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Research Article

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Chemical profiling and antioxidant activity of commercial milk thistle food supplements

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ABSTRACT

Milk thistle dietary supplements that contain silymarin are widely marketed and used in the U.S.A and other countries for liver enhancement and recovery. To assess different brands of commercially sold milk thistle, 45 products were collected from local stores and analyzed for their silymarin content and antioxidant activities. High performance thin layer chromatography and high performance liquid chromatography coupled with diode array detector and electrospray mass spectrometry were used for establishing a finger printing and for the determination of all of seven major constituents of silymarin in each product. Antioxidant activity was measured as radical scavenging activity using DPPH and by estimating their antioxidant capacity as trolox equivalent. Samples were found to vary widely in their silymarin content, with some samples having none or very low concentrations while silymarin represented higher than 80% of other samples. Antioxidant activity correlated with the overall level of silymarin in each sample, with samples containing the highest relative concentration of taxifolin exhibiting the most effective antioxidant activity.

Keywords: *Silybum marianum*, radical scavenger, foodsupplement, over the counter drugs.

INTRODUCTION

Over-the-counter nutritional or dietary supplements are becoming extremely popular in the United States, Europe and many other countries. As defined by the USA Food and Drug Administration (FDA), a dietary supplement is a product taken by mouth that contains a “dietary ingredient,” which can be vitamin, mineral, herb, amino acid, enzyme, or metabolite. Traditional medicines, including medicinal herbs and their preparations, are used as part of the primary health care for 70-95% of the population in the developing world, while over 70% of the population in developed nations use some form of complementary/alternative medicines [1]. Nearly 50% of older adults regularly use dietary aids [2]. As a result, one recent estimate of the global market for traditional medicines was $83 billion annually with the expectation that this will grow considerably in the coming years [3].

One of the products that have gained tremendous popularity in recent years is milk thistle seed extract, also known as silymarin, which is sold under many different brand names. Silymarin is isolated from the milk thistle plant *Silybum marianum* of the family Asteraceae. The product is advertised as a hepatoprotective, antioxidant, antiradical, and free radical scavenging food supplement and has been used widely for centuries for the protection of the liver from toxic substances, treating liver damage and for the therapy of hepatitis and cirrhosis [4-7]. In addition to its antioxidant properties, it has been reported to have high anti-tumor promoting activity [8] and has been linked to the prevention of skin carcinogenesis [9]. Recent studies have also reported that silymarin is an effective antiviral treatment for hepatitis C virus (HCV) [10]. Silymarin is a mixture of seven major compounds: taxifolin, silychristin,

silydianin, silybin A, silybin B, isosilybin A and isosilybin B [11, 12]. The chemical structures of the seven main active constituents of silymarin are shown in (Fig. 1).

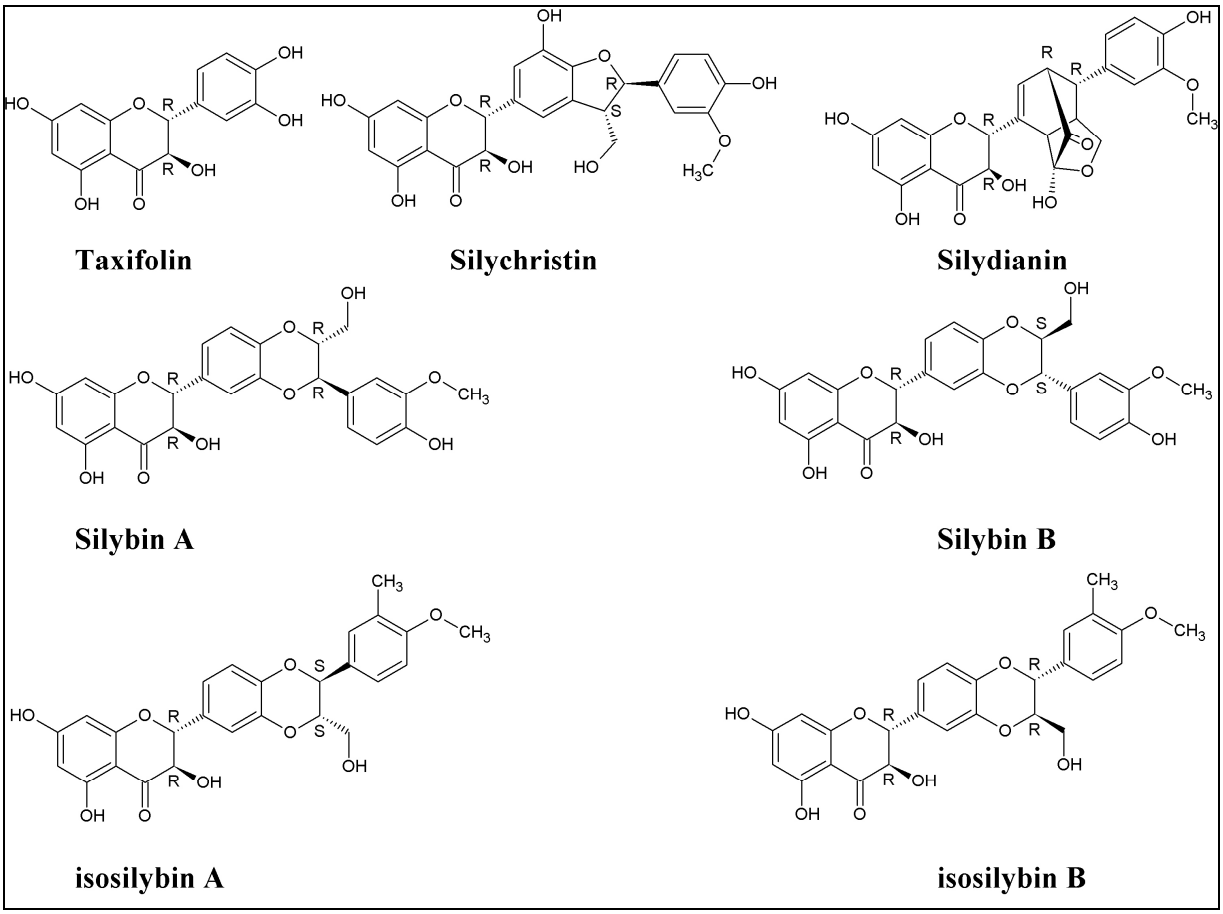


Figure 1. Chemical structure of the major constituents of silymarin.

The complexity of the silymarin product combined with its unregulated manufacturing process has made it difficult to judge the role of silymarin in the treatment of chronic liver diseases. This has been further compounded by the poor documentation of the ingredients in these products, the source of the silymarin or the specific extraction processes used. For example, harvesting herb plants in different geological regions and seasons is well known to affecting the quantities of the chemical components of the plants and potentially the efficacy of the extracts [13-15]. In the absence of specific criteria or guidelines for the judging the quality of silymarin extracts, it is difficult to interpret the majority of previous clinical efficacy studies or determine the individual active components [16, 17]. Therefore, there is an urgent need to establish an analytical protocol for monitoring chemical composition and concentration of each individual constituent in the different silymarin commercial products allowing for the implementation of quality control and standardization of the final extracts. As one effort to initiate this process, we compared the chemical composition of commercially available brands of milk thistle extract for their radical scavenging, antioxidant and the concentration of the seven silymarin constituents in each sample.

Table 1. Commercial milk thistle samples: their identification numbers, sources and brand names.

1
0.5504
g/tablet

2
0.4502
g/tablet

3
0.4975
g/tablet

4
0.3319
g/tablet

5
0.2430
g/tablet

6
0.6789
g/tablet

7
0.2450
g/tablet

8
0.3768
g/tablet

9
0.2786
g/tablet

10
0.5696
g/tablet

11
0.5696
g/tablet

12
0.3466
g/tablet

13
0.5063
g/tablet

14
0.3262
g/tablet

15
0.4867
g/tablet

16
1.0514
g/tablet

17
0.7060
g/tablet

18
0.3443
g/tablet

19
0.4508
g/tablet

20
0.9847
g/tablet

21
0.5090
g/tablet

22
0.6765
g/tablet

23
0.1610
g/tablet

24
0.3939
g/tablet

25
0.3939
g/tablet

26
0.7944
g/tablet

27
0.4569
g/tablet

28
1.3158
g/tablet

29
0.6589
g/tablet

30
1.2484
g/tablet

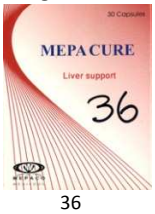
31
0.9610
g/tablet

32
1.5340
g/tablet

33
1.3597
g/tablet

34
0.4228
g/tablet

35
0.3351
g/tablet



36
0.3907
g/tablet

37
1.3371
g/tablet

38
1.2695
g/tablet

39
0.5625
g/tablet

40
0.4259
g/tablet

41
0.3124
g/tablet

42
0.0518
g/tablet

43
0.5075
g/tablet

44
0.4775
g/tablet

45
1.2624
g/tablet

Samples 1 – 24 were collected from USA Markets

Samples 25 to 45 were collected from Egyptian Markets in Cairo.

EXPERIMENTAL SECTION

Chemicals and reagents

All solvents used for HPLC and MS analyses were of chromatographic grade, formic acid and, dimethyl sulfoxide (DMSO) were purchased from VWR International Co. (Sugar Land, Texas). Technical silymarin (>96% pure) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich Inc., Atlanta GA. All commercial over-the-counter milk thistle food supplements samples were obtained from USA and International markets. Samples identification numbers, sources and brand names are shown in (Table1).

Preparation of samples

10 tablets of each commercial sample were randomly taken, crushed and homogenized.Weight of each 10 tablets was recorded and is presented for each brand sample (Table 1).

20 mg of each crushed product were extracted in 5mL of methanol (3 replicate each) and a second batch of 20 mg of each product were separately extracted in 5 mL of DMSO (3 replicate). Extractions were performed in 10 mL sealed tubes at room temperature rotated constantly using a Labnet Labroller II, (Optics Planet Inc. 3150 Commercial Avenue Northbrook Illinois), at maximum speed for 24 hours. Extracts were then filtered and stored in the refrigerator. External calibrated standards were made under the same condition for technical silymarin (SigmaProducts).

Isolation and purification of the individual silymarin compounds

All of the individual compounds of silymarin (Fig. 1) were isolated from silymarin (Sigma Products) using high resolution preparative HPLC Dionex Summit systems (Sunnyvale, CA, USA) equipped with P680 HPLC pump, solvent delivery module, auto sampler, automatic sampler injector, (117- well capacity), controller module, column oven, photodiode array detector (PDA), with data collected and analyzed using Star Chromeleon Chromatography Managing System software (version 6.80). Phenomenex (Luna C18 AXIAP 5 micron) column of 250 mm in length and 21.2 mm in diameter was used for the separation. Fractionation was carried out with a isocratic mobile phase of methanol: 0.1% formic acid in water (60:40 v/v) at a flow rate of20mL/min. Column effluent were split to 1:100 using an QuickSplitTM Flow Splitter (Analytical Scientific Instruments, Richmond, CA 94806), 1 % of the column effluent was directed to the detector while 99 % of the effluent went to the collector. Peaks were detected at 288 nm. Aliquots of silymarin (Sigma) dissolved in DMSO were repeatedly injected in the HPLC using 300 µL per injection containing 100 mg of crude product. To achieve the highest purity each individual peaks were collected manually at its half highest for peak front to half heights of peak ends. Each individual peak collected was examined by analytical LCMS (system described below) to ensure purity of 95% or higher. Purity of individual compounds was evaluated using mass, ultraviolet spectral data, retention times and co-chromatography. Taxifolin (50 mg), silychristin (25 mg), silydianin (20 mg), silybin A (160 mg), silybin B (250 mg), isosilybin A (15 mg), and isosilybin B (10 mg) were obtained.

Analytical HPLC/MS technique

High Performance Liquid chromatography and Mass Detection HPLC/MS were used to determine the chemical composition of each the commercial products. HPLC of Silymarin and commercial samples was performed on Agilent 1100 HPLC/MSD VL using PhenomenexKinetic 2.6µ C_{18} 100A 100x 4.16mm column with electrospray (ES) ionization. Methanol, water, and formic acid(90:10:1)was used as mobile phase A and 0.1% formic acid for mobile phase B at a gradient flow rate of 0.5 mL/min. Solvent A = 55% 0.1 formic acid, solvent B = 45% 90:10:1MeOH: H_2O: Formic acid. Starting at time 0, 45% B, at 15 min, increase to 65% B, at 15.5min decrease to 45% B and hold at 45% B for 5min run end 20.5 minute and diode array detection at 288 nm. Mass Spectroscopy was performed using single ion monitoring in the positive ESI mode for ions of m/z 327 (M+Na) for taxifolin and m/z 505 (M+Na) for all other compounds. Mass detection conditions were: quasi molecular ions dwell time of 294ms, nitrogen was used both as drying gas and nebulizing gas at flow rates of 12 L/min and 35 (psig). The temperature of the drying gas was set to 350ºC. Data collection was handled using Chemstation V. B.04.02. All samples (45 x 3 replicate) were analyzed by injecting 5 µL of sample in DMSO and the analysis was repeated three times to calculate the average and standard deviations.

Quantitative analysis and calibration of the silymarin constituents

Standard calibration solutions of all of the seven individual compounds were prepared separately at concentrations of 10, 20, 30, 40, 50 and 100 µg/mL. 5 µL of each compound were injected in the LCMS system at the condition

described above. Calibration curves were prepared by plotting peak areas versus amount of chemical to calculate detector response factors both at UV absorbance at 288nm and at mass ion current peak area of M+Na single ion respond.

High performance thin layer chromatography (HPTLC)

Solutions of the commercial silymarin in methanol were applied to silica gel plates in 10 mm bands at 40µg/band (HPTLC Silica Gel 60F254 20x10 Merck K GaA 6427 Darmstadt, Germany). Each plate was developed with chloroform, acetone and formic acid 75:16.5:8.5 v/v. Plates were dried and observed at a UV of 254 and 366 nm. Plates were also derivatized in 1% vanillin reagent, (1g of Vanillin, 100mL of ethanol mixed with 5mL of conc. Sulfuric acid and 95mL of ethanol), and the plate was dried in the oven at 100ºC for 5 minutes, after which spots were visualized under white light and 366nm wavelengths. All applications including spotting, development, derivatization and imaging were performed using the Camag automated instruments (Automatic TLC Sampler 4, Rep Pro Star 3 and ADC 2 Automatic Developer) and win Cats-Planar Chromatography manager software version 1.4.4.6337 (CAMAG USA. Denver CO).

Free radical-scavenging activity: DPPH Test

Free radical-scavenging activity of each commercial sample was carried out using the DPPH scavenging method [20]. The antioxidant activity was carried out using Perkin Elmer Victor 4X micro plate reader performed in a 96 well plate using a total volume of 200 µL methanol containing 0.004 µg DPPH and samples aliquots at a series of concentrations of 1, 10, 20, 40, 60, 80, 200, 400, 800 and 2000 µg/mL. The test was repeated at all concentration of each sample in triplicate. DPPH solutions at the same concentration without the tested samples were used as control. Each sample, as well as each control was analyzed in triplicates. After filling the well plates, they were incubated in the dark with continuous shaking for 30 minutes followed by reading the absorbance at 520nm. The free radical scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

$$\% \text{ inhibition} = 100(A_{blank} - A_{sample}) / A_{blank}$$

Where A_{sample} is the absorbance of the sample and A_{blank} is the absorbance of the blank. Inhibition % was plotted against concentration and the EC_{50} was calculated graphically.

Trolox-equivalent antioxidant capacity assay

Trolox-equivalent antioxidant capacity (TEAC) of the commercial silymarin samples was carried out using the procedure from Antioxidant Assay Kit item No. 709001 from Cayman Chemical Company1180 E. Ellsworth Rd. Ann Arbor, MI 48108. The 45 commercial silymarin samples were prepared by removing 100µL of the stock preparation (20mg commercial silymarin / 5mL of DMSO) and adding it to 400µL HPLC grade water. On a 96 well plate, 10 µL of this preparation was removed and added to 10 µL of metmyoglobin, 150 µL of chromogen and 40 µL of hydrogen peroxide mixture for a total of 210 µL in each well. The plate was covered and place on a shaker for five minutes and read at 750 nm on a Perkin Elmer Victor X4 2030 Multilabel Reader (710 Bridgeport Avenue Shelton, Connecticut 064844794). The absorbance was plotted as a function of the final Trolox concentration (mM) according to the assay.

Antioxidant (mM) = Sample absorbance- (y- intercept)/ Slope x Dilution

RESULTS AND DISCUSSION

Determination of total silymarin and concentration of the individual constituent

All of the seven major components of silymarin were isolated and purified (Fig. 2). Detector response curves for UV absorbance at 288 nm and SIM of *m/z* (M+Na ion) are shown in (Fig. 3) and both had a coefficient of determination (r^2) values exceeding 0.999.

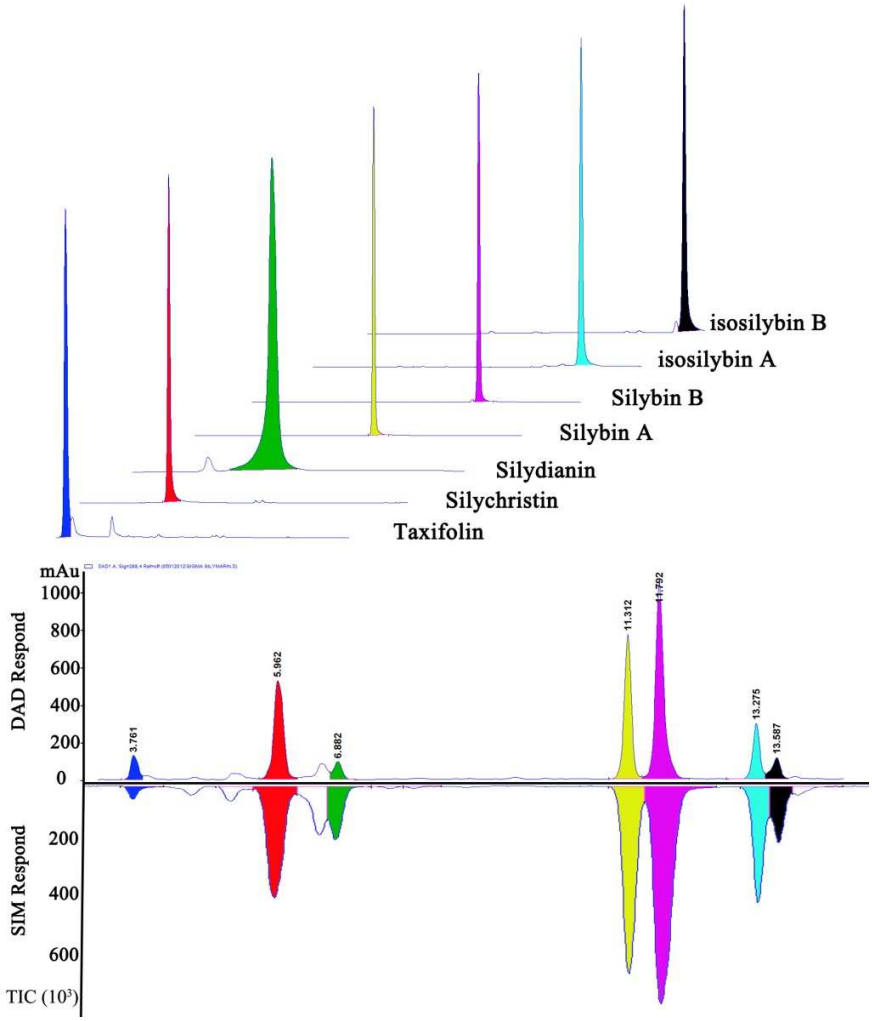


Figure 2. HPLC chromatograms of silymarin and all of the individually purified compounds.

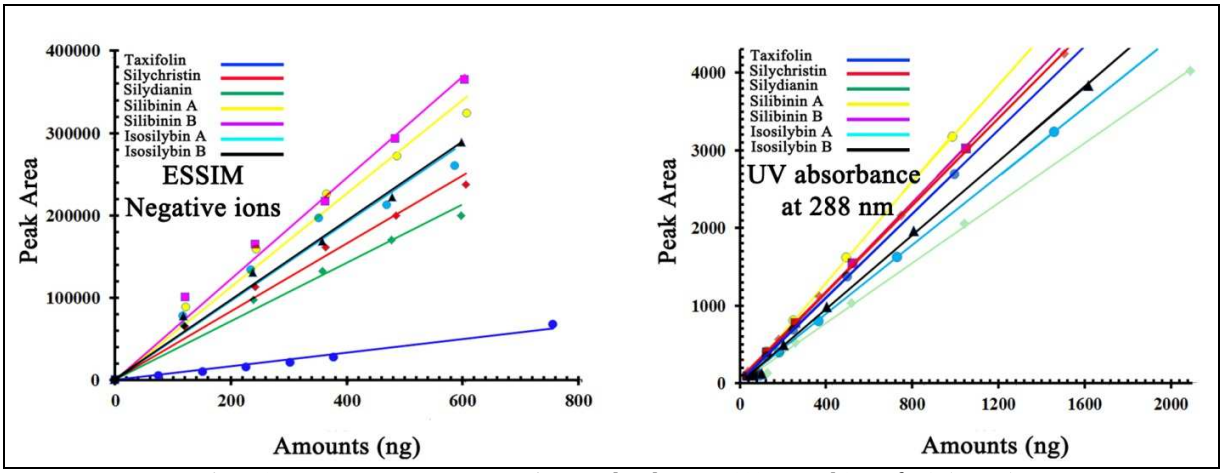


Figure 3. Detector responses curve using UV absorbance at 288 nm and SIM of m/z (M+Na).

Quantitative analysis of all of the seven compounds in all of the selected commercial samples was performed with 3 replicate extracts and three analytical measurements. Average concentrations and standard deviation of all compounds in each sample are shown in Table 2 as mg of isomer per gram of tablet.

The results also show that the different silymarin constituents exhibited different responses to each method of measurement as injecting the same amounts of individual compounds produced different peak areas depending on the method of measurement. These results suggest that previous analyses of silymarin extract composition may have been misleading as the calculations were based solely on relative peak areas without consideration that the specific factors may exhibit varying responses.

HPTLC profiles of commercial preparations

The TLC profiles of all 45 collected samples are shown in (Fig. 4). Taxifolin, silychristin and silydianin were fully resolved in the TLC, however the silybin and isosilybin isomers did not separate and co-chromatogramed as one band. Samples manufactured in the USA all showed theabsence or very small concentrations of non-silymarin bands with R_f values higher than the R_f value of the silybin or isosilybin isomers, on the other hand samples from Egypt showed high level of bands with higher R_f values and bands near solvent front indicating that theirpreparations contain other chemicals than silymarin. One sample from the USA market (sample 8 in Fig. 4) and 3 samples from other countries (29, 42 and 45) did not contain any silymarin. The cluster presenting taxifolin, silychristin and silydianin is greatly different among all samples. The variation in the TLC profiles among different samples could be attributed to sources of milk thistle, geographical locations, the techniques for extraction, and/or the formulation of the products.

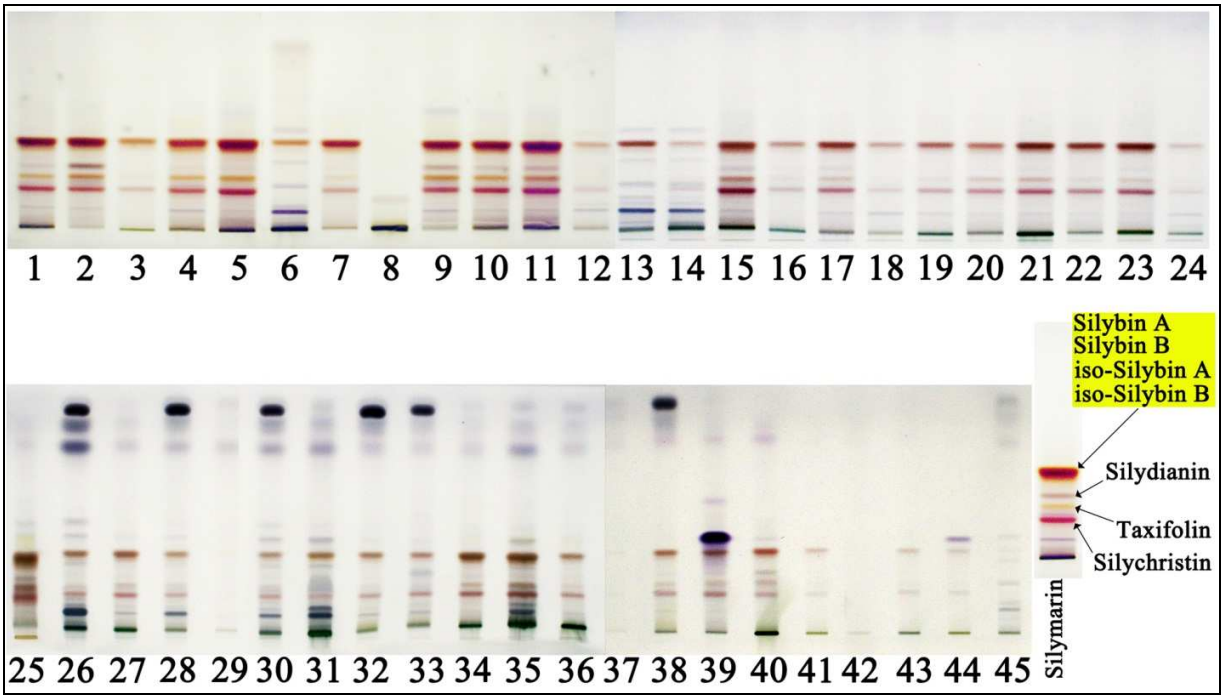


Figure 4. HPTLC profiles of all collected samples

Total amounts of silymarin vary greatly among the different samples with 4 samples containing no silymarin at all, 8 samples containing less than 100 mg/g, 9 samples containing between 100 and 200 mg/g, 6 samples containing between 200 and 300 mg/g, 8 samples containing between 300 and 400 mg/g, 5 samples containing between 400 and 500 mg/g, 2 samples containing between 500 and 600 mg/g and 3 samples containing higher than 600 mg/g as shown in Table 3. The relative ratios between the seven major compounds were also found to vary greatly among different samples.

DPPH free radicals scavenging activity

The change in absorbance produced by reduced DPPH was initially used to evaluate the ability of the silymarin samples to act as free radical scavengers with the lower the value of EC_{50} indicating higher anti-radical power. Thirteen samples showed an $EC_{50}$of less than 1 µg/mL, 14 samples were shown to have an EC_{50} of 1 to less than 2 µg/mL, 11 samples had an EC_{50}greater than 2 but less than 4µg/mL, and 7 samples of had an EC_{50}greater than 4

µg/mL (Table 3). Notably, the EC_{50} in all cases was found to be closely related to the total amount of silymarin in each tablet (r^2=0.9189; Fig. 5).

Table 2. Concentration of silymarin compounds in the tested commercial samples.

ID#	Concentration of silymarin constituents expressed as mg/g of tablets						
	Taxifolin	Silychristin	Silydianin	Silybin A	Silybin B	Isosilybin A	Isosilybin B
1	13.1 ± 0.3	67.9 ± 1.1	13.8 ± 0.6	56.2 ± 0.8	97.0 ± 1.3	34.9 ± 0.8	15.1 ± 0.7
2	13.2 ± 0.2	71.0 ± 4.4	65.6 ± 1.0	58.4 ± 0.4	103.9 ± 0.9	48.4 ± 1.0	28.5 ± 0.4
3	3.4 ± 0.0	19.8 ± 0.2	2.5 ± 2.7	17.0 ± 0.2	29.4 ± 0.3	10.9 ± 1.0	4.0 ± 0.1
4	14.6 ± 0.2	92.7 ± 0.8	19.5 ± 0.7	63.2 ± 1.3	118.1 ± 2.4	48.8 ± 1.3	22.5 ± 1.1
5	37.8 ± 0.7	155.5 ± 0.4	11.4 ± 0.3	144.9 ± 0.5	237.9 ± 0.8	78.0 ± 1.8	25.7 ± 0.7
6	1.3 ± 0.2	1.4 ± 1.3	3.9 ± 1.7	15.4 ± 0.4	21.0 ± 0.5	2.8 ± 2.5	0.8 ± 0.1
7	5.8 ± 0.7	37.4 ± 0.2	5.0 ± 3.9	72.8 ± 0.3	111.3 ± 0.9	22.9 ± 0.6	9.8 ± 0.4
8	Not Detected						
9	16.7 ± 0.2	80.2 ± 0.7	30.3 ± 9.6	65.4 ± 0.8	115.9 ± 1.5	46.6 ± 0.4	25.3 ± 0.3
10	20.3 ± 0.3	106.6 ± 1.5	26.9 ± 2.7	87.6 ± 1.5	147.0 ± 2.8	51.7 ± 2.0	21.8 ± 1.3
11	27.1 ± 0.2	146.5 ± 0.7	39.7 ± 2.7	137.6 ± 0.9	228.0 ± 0.9	79.2 ± 1.6	32.3 ± 0.6
12	1.9 ± 0.0	13.4 ± 0.0	3.6 ± 1.2	9.8 ± 0.3	16.9 ± 0.4	6.8 ± 0.3	2.8 ± 0.3
13	0.9 ± 0.0	1.1 ± 0.1	1.4 ± 0.8	51.4 ± 0.9	70.9 ± 1.3	3.7 ± 0.3	2.5 ± 0.4
14	02.1 ± 0.1	12.9 ± 0.1	3.9 ± 0.9	11.3 ± 0.1	19.5 ± 0.1	8.2 ± 1.0	3.4 ± 0.1
15	46.3 ± 5.2	183.5 ± 0.7	38.2 ± 13.7	43.7 ± 0.0	118.0 ± 0.6	90.0 ± 1.6	36.7 ± 1.0
16	3.1 ± 0.1	14.2 ± 0.1	3.9 ± 0.8	11.7 ± 0.0	19.9 ± 0.1	9.1 ± 1.4	3.7 ± 0.0
17	12.1 ± 0.1	58.8 ± 0.1	17.9 ± 0.7	43.9 ± 0.6	78.4 ± 0.7	30.3 ± 1.0	14.2 ± 0.5
18	2.5 ± 0.0	15.5 ± 0.1	3.7 ± 1.7	12.5 ± 0.0	21.3 ± 0.1	8.2 ± 1.0	3.1 ± 0.0
19	3.2 ± 0.1	23.4 ± 1.0	5.2 ± 2.2	17.0 ± 0.0	29.0 ± 0.1	6.5 ± 2.8	4.5 ± 0.2
20	11.2 ± 0.1	56.1 ± 0.1	14.1 ± 0.1	26.1 ± 0.0	52.1 ± 0.2	31.0 ± 0.3	11.4 ± 0.1
21	12.2 ± 0.3	75.3 ± 0.2	11.1 ± 0.1	47.9 ± 0.5	85.5 ± 1.0	31.4 ± 0.3	15.0 ± 0.7
22	9.0 ± 0.1	53.1 ± 0.4	4.9 ± 0.1	45.5 ± 0.5	78.2 ± 0.9	26.7 ± 0.6	7.7 ± 5.1
23	16.2 ± 0.3	82.1 ± 0.3	8.0 ± 0.1	63.9 ± 0.5	111.4 ± 0.7	36.4 ± 0.7	14.4 ± 0.5
24	2.7 ± 0.0	12.8 ± 0.1	4.2 ± 0.3	10.6 ± 0.4	18.3 ± 0.8	7.4 ± 0.7	3.2 ± 0.4
25	12.7 ± 0.0	87.5 ± 0.3	54.6 ± 0.4	70.2 ± 0.9	120.7 ± 1.9	51.9 ± 1.8	27.2 ± 0.8
26	5.1 ± 0.1	16.0 ± 0.0	1.6 ± 0.2	13.6 ± 0.3	23.8 ± 0.6	8.4 ± 0.3	3.6 ± 0.3
27	5.8 ± 0.1	48.2 ± 0.0	11.2 ± 3.2	39.1 ± 0.2	68.4 ± 0.6	27.3 ± 0.2	11.6 ± 0.2
28	8.7 ± 0.1	28.5 ± 0.2	7.9 ± 0.1	18.6 ± 0.1	33.3 ± 0.3	14.7 ± 1.0	6.3 ± 0.1
29	Not Detected						
30	3.5 ± 0.0	27.4 ± 0.3	3.9 ± 0.1	11.2 ± 0.1	23.9 ± 0.2	14.7 ± 1.0	6.0 ± 0.0
31	2.0 ± 0.0	24.2 ± 0.1	6.3 ± 0.1	21.7 ± 0.0	37.3 ± 0.1	11.9 ± 1.0	4.7 ± 0.0
32	4.5 ± 0.1	19.9 ± 0.1	2.6 ± 0.1	19.0 ± 0.4	31.7 ± 0.8	10.1 ± 0.4	3.9 ± 0.3
33	7.1 ± 0.2	36.4 ± 0.1	11.0 ± 0.1	22.0 ± 0.2	40.9 ± 0.3	18.6 ± 1.0	7.9 ± 0.1
34	10.0 ± 0.1	63.8 ± 0.1	14.4 ± 0.4	89.9 ± 0.9	139.2 ± 1.5	33.6 ± 1.2	15.0 ± 0.2
35	14.9 ± 0.1	65.1 ± 0.2	9.3 ± 0.1	51.0 ± 0.2	88.2 ± 0.4	33.2 ± 0.3	14.3 ± 0.1
36	3.0 ± 0.1	19.8 ± 0.2	2.1 ± 0.1	11.7 ± 0.2	20.5 ± 0.4	7.1 ± 2.4	3.6 ± 0.1
37	5.4 ± 0.1	20.4 ± 0.1	5.3 ± 0.1	15.6 ± 0.0	28.3 ± 0.1	12.1 ± 0.2	5.2 ± 0.0
38	11.3 ± 0.2	44.7 ± 0.1	7.5 ± 0.2	32.3 ± 0.1	58.2 ± 0.1	22.5 ± 0.0	8.4 ± 0.1
39	8.8 ± 0.1	49.8 ± 0.4	7.8 ± 0.2	29.1 ± 0.1	53.2 ± 0.1	24.1 ± 0.5	10.9 ± 0.4
40	6.3 ± 0.1	40.9 ± 0.4	33.7 ± 0.8	35.8 ± 0.5	61.6 ± 0.8	28.5 ± 0.6	15.7 ± 0.6
41	10.8 ± 0.1	75.5 ± 0.2	15.7 ± 0.3	54.0 ± 0.1	97.6 ± 0.5	40.9 ± 0.6	17.5 ± 0.4
42	Not Detected						
43	10.9 ± 0.1	74.4 ± 0.3	19.7 ± 3.2	41.7 ± 0.2	80.1 ± 0.8	38.5 ± 1.3	16.8 ± 0.3
44	12.2 ± 0.1	61.0 ± 0.6	23.9 ± 0.5	36.0 ± 0.6	67.7 ± 1.2	32.3 ± 1.0	13.9 ± 0.5
45	Not Detected						

Total antioxidant capacity astrolox equivalent(TEAC)

Silymarin samples were also assayed for their total antioxidant capacity as equivalent to trolox (TEAC). TEAC values of all of the commercial samples varied greatly with samples showing no antioxidant capacity to samples with 9 or higher mmoles trolox equivalent/g (Table 3). The average concentration of silymarin in the samples when plotted against their average TEAC (Fig. 5) also showed good correlation (r^2=0.9796). Both the DPPH and TEAC assays appear to give similar results: lowest DPPH EC_{50} values correlated with the highest TEAC values, while highest DPPH EC_{50} values correlated with the lowest TEAC values (Table 3, Fig. 5). However, the DPPH test showed a broader range of linearity, especially at the highest silymarin concentrations (Fig. 5). Importantly, when

correlating the isomer components of silymarin to the antioxidant activity using the DPPH values, samples with the highest relative concentration of isomertaxifolin were found exhibit the most effective antioxidant activity.

Table 3.Total silymarin concentration and antioxidant activity in the tested samples.

ID #	Total Silymarin mg/gram tablet	Antioxidant Activity	
		DPPH EC_{50} µg/mL	Trolox Equivalent mmole/g
1	366.7 ± 0.8	1.08 ± 0.02	9.0 ± 0.1
2	478.5 ± 1.1	0.98 ± 0.02	9.3 ± 0.3
3	107.0 ± 0.5	2.97 ± 0.02	5.3 ± 0.2
4	467.4 ± 1.1	1.00 ± 0.00	8.9 ± 0.5
5	849.9 ± 0.8	0.45 ± 0.00	9.0 ± 0.7
6	57.8 ± 0.9	0.98 ± 0.13	4.1 ± 2.2
7	326.0 ± 1.0	2.00 ± 0.00	7.7 ± 0.8
8	Not detected	0.20 ± 0.00	9.5 ± 0.5
9	467.8 ± 1.9	1.03 ± 0.02	8.7 ± 0.4
10	568.3 ± 1.7	0.92 ± 0.02	9.2 ± 0.4
11	848.7 ± 1.1	0.25 ± 0.01	9.1 ± 0.5
12	67.7 ± 0.4	4.40 ± 0.00	3.4 ± 0.5
13	162.4 ± 0.5	6.13 ± 0.09	4.8 ± 0.7
14	75.0 ± 0.2	1.20 ± 0.00	5.8 ± 0.9
15	685.1 ± 3.3	0.30 ± 0.00	9.4 ± 0.4
16	81.2 ± 0.3	2.37 ± 0.12	4.0 ± 0.3
17	314.9 ± 0.5	1.08 ± 0.02	8.3 ± 0.5
18	82.4 ± 0.3	3.03 ± 0.05	6.6 ± 1.4
19	109.5 ± 0.9	2.17 ± 0.05	7.6 ± 1.5
20	248.5 ± 0.1	1.00 ± 0.00	7.5 ± 0.6
21	341.9 ± 0.5	0.90 ± 0.00	8.9 ± 0.5
22	276.8 ± 1.1	1.20 ± 0.00	7.7 ± 0.1
23	408.4 ± 0.4	1.10 ± 0.00	9.2 ± 0.1
24	72.6 ± 0.4	4.23 ± 0.12	4.0 ± 0.8
25	522.8 ± 0.9	0.95 ± 0.04	9.0 ± 0.2
26	88.6 ± 0.3	3.13 ± 0.19	1.9 ± 1.2
27	260.8 ± 0.4	1.20 ± 0.08	7.4 ± 0.3
28	145.1 ± 0.1	2.80 ± 0.49	3.8 ± 1.0
29	Not detected	>10.00	0.0
30	111.9 ± 0.1	2.27 ± 0.25	3.9 ± 1.4
31	132.8 ± 0.1	4.08 ± 0.23	2.6 ± 1.1
32	113.2 ± 0.3	3.87 ± 0.57	1.7 ± 0.9
33	177.1 ± 0.1	0.31 ± 0.00	4.2 ± 0.7
34	450.2 ± 0.6	1.10 ± 0.07	8.0 ± 1.1
35	339.5 ± 0.2	0.95 ± 0.04	7.5 ± 1.0
36	83.6 ± 0.5	2.17 ± 0.05	5.3 ± 0.8
37	113.2 ± 0.1	1.18 ± 0.44	2.8 ± 0.9
38	227.6 ± 0.1	2.07 ± 0.09	5.4 ± 0.7
39	226.3 ± 0.3	1.67 ± 0.26	5.8 ± 0.8
40	274.3 ± 0.5	2.13 ± 0.05	7.4 ± 0.5
41	383.8 ± 0.3	1.03 ± 0.05	8.2 ± 0.8
42	Not detected	>10.00	0.0
43	346.9 ± 0.9	1.13 ± 0.09	7.6 ± 1.3
44	303.8 ± 0.6	1.15 ± 0.04	7.4 ± 0.9
45	Not detected	>10.00	0.0

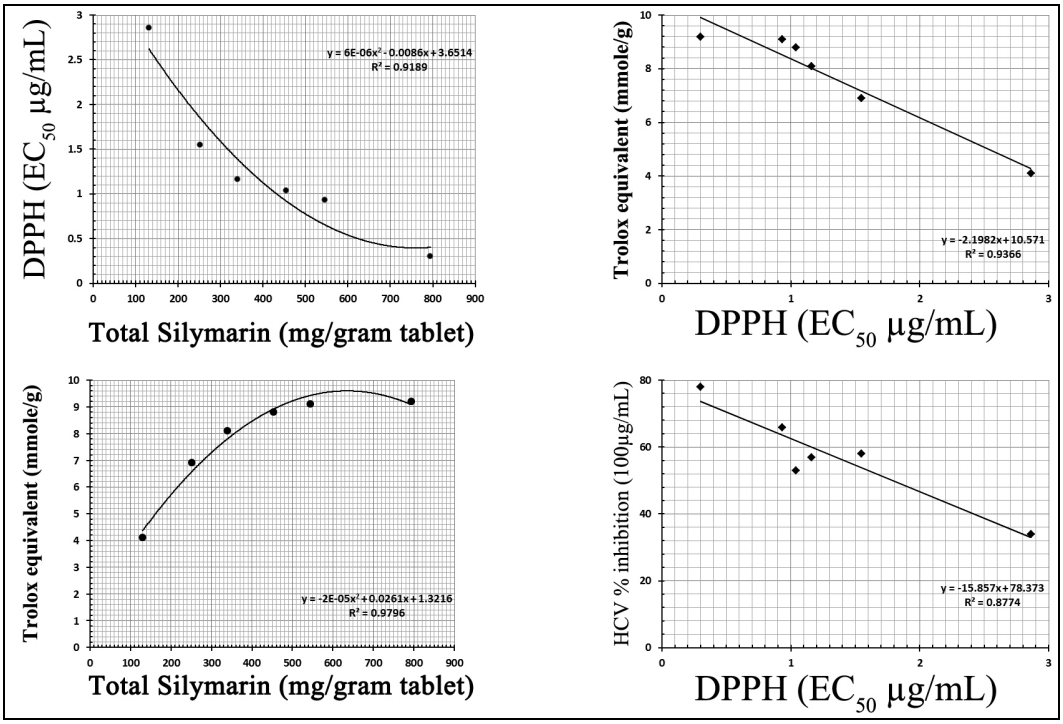


Figure 5. Correlation between total amounts of silymarin in the tested samples verses their trolox equivalent antioxidant capacity and DPPH radical scavenging EC_{50}.

CONCLUSION

Our findings on different commercial preparations of silymarin are significant in light of the fact that oxidative stress is a secondary effect of many human diseases [24]. As such, consumption of antioxidant-containing foods can potentially reduce oxidative damage to cells and could have the general effect of protecting the immune system. However, the different commercial sources tested varied greatly in overall silymarin levels and levels of the key compounds, which also varied by the method of measurement. Anti-oxidant activity exhibited significant correlation with silymarin levels and thus likewise varied greatly among the different silymarin products. While the ratio of the different silymarin compounds did vary among the various products, the level of the different compounds was still highly correlative making the assessment of activity of individual compounds difficult, but preliminarily it appears that measurement of taxifolin concentrations in silymarin products may be an effective way of measuring the antioxidant potency of products from different suppliers. However, until standardized isomer quantification and biological activity assays are implemented, critical evaluation of commercial sources of silymarin must be carefully considered in order to find the most effective.

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