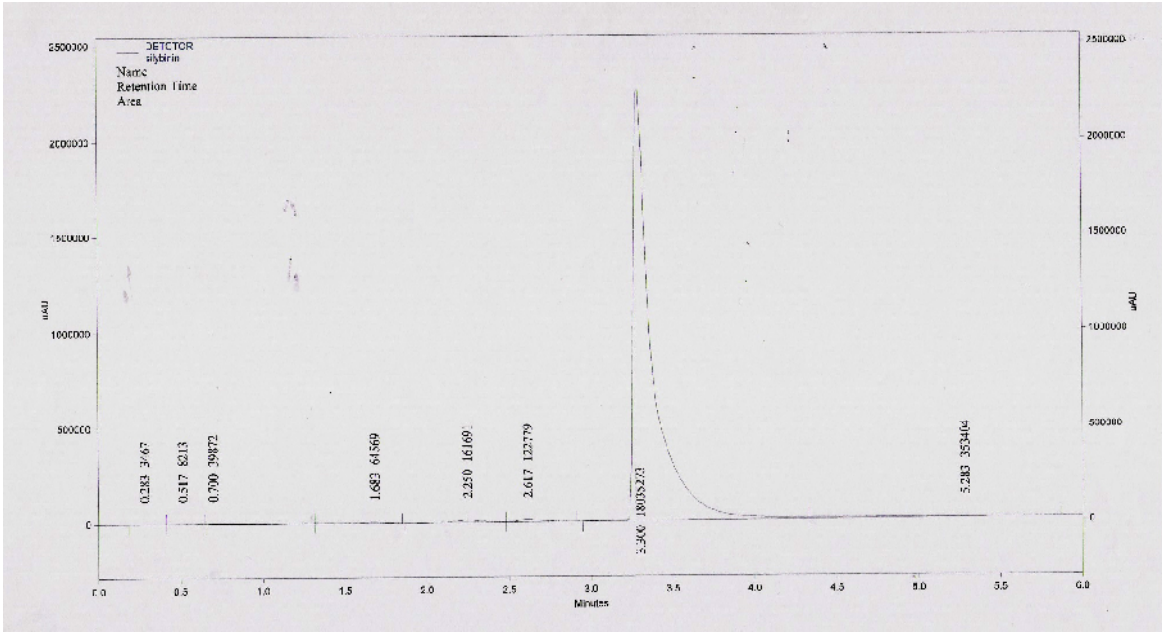


Figure (8): [HPLC of standard Silybinin]

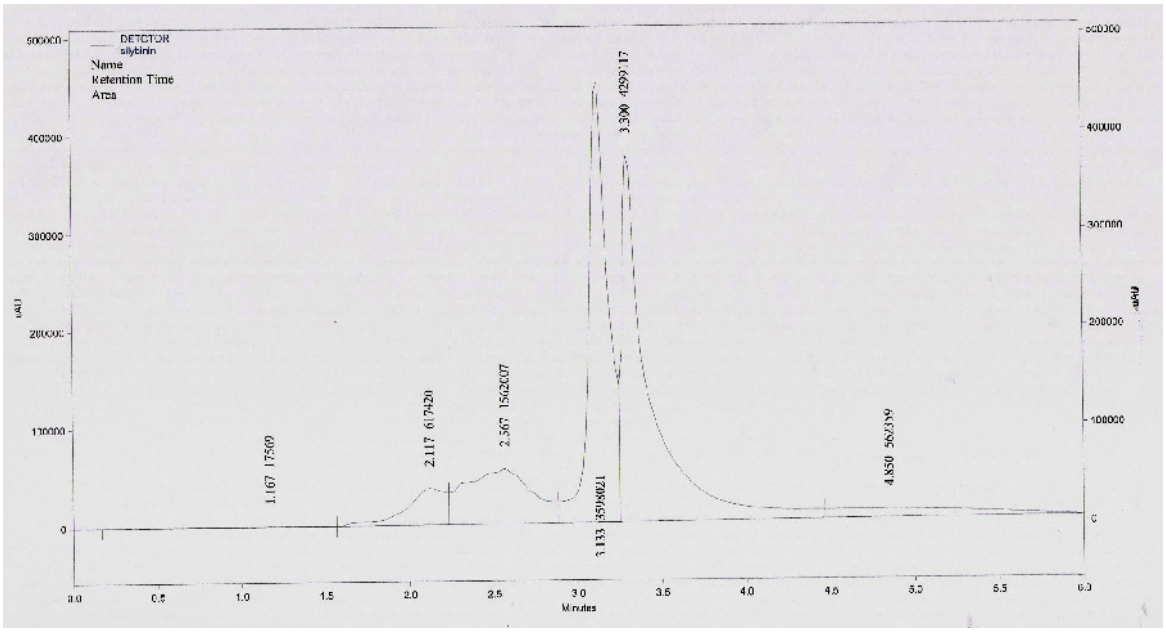


Figure (9): [HPLC of aerial plant extract]

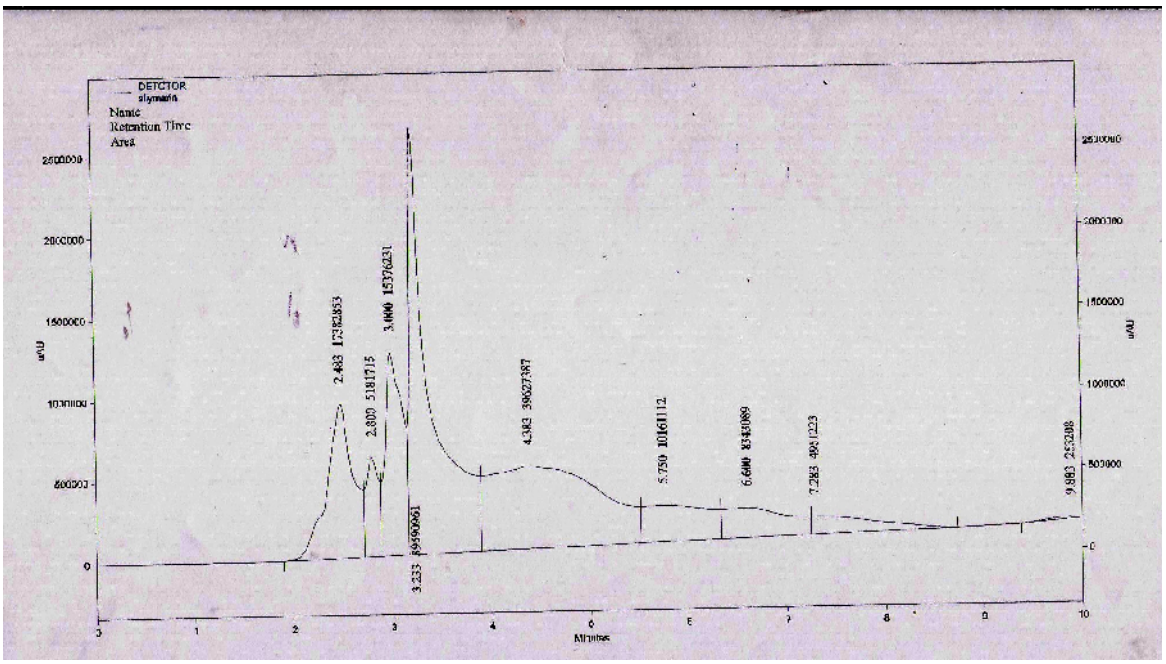


Figure (10): [HPLC of seed extract]

It is obvious from the previous charts that both the aerial plant extract and the seed extract contain silymarin (Silybinin) Flavonoid glycoside.

Calculations:

The quantitative analysis of silymarin and Silybinin in the extracts from the HPLC chart represented as follow:

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

[AUC of the sample/AUC of the standard] × conc. of the standard.

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

The AUC of the aerial plant part extract Silybinin in the HPLC chart = 4299117

The AUC of the standard Silybinin in the HPLC chart = 18035273

Therefore the equation will be:-

[4299117/18035273] ×10 mg/ml = 2.3837mg/ml

2.3837mg/ml × dilution factor (20) = 47.6745mg/ml → 0.04767g/ml

The % of the silymarin content in the aerial plant part extract = [0.04767/50] ×100=0.0953%.

2. Percent yield in the seed extract:-

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

The AUC of the seed extract Silybinin in the HPLC chart = 39590961

The AUC of the standard Silybinin in the HPLC chart = 18035273

Therefore the equation will be:-

[39590961/18035273] ×10 mg/ml = 21.9519mg/ml

21.9519mg/ml ×dilution factor (20) = 439.038mg/ml → 0.439038 g/ml

The % of the silymarin content in the seed extract = [0.439038/50] ×100=0.878%.

Conclusion

1. *Echinopus tenuisectus*) serves as another source of silymarin production.

2. Both the aerial plant parts and the seed part contain silymarin (Silybinin) Flavonoid glycoside.

3. The percentage of silymarin content in the seed extract is higher than that in the aerial plant part.

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