

(RESEARCH ARTICLE)



Detection of total flavonoid, antioxidant activity and HPLC analysis of methanolic extract of *Silybum marianum* seeds

Farah TO AL-Jumaili ^{1,*}, Asmaa Obaid Ismeel ¹ and Linda fawzi abdulattar ²

¹ Collage of Biotechnology, Al-Nahrain University, Baghdad, Iraq.

² Ministry of science and technology, Baghdad, Iraq.

International Journal of Biological and Pharmaceutical Sciences Archive, 2022, 03(02), 102–107

Publication history: Received on 03 March 2022; revised on 17 April 2022; accepted on 17 April 2022

Article DOI: <https://doi.org/10.53771/ijbpsa.2022.3.2.0034>

Abstract

Silybum marianum considered as an ancient herb promising plants. In this work, these study was design to assess total flavonoids, anti-oxidant and HPLC analysis of *Silybum marianum* plant extract by methanol *in vitro*. Total flavonoids had been measured in the extract that about 201.6667 ± 1.52753 mg/ml. anti-oxidant activity of methanolic plant extract *Silybum marianum* *in vitro* was estimated by assesment of reductive ability, in concentrations that be tested (0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mg/ml) , the absorbance had been increased significantly , results of HPLC analysis of plant contain (Ascorbic acid ,Gallic acid Catechin, Rutin, Quercetin, Apigeninand Kaemp ferol) in concentration (0.839, 0.0918, 0.628, 2.989, 0.010, 0.0680, 0.228) ppm respectively.

Keywords: *Silybum marianum*; HPLC; Total flavonoids; anti-oxidant activity; Ascorbic acid

1. Introduction

Silybum marianum fig (1) had general names cardus marianus, milk thistle [1]. Plant species was a biennial or annual plant of Asteraceae family. This fully typical thistle had been purple to red flowers .Originally it was now found in all world [2].



Figure 1 *Silybum marianum* plant

* Corresponding author: Farah TO AL-Jumaili
Collage of Biotechnology, Al-Nahrain University, Baghdad, Iraq.

Silybum marianum extract was prepared from the seeds, which had been contained about 4–6% silymarin and 20–35% fatty acids [3]. Silymarin was a mixture of polyphenolic molecules, including 7 related flavonolignans (silybin a, silybin b, isosilybin a, isosilybin b, silychristin, isosilychristin, silydianin) and one flavonoid Silibinin [4].

This plant had been grown in a straight herb 30 to 200 cm and have a total conical shape. It had been estimated in maximum base diameter was 160 cm. The stem had been splined and might covered in a cottony light pile.[5].

The leaves had been long to lanceolate and 15–60 cm and typically lobed pinnately. They were flowered from June to August. The flower head was rounded by bract like hair, appendages was spine-ended, tipped with a yellow thick spine, [6].

2. Material and methods

2.1. Plant collection and identification

The aerial parts of the plant had been collected from the local markets during September (2020), and was previously identified by National Herbarium of Iraq.

2.2. Preparation of plant extract

Plant extract of *Silybum marianum* by methanol was prepared according to [7] 50 grams of the seeds from plant powdered and extracted with eighty % methanol (using 250 ml) at 65°C for three hours that by used Soxhlet device. The extract solution had been concentrated to dryness under lower pressure in a rotary evaporator to become crude dried extract, it had been frozen at -20°C until used experimental doses.

2.3. Determination of Total Flavonoids

Total flavonoids contented evaluated by spectrophotometrically resolute in *Silybum marianum* methanolic extract by way of rutin standard equal used $AlCl_3$ colorimetric method [8].

The methanolic plant extract in weight (3.2 mg) was once as soon as dissolved among five ml in regard to fifty % methanol, accompanied with the aid of addition concerning one ml concerning a five percentage (w/v) ($NaNO_3$) solution. After six minutes, one ml involving a ten percentage (w/v) aluminium chloride answer was once brought yet the combination used to be allowed in accordance with remain because of a in addition five minutes before ten ml in regard to a ten % (w/v) NaOH reply used to be added. The mixture used to be committed up in imitation of fifty ml along DW but mixed well. Then the absorbance used to be modest at 450 nm with a spectrometer below fifteen min. A similar process used to be applied in accordance with vii after concentrations (two and half, five, ten, twenty, forty since eighty μg) concerning rutin as like standard, yet ancient in imitation of put together standard curve

2.4. Determination of free radical scavenging assay method

This method was complete by estimate the reductive ability by using [9], used to be adopted in conformity with consider the reductive ability, inside namely 1 ml regarding every attention of the drive into put off [0.02, 0.04, 0.08, 0.16, 0.32 yet 0.64 mg/ml] used to be combined collectively along one ml as regards 0.2M phosphate clink (pH 6.6) after one half ml concerning one % potassium ferricyanide, afterward then incubated at 50°C due to the fact on 20 minutes. Then, 1ml related to ten % trichloroacetic acid style was once introduced below the combination after quit the reaction. The mixture was once centrifuged due to the fact x min at 3000 rpm, but 2.5 ml above the supernatant used to be blended including two ml of distilled water or 0.5 ml over anew.

2.5. HPLC analysis of methanolic extract of *Silybum marianum*

Hplc analysis was done to detect the most active compound found in methanolic extract of *Silybum marianum* seeds as compared with standard ascorbic acid, gallic acid, catechin, rutin, quercetin, apigenin, and kaempferol.[10]

Hplc analysis was done using column ODS(250*4.6 ID)mm, 5 mm particle size; the mobile phase A=0.5 % formic acid B=Acetonitrile, Volume Inj =20 μl

With Flow Rate =1.0 ml/min.

2.6. Gradient programme

| | | |
|----------|-------|-----|
| 0.01 min | B.con | 10% |
| 5 min | B.con | 40% |
| 10 min | B.con | 80% |
| 12 min | B.con | 40% |
| 14 min | B.con | 10% |
| 15 min | B.con | 10% |
| 15.1 | stop | |

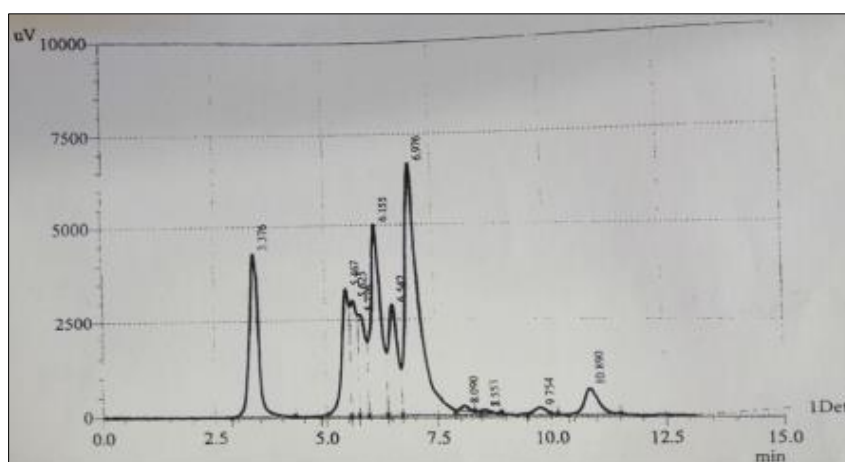


Figure 2 Standard curve for determination of HPLC analysis

3. Results and discussion

3.1. Total Flavonoid

Total flavonoids of methanolic extract of *Silybum marianum* seeds are presented in the table(1) Total flavonoids had been spectrophoto-chemically estimated extract of *Silybum marianum* as rutin equivalent. The methanolic extract presented to contain 201.6667 ± 1.52753 $\mu\text{g/ml}$ flavonoids.

Table 1 Total Flavonoide of *Silybum marianum* methanolic extract

| Total Flavonoid | Mean \pm Std |
|-------------------------|------------------------|
| <i>Silybum marianum</i> | 201.6667 ± 1.52753 |

3.2. Free radical scavenging (Reductive ability)

At six concentration had been tested (0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mg/ml), of *Silybum marianum* extract then absorbance was matured that presented increase significantly ($P \leq 0.001$) as compared with trolox (vitamin E), result showed that the methanolic plant extract was more active than trolox in the reductive ability, that in 0.64 was 4.24667 ± 0.001528 mg/ml while in trolox was 0.211 ± 0.015 mg/ml and in concentration 0.02 was 1.07333 ± 0.006028 mg/ml while in trolox was 0.100 ± 0.001 mg/ml.

Table 2 Reductive ability of *Silybum marianum* methanolic extract

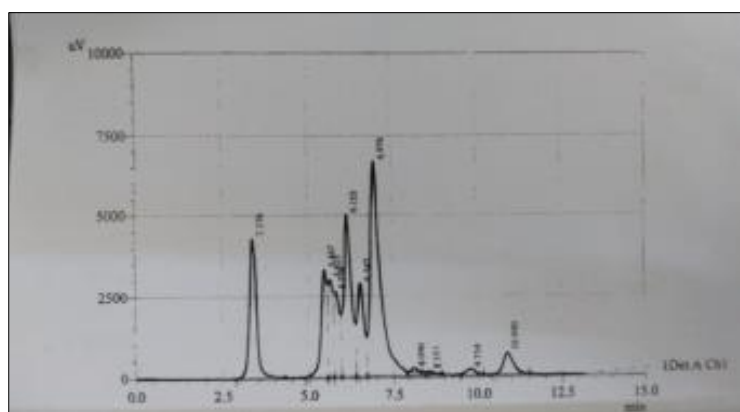
| Con. (mg/ml) | Absor. (Mean \pm SD) | |
|--------------|-------------------------|-------------------|
| | <i>Silybum marianum</i> | Trolox(Vitamin E) |
| 0.64 | 4.24667 \pm 0.001528 | 0.211 \pm 0.015 |
| 0.32 | 3.55233 \pm 0.180736 | 0.132 \pm 0.007 |
| 0.16 | 2.62667 \pm 0.003055 | 0.114 \pm 0.004 |
| 0.08 | 1.60433 \pm 0.001155 | 0.108 \pm 0.001 |
| 0.04 | 1.24367 \pm 0.009074 | 0.101 \pm 0.001 |
| 0.02 | 1.07333 \pm 0.006028 | 0.100 \pm 0.001 |

4. HPLC analysis

HPLC was played important role in the branch of pharmaceutical industries and analysis, it was used to analysis detect and products rawing resident that had been used to make them. HPLC analysis of plant contain (Ascorbic acid, Gallic acid Catechin, Rutin, Quercetin, Apigenin and Kaempferol) in concentration 0.839, 0.0918, 0.628, 2.989, 0.010, 0.0680, 0.228 ppm respectively.

Table 3 HPLC analysis of *Silybum marianum* methanolic extract

| Name of | Ret. time of standard | Area of standard | Ret. time of Sample | Area of Sample | Conc. of sample ppm |
|---------------|-----------------------|------------------|---------------------|----------------|---------------------|
| Ascorbic acid | 3.322 | 36541 | 3.376 | 61372 | 0.839 |
| Gallic acid | 5.735 | 158938 | 5.776 | 29200 | 0.0918 |
| Catechin | 6.276 | 64832 | 6.155 | 81550 | 0.628 |
| Rutin | 6.841 | 23772 | 6.976 | 142146 | 2.989 |
| Quercetin | 8.079 | 8160 | 8.090 | 1486 | 0.0910 |
| Apigenin | 10.222 | 26691 | 9.754 | 3635 | 0.0680 |
| Kaempferol | 11.180 | 31184 | 10.890 | 14244 | 0.228 |

**Figure 3** Standard curve for determination the concentration chemical composition of sample in HPLC analysis

The plant was cultured in all over the world for the therapeutic potential of its seeds. The essential active compound in the plant seeds was flavonoid known as silymarin, which was broadly used in redeveloping damaged hepatic tissues

(11, 12). Polysaccharides are the main agent that had been used as antioxidant of medicinal plants and the antioxidant effective of polysaccharides that had been extracted from many plants had been studied [13, 14]. In addition flavonoids, existed in several eaten of plant sources and comprised vital secondary metabolites with antioxidant,

Antioxidant and hepatoprotective effectiveness of *Silybum marianum L.* plants had been studied [15]. the antioxidative activity of *Silybum marianum L.* and its relationship with plant growth and development. The results showed that the maximum antioxidant effectiveness of leaves could be acquired from 80-day-old plants [16].

Results suggested that the chance antioxidant activity of poly phenolic compounds might be related to regulate ROS resone for free radicals in tumors [17,18]. Flavonoids are generally thought to be having free radical scavenging and antioxidant effects.

Milk thistle, due to their antioxidant work, has been found for preventing a rise in both pancreatic lipid peroxidation and plasma glucose in rats with hyperglycemic. Similarly, hyperplasia of islet of langerhans were reported in mice treated with alcohol after treatment with methanolic extract milk thistle [19].

The phenolic and flavonoid compounds were among the chief pharmaceutical components of therapeutic plants. These compounds are considered as effective anti-oxidant sources .Further studies exposed that natural products like phenolics and flavonoids had been observed to be effective scavengers of free radicals and inhibitor lipid peroxidation [20].

5. Conclusion

Silybum marianum plant extract with Total flavonoids about 201.6667±1.52753 mg/ml. anti-oxidant activity of methanolic plant extract *Silybum marianum* in vitro was tested in all concentration (0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mg/ml) the absorbance had been increased significantly, results of HPLC analysis of plant contain (Ascorbic acid, Gallic acid ,Catechin, Rutin, Quercetin, Apigenin and Kaempferol) in concentration (0.839, 0.0918, 0.628, 2.989, 0.010, 0.0680, 0.228) ppm respectively.

Compliance with ethical standards

Acknowledgments

The authors would like to thank all the authors who were involved in the research and compiled the results of this research.

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

References

- [1] Patel CJ, Tyagi S, Kumar U, Patel S, Patel Ph, Bharat C. Clinical benefits of milk thistle (*silybum marianum*): a recent review. *Journal of Drug Discovery and Therapeutics*. 2013; 1(1): 08-11.
- [2] Karkanis, Anestis, Dimitrios Bilalis, und Aspasia Efthimiadou. „Cultivation of Milk Thistle (*Silybum Marianum L. Gaertn.*), a Medicinal Weed. *Industrial Crops and Products* 34, Nr. 1 .2011: 825–30.
- [3] Greenlee, H.; Abascal, K.; Yarnell, E.; Ladas, E. "Clinical Applications of *Silybum marianum* in Oncology". *Integrative Cancer Therapies*.2007; 6 (2): 65–158.Abenavoli L, Spagnuolo R, Luppino I, Luzzza F. Recent progress in medicinal plants. *Spilc Press*. 2010; 387-409.
- [4] Kroll, D. J.; Shaw, H. S.; Oberlies, N. H. "Milk Thistle Nomenclature: Why It Matters in Cancer Research and Pharmacokinetic Studies". *Integrative Cancer Therapies*.2007; 6 (2): 9–110
- [5] Khan FA, Zahoor M, Ullah N, Khan S, Khurram M, Khan S, Ali J. A general introduction to medicinal plants and *silybum marianum*. *Life Science Journal*. 2014; 11(9s): 471-481.
- [6] Andrzejewska J, Sadowska K, Mielcarek S. Effect of sowing date and rate on the yield and flavonolignan content of the fruits of milk thistle (*Silybum marianum L. Gaertn.*) grown on light soil in a moderate climate. *Industrial Crops and Products*. 2011; 33(2): 462- 8.

- [7] Taskeen A, Naeem I, Mubeen H, Mehmood T. Reverse Phase High Performance Liquid Chromatographic Analysis of Flavonoids in Two Ficus Species. *New York Sci. J.* 2009; 2: 32-5.
- [8] Sakanaka S, Tachibana Y, Okada Y. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). *Food chemistry.* 2005; 89(4): 569-575.
- [9] Fu W, Jinglou Chen, Yaling Cai, Yongfang Lei, Liming Chen, Lei Pei, Daonian Zhou, Xiaofei Liang, Jinlan Ruan, Antioxidant free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from *Parathelypteris nipponica* (Franch. et Sav.) Ching. *Journal of ethnopharmacology*, 2010; 130(3): 521-528.
- [10] Gerber F, Krummen M, Potgeter H, Roth A, Siffrin C, Spöndlin C. Practical aspects of fast reversed-phase high-performance liquid chromatography using 3µm particle packed columns and monolithic columns in pharmaceutical development and production working under current good manufacturing practice. *Journal of Chromatography A.* 2004; 1036(2): 127–133.
- [11] Al-Anati L, Essid E, Reinehr R. *Molecular Nutrition and Food Research. Letters.* 2009; 53: 460–466.
- [12] Jayaraj R, Deb U, Bhaskar ASB. *Environmental Toxicology. Letters.* 2007; 22: 472–479.
- [13] Hammi, Khaoula Mkadmini, Hammami Majdi, Rihouey Christophe. *Food Chemistry. Letters.* 2016; 212: 476- 484.
- [14] Yan Qu, Chunxue Li, Chen Zhang, *Carbohydrate Polymers. Letters.* 2016; 150: 345-353.
- [15] Amand N, Abbasi BH, Fazal H. *Industrial crops and products. Letters.* 2013; 49: 164-168.
- [16] Amand N, Fazal H, Abbasi BH, Anwar S, Basir A. *Toxicology and industrial health. Letters.* 2013; 29: 460- 467.
- [17] Feng R, Ni HM, Wang SY, Tourkova IL, Shurin MR, Harada H, Yin XM. Cyanidin-3- rutinoside, a natural polyphenol antioxidant, selectively kills leukemic cells by induction of oxidative stress. *J. Biol. Chem.* 2007; 282: 13468-13476.
- [18] Viktorova J, Stranska-Zachariasova M, Fenclova M, Vitek L, Hajslova J, Kren V, Ruml T. Complex Evaluation of Antioxidant Capacity of Milk Thistle Dietary Supplements. *Antioxidants.* 2019; 8: 317.
- [19] Al-taei EH, Al-Naimi RA, Faleh EB. Study the effect of *Silybum marianum* on the toxicopathological changes in mice treated with alcohol. *Proceeding of the ninth veterinary scientific conference.* 2009; V(2): 162-171.
- [20] Egert S, Rimbach G. Which Sources of Flavonoids: Complex Diets or Dietary Supplements? *Advances in Nutrition.* 2011; 2(1): 8–14.