Determination of the Phytochemicals and Antioxidant Properties of Ethanol and Water Extracts Respectively from Leaves and Flowers of *Silybum marianum* L.

Dr. Mohamad Jawad Khubeiz*

Abstract

This study aims to determine and compare the total phenolic (TPC), flavonoids (TFC) components and antioxidant activities, upon extraction using two different polar solutions: ethanol and boiling water respectively for the extraction of *Silybum marianum* L. leaves and flowers used maceration method. The TPC and TFC were evaluated using Folin–Ciocalteu reagent and aluminum chloride colorimetric method, respectively; while antioxidant activities were determined by DPPH radical scavenging and reducing power activity. These various antioxidant activities were compared with standard antioxidants as ascorbic acid. The highest TPC was determined in the ethanol extract of leaves (88.53 ± 0.57 mg GAE/g ext.), followed by aqueous extract of flowers (81.39 ± 1.13 mg GAE/g ext.), while, ethanol flowers and aqueous leaves extracts were (72.34 ± 1.16 mg GAE/g ext.) and (71.92 ± 0.73 mg GAE/g ext.) respectively. The highest TFC was found in ethanol extract of leaves (61.61 ± 0.52 mg QUE/g ext.), followed by ethanol extract of flowers (37.79 ± 0.12 mg QUE/g ext.), while extracted with hot water

*Department of Chemistry, faculty of Science, Damascus University.

was (18.86 ± 0.13 mg QUE/g ext.) for leaves, and (27.54 ± 0.62 mg QUE/g ext.) for flowers. The extracts of *S. marianum* showed Average strength antioxidant activity. where the antioxidant activity assessed by DPPH radical scavenging assay was the highest in aqueous flowers extract (IC_{50} = 189.63 µg ASC.acid/ml), and lowest in aqueous leave extracts (IC_{50} = 401.61 µg ASC.acid/ml). While the antioxidant activity assessed by reducing power was the highest in ethanol leaves extract (IC_{50} = 318.90 µg Fe^{2+}/mL), and the lowest was in aqueous leaves extract (IC_{50} = 433.74 Fe^{2+}/mL). The present study enumerates the total phenols, total flavonoid and antioxidant activity of the *S. marianum*, which may help the researchers to set their minds for approaching the utility and efficacy of the plant extracts.

Keywords: *Silybum marianum*, phenolic compounds, total lavonoid, DPPH, reducing power.

تحديد الخواص الكيميائية النباتية ومضادات الأكسدة للمستخلصات الإيتانولية والمائية على التوالي لأوراق وأزهار *Silybum marianum* L.

د. محمد جواد خبيز*

الملخص

تهدف هذه الدراسة إلى تحديد ومقارنة المحتوى الكلي للفينولات والفلافونيدات لمستخلصات أوراق وأزهار نبات *Silybum marianum* L. عند استخدام محلين مختلفي القطبية هما: الإيتانول، ثم الماء الغالي على التعاقب، اعتمدت الطريقة اللونية لتحديد الفينولات الكلية والفلافونيدات باستخدام كاشف فولن سيكالتو وكلوريد الألمنيوم على التوالي، بينما حُدّدت الفعالية المضادة للتأكسد باستخدام تثبيط الجذور الحرة DPPH والقوة الإرجاعية. تمت مقارنة الفعالية المضادة للتأكسد بمضادات أكسدة مرجعية مثل حمض الأسكوربيك. وجدت أعلى قيمة للمحتوى الكلي للفينولات في المستخلص الإيتانولي للأوراق (88.53±0.57 mg GAE/g ext.)، يليه المستخلص المائي للأزهار (81.39±1.13 mg GAE/g ext.)، بينما كان مستخلص الأزهار الإيتانولي والأوراق المائي: (72.34±0.57 mg GAE/g ext.) و(71.92±0.73 mg GAE/g ext.) على التوالي. وجدت أعلى قيمة للفلافونيدات في المستخلص الإيتانولي للأوراق (61.61±0.52 QUE/g ext.)، يليها المستخلص الإيتانولي للأزهار (37.79±0.12 QUE/g ext.)، وعند الاستخلاص

* قسم الكيمياء– كلية العلوم – جامعة دمشق.

بالماء الغالي كانت (18.86±0.13 QUE/g ext.) للأوراق و (27.54±0.62 QUE/g ext.) للأزهار. أظهرت مستخلصات *S. marianum* فعالية مضادة للتأكسد متوسطة القوة، حيث كانت الفعالية المضادة للتأكسد التي تم تقييمها بوساطة تثبيط الجذور الحرة DPPH هي الأعلى في المستخلص المائي للأزهار (IC_{50} = 189.63 µg ASC.acid/ml) والأخفض في المستخلص المائي للأوراق (IC_{50} = 401.61 µg ASC.acid/ml)، في حين كانت الفعالية المضادة للتأكسد الأعلى بالقوة الإرجاعية في المستخلص الإيتانولي للأوراق (IC_{50} = 318.90 µg Fe^{2+}/mL) والأخفض في المستخلص المائي للأوراق (IC_{50} = 433.74 µg Fe^{2+}/mL). تسرد الدراسة الحالية محتوى الفينولات والفلافونيدات الكلي، والفعالية المضادة للتأكسد لنبات *S. marianum* والتي قد تساعد الباحثين على تركيز أفكارهم للوصول إلى فائدة وفعالية مستخلصات هذا النبات.

الكلمات المفتاحية: *Silybum marianum*، المكونات الفينولية، الفلافونيدات الكلية، DPPH، القوة الإرجاعية.

INTRODUCTION

Since very old times, herbal medications have been used for relief of symptoms of disease [1]. Despite the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. Much interest, in medicinal plants however, emanates from their long use in folk medicines as well as their prophylactic properties, especially in developing countries. Large number of medicinal plants has been investigated for their antioxidant properties. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress [2]. Although the toxicity profile of most medicinal plants have not been thoroughly evaluated, it is generally accepted that medicines derived from plant products are safer than their synthetic counterparts [3,4].

Plant-derived antioxidants, especially polyphenolic compounds, have gained considerable importance due to their potential health benefits. Antioxidants are important compounds, which protect organisms from damage caused by free radical-induced oxidative stress [5]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators [5–7]. Many plants respond to environmental stressors by producing antioxidants such as polyphenols. These absorb and neutralize free radicals, quenching singlet and triplet oxygen, or inducing expression of peroxides and other toxic metabolites [8, 9]. The medicinal value of plants is related to their phytochemical component content and secondary metabolites, including: phenolic compounds, flavonoids, alkaloids, tannins, and other stress gene response products [9, 10].

The increasing amount of free radicals on human will lead to oxidative stress of the cells continuously directed to the emergence of degenerative diseases such as cancer, diabetic,

inflammation and cardiovascular [11,12]. To overcome this, an antioxidants are indispensable to protect the cells from the negative effect of free radicals [13]. However, the use of synthetic antioxidants such as butyl hydroxyl anisole (BHA) and butyl hydroxyl toluene (BHT) cause some side effects which is cytotoxic for the lungs and liver and also carcinogenic [14]. Accordingly, many studies have been conducted on antioxidant compounds derived from plant sources which have more benefits than the synthetic [15]. Free radicals are produced either from by normal cellular processes or from external sources such as exposure to radiation, cigarette smoking, air pollutants and industrial chemicals. The most common reactive oxygen species (ROS) include: the superoxide anion ($O2^{\bullet-}$), the hydroxyl radical ($OH^{\bullet}$), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2). The oxidation induced by ROS may result in cell membrane disintegration, membrane protein damage and DNA mutations which play an important role in aging and can further initiate or propagate the development of many diseases [16]. Therefore, antioxidants with free radical scavenging activities may have great significance in the protection and therapeutics of diseases involving free radicals.

One of the medicinal plants that have been widely used in the world wide, is *Silybum marianum*, is an annual biennial plant belonging to the family of *Asteraceae*, used for more than two thousand years in traditional medicine. commonly known as milk thistle, is one of the oldest herbs used for centuries as an herbal medicine. *S. marianum* was a native of Asia and Southern Europe, but now it is found throughout the world. It grows of three to ten feet with an erect stem that bears large, alternating, prickly-edged leaves. Its flowering season is from June to August and each stem bears a single, large, purple flower ending in sharp spines. The fruit portion of the plant is glossy brown or grey with spots [17]. The extracts of the flowers and leaves of *S. marianum* have been used for centuries to treat liver, spleen and

gallbladder disorders [18]. The Food & Drug Administration in Germany has proposed this medicinal plant to treat digestive disorders, intoxication, and alcoholic liver and as a complement drug to treat enlarged liver. To the best of our knowledge in Syria, there are no studies related to the phytochemical from leaves of this plant. Then there is great need of increasing to investigation this herb, and used for in vitro studies of the secondary metabolites from therapeutically important medicinal plants.

The main objective of the present study was to evaluate the antioxidant activities of ethanol extracts from *Silybum marianum* L. by DPPH radical scavenging and reducing power activity. The total flavonoid content (TFC) and polyphenols content (TPC) in the leaves, flower receptacles were determined in order to assess their roles as potential sources of natural antioxidants.

MATERIALS AND METHODS

Plant collection

Leaves and flowers of *Silybum marianum* (Fig. 1) were collected from the Qudsaya Suburb, west of Damascus - Syria, in July, 2020 and were identified by an expert taxonomist at the Department of Botany, University of Damascus. Then, the collected plant materials were washed separately with fresh water to remove dirty materials and were shade dried for several weeks in the laboratory conditions. The dried materials were ground into fine powder by grinding machine and then stored at 4 ^{o}C temperature for future used.

Fig 1 Leaves and Flowers of *S. marianum*

Preparation of *S. marianum* Leaves and Flowers Extracts

50g powdered of plant leaves and flowers separately was defatted for 72 hours with 500 ml petroleum ether on a mechanical shaker. The petroleum ether solvent was discarded, then the defatted samples air-dried. Exactly 40 g of the defatted plant samples was mixed with 400 ml of ethanol at room temperature for 24 hours. The mixture was filtered through Whatman No.1 filter, and resulting ethanol extract (Eth.). The procedure was repeated twice with same condition. The crude residue was dried and suspended in boiling distilled water (400 mL) for 24 hours. The mixture was filtered through Whatman No.1 filter. and resulting aqueous extract (Aqu.). The extracts were filtered and the filtrates were evaporated under reduced pressure using rotatory evaporator. The dried extracts were stored at -20 ^{o}C until used.

Determination of Total Phenolic Content (TPC)

Total phenolic content was estimated as Gallic acid (GA) equivalents per gram of dried plant extract, according to the Folin-Ciocalteu phenol reagent method [19] with slight modification. First, a standard curve was generated using Gallic acid as a standard. For this purpose, the calibration curve of Gallic acid was drawn (Figure 2 a). Different concentrations of Gallic acid (7.813, 15.625, 31.25, 62.5, 125, 250 and 500 µg/ml)

were prepared in methanol, and their absorbance values were measured at 765 nm. For sample measurement, 0.5 mL (1/10 dilution) of Folin-Ciocalteu phenol reagent and 1000 mL of distilled water were added to 100 µL of plant extract. The solutions were mixed and incubated at room temperature for 5 min. After 5 min, samples were combined with 1500 mL of 20% sodium carbonate (Na_2CO_3) solution, mixed, and incubated for an additional 90 minutes at room temperature. Absorbance at 765 nm was measured with a Double beam UV-visible Spectrophotometer (SYSTRONICS 2201). Data presented are average values of three measurements for each sample.

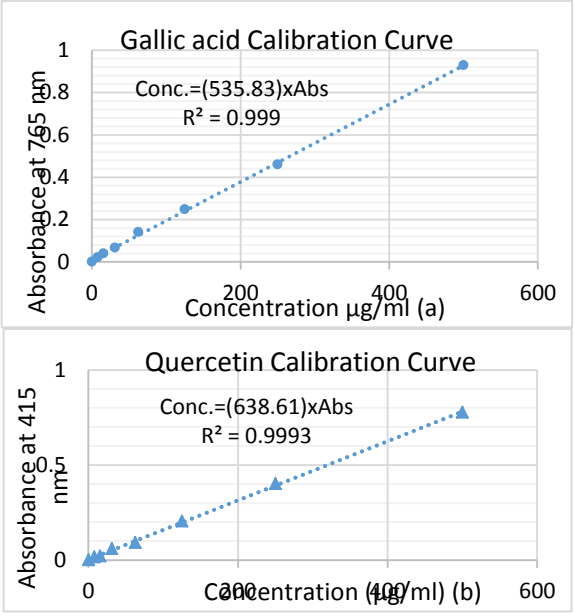

Fig 2 (a): Calibration Curve of Gallic Acid, (b): Calibration Curve of Quercetin

Determination of Total Flavonoid Content (TFC)

The aluminum chloride colorimetric method was used for the determination of the total flavonoid content of the sample [20]

with slight modification. For total flavonoid determination, quercetin was used to make the standard calibration curve, for this purpose, the calibration curve of quercetin was drawn (Figure 2 b). Stock quercetin solution 1mg/ml was prepared by dissolving in methanol, then the standard solutions of quercetin were prepared by serial dilutions using methanol (7.813, 15.625, 31.25, 62.5, 125, 250 and 500 μg/mL). 50 mg of extracts were dissolved in 50 mL of methanol to get 1 mg/mL solutions. 0. 1 mL from the above stock solution were taken in different tubes. To each tube 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.2 mL of distilled water was added. The reaction mixture was kept at room temperature for 30 min. The absorbance of the resulting solutions was measured at 415 nm against reagent blank. The calibration curve was prepared by plotting absorbance against concentration and it was found to be linear over this concentration range. 10 mg of extracts were dissolved in 10 mL of methanol to get 1 mg/mL solutions. The concentration of total flavonoid in the test sample was determined from the calibration curve. The total flavonoid content in the extract was expressed as quercetin equivalent (mg QUE/g extract). Measurements were performed in three replicates and values It is expressed as mean ± SD.

Evaluation of DPPH Scavenging Activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical. On accepting hydrogen from a corresponding donor, its solutions lose the characteristic deep purple (λ_{max} 517 nm) color. DPPH is very popular for the study of natural antioxidants. The assay was carried out according to the modified method of Blois, 1958 [21]. DPPH (30 mg) was dissolved in methanol (1000 mL) to obtain the concentration of 30 µg/mL. 2 ml of DPPH solution was mixed with 1 ml of the extracts of *S. marianum* at different concentrations (50, 100, 200, 400, 600, 800 µg/ml of ethanol, aqueous). The mixture was then incubated

at room temperature for 30 min in the dark. The control was prepared by mixing 2 ml of DPPH solution with the respective solvent. The absorbance was measured against a blank at 517 nm using spectrophotometer. Lower absorbance of the reaction mixture indicates higher DPPH free radical scavenging activity. The antioxidant activity of the extract was expressed as IC_{50}, which was defined as the concentration (µg/ml) of extract that inhibits the formation of DPPH radicals by 50 %. IC_{50} values were estimated from the % inhibition versus concentration sigmoidal curve, using a non-linear regression analysis by the software GraphPad prism 8.0.2 . Ascorbic acid at different concentrations (6.25, 12.50, 25, 50, 100 and 200 µg/ml) was used as the standard. Samples were prepared and measured in triplicates. The percentage of scavenging activity of each extract on DPPH radical was calculated as % inhibition of DPPH (I %) using the following equation:

DPPH scavenging activity (I %) = $[(A_C - A_S / A_C) \times 100]$

where A_C = absorbance of the control and A_S = absorbance of tested samples.

Estimation of Reducing Power

The reducing power of the extract was determined according to the method of [22]. Briefly, 1 ml of different concentration sample (31.5, 62.5, 125, 250, 500, 1000 µg/ml) was mixed with 2.5 ml of a 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of a 1% (w/v) solution of potassium ferricyanide $K_3Fe(CN)_6$. The mixture was incubated in a water bath at 50°C for 20 min. Afterwards, 2.5 ml of a 10% (w/v) trichloroacetic acid solution was added and the mixture was then centrifuged at 3000 rpm for 10 min. A 2.5 ml aliquot of the upper layer was combined with 2.5 ml of distilled water and 0.5 ml of a 0.1% (w/v) solution of ferric chloride $FeCl_3$, and absorbance was measured spectrophotometrically at 700 nm against a blank sample. Ascorbic acid at different concentrations (7.863, 15.625, 31.25, 62.5, 125 and 250 µg/ml) was used as standard, and phosphate

buffer was used as blank solution. Increase in the absorbance of the reaction mixture indicated increase in the reducing power. The percentage inhibition was calculated by using the following equation:

$$\text{I\% Reducing power activity} = \left[\frac{A_{sample} - A_{control}}{A_{sample}}\right] \times 100$$

where A_{sample} and $A_{control}$ are the absorbance of the sample and the control, respectively. The IC_{50} value (μg Fe^{2+}/mL) is the effective concentration giving an absorbance of 0.5 for reducing power and was estimated from the % inhibition versus concentration sigmoidal curve, using a non-linear regression analysis by the software GraphPad prism 8.0.2 .

Statistical analysis

The experiments were carried out in triplicate and results are given as the mean ± standard deviation SD. Average, standard deviation calculation and graphs were performed with Microsoft Excel (2013). IC_{50} values were generated with GraphPad Prism v 8.0.2 .

Results and Discussion

Regarding the selection of water and ethanol as a solvent for extraction procedure attention has been given to the safety of the extract for consumption by human and cost effectiveness. Literature revealed the fact that water can be used as a suitable solvent for extraction of polyphenols [23,24], and also support regarding the presence of flavonoids, steroids and terpenoids in aqueous extract that being evaluated through phytochemical screening [25]. These extractions were preceded by a degreasing process, which is a fundamental step in the success of the extraction of phenolic compounds since the presence of interfering substances such as waxes, fats, terpenes and chlorophylls is an aspect that directly affects the efficiency of the extractive process of this class of compounds, as this petroleum ether extract was neglected due to its low content of phenols and flavonoids [26].

Percent yield (%) was calculated using Eq.: yield (%) = (weight of extract g)/ (weight of plant material set for maceration g) ×100. The extractive yields of the ethanolic leaves and flowers were 8.47% and 15.32% respectively, while the yields of leaves and flowers aqueous extracts were 13.29% and 19.83% respectively (Table 1). The highest amount of total extractable compounds was in the flowers aqueous extract, and the lowest was in leaves ethanolic extract.

Quantitative Estimation of Total Phenolic Content (TPC)

Plants sources are effective antioxidant agents due to the presence of polyphenolics compounds. These compounds are ubiquitously present in plants and some of their parts have high content of phenols. Phenolic compounds are good antioxidant because of presence of hydroxyl and hydrogen groups which effectively scavenge free radicals including reactive oxygen species[27]. The concentration of phenolics in the extracts was dependent on both the solvent and the part of the plant used in the extraction. The TPC of the extracts was determined by extrapolation from the calibration curve (Conc. = (535.83) × Abs R^2 = 0.999) prepared from the gallic acid concentrations (Figure 3 c) and expressed in mg of Gallic acid equivalence (GAE) per gram extracts. The amount of phenolic compounds in the various extracts was obtained from regression equation and the values were expressed in Gallic acid equivalence.

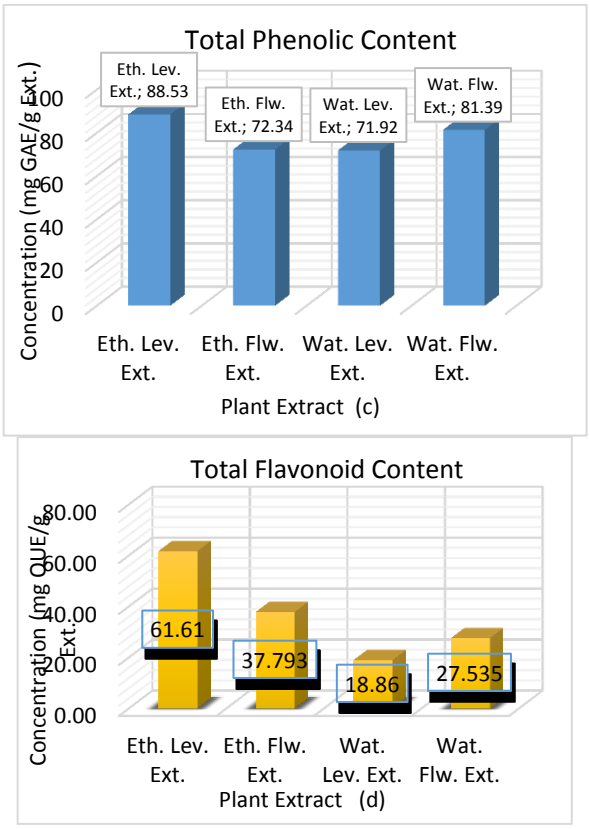

Fig. 3 Total Phenolic and Flavonoid contents

The results obtained in this study showed a significant level of phenolic compounds in ethanol and aqueous extracts of the leaves and flowers of *S. marianum* (Table. 1). The ethanolic extracts contents of phenolic compounds were greater in leaves than in flowers (88.53 ± 0.57 and 72.34 ± 1.16 respectively). While the aqueous extract showed different results, the amount of phenolic contents in the aqueous extract was greater than in the ethanolic extract (81.39 ± 1.13 and 71.92 ± 0.73 respectively).

Table. 1 TPC, TFC Content, Ratio TFC/TPC and Yield of Leaves and Flowers Extracts

Sample	TPC mg GEA/g Ext.	TFC mg QUE/g Ext.	Ratio TFC/TPC	Yield (%)
Eth. Lev. Ext.	88.53 ± 0.57	61.61 ± 0.52	0.70	8.47
Wat. Lev. Ext.	71.92 ± 0.73	18.86 ± 0.13	0.26	13.29
Eth. Flw. Ext.	72.34 ± 1.16	37.79 ± 0.119	0.76	15.32
Wat. Flw. Ext.	81.39 ± 1.13	27.54 ± 0.618	0.34	19.83

Total flavonoid content (TFC)

The total flavonoid content was estimated by a colorimetric method, using quercetin as standard flavonoid. The total flavonoid content of ethanol and aqueous extracts of leaves and flowers of *S. marianum* is shown in Table 1. The TFC content extracts was also determined by extrapolation from the calibration curve (Conc.= (638.61) x Abs R^2 = 0.9996) prepared from the quercetin concentrations and expressed in mg of quercetin equivalence (QUE) per gram. The amounts of flavonoid compounds in the various extracts were obtained from regression equation and the values were expressed in quercetin equivalence (Fig. 3 d).

As in the case of total phenolics, the concentration of flavonoids in the extracts was dependent on both the solvent and the part of the plant used in the extraction, the total flavonoid content ranging from 27.54 to 61.61 mg QUE/g of extract. In this case too, the ethanolic extracts from leaves showed a higher flavonoid content than the flowers extract (61.61 ± 0.52 and 37.79 ± 0.119 respectively), while the aqueous extracts showed opposite results, it was found that the flowers extract showed the highest flavonoid content than leaves extract (27.54 ± 0.618 and 18.86 ± 0.13 respectively).

DPPH Free Radical Scavenging Activity

Antioxidant properties, especially radical scavenging activities, are very important due to the deleterious role of free radicals in foods and in biological systems. When DPPH accepts an

electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The characteristic feature of antioxidants to scavenge DPPH free radical is well accepted and is therefore most often selected as a reliable tool to evaluate the free radical scavenging capacity of different plant extracts. Interestingly, the DPPH radical assay is incredibly sensitive towards active ingredients even at lower concentrations. Another beneficial aspect is that this test is time saving and can be used to analyze a batch of samples in a shorter time.

The ethanolic and aqueous extracts of *S. marianum* leaves and flowers exhibited moderate antioxidant activity, but scavenged the free radicals of DPPH in a concentration dependent manner (Fig. 4). Among the extracts tested, the ethanolic leaves extract exhibited better activity than aqueous extract. The IC_{50} valves for ethanolic and aqueous leaves extracts were 204.52 and 401.61 µg\ml respectively (Table 2 and Fig. 4 a). While the tests of flower extracts showed that the aqueous extract gave a greater scavenging activity rate than the ethanolic extract, IC_{50}: 189.63 and 390.41 µg\ml respectively (Table 2 and Fig. 4 b).

Table 2 DPPH free radical scavenging activity and IC_{50} values of the plant extracts and standard

Asc. Acid	Asc. Acid Con. µg/ml	Aqu.Flw. Ext.	Eth.Flw.Ext.	Aqu.Lev.Ext.	Eth.Lev.Ext.	Plant Ext.Con. µg/ml
96.437±0.25	200	90.23±0.98	77.72±1.04	76.79±2.07	85.46±1.23	800
96.092±0.83	100	89.67±0.54	76.87±1.26	66.67±0.65	84.48±0.65	600
69.540±0.17	50	77.62±1.21	48.68±0.76	55.67±0.73	77.21±0.2.16	400
39.770±0.25	25	54.90±0.78	23.43±0.44	38.57±1.84	46.56±1.25	200
24.253±0.33	12.5	23.61±0.84	15.18±1.05	17.98±0.33	23.77±0.09	100
16.00	6.25	12.61±1.06	6.77±1.30	9.42±0.95	10.61±0.86	50
37.98	IC_{50}	189.63	390.41	401.61	204.52	IC_{50}

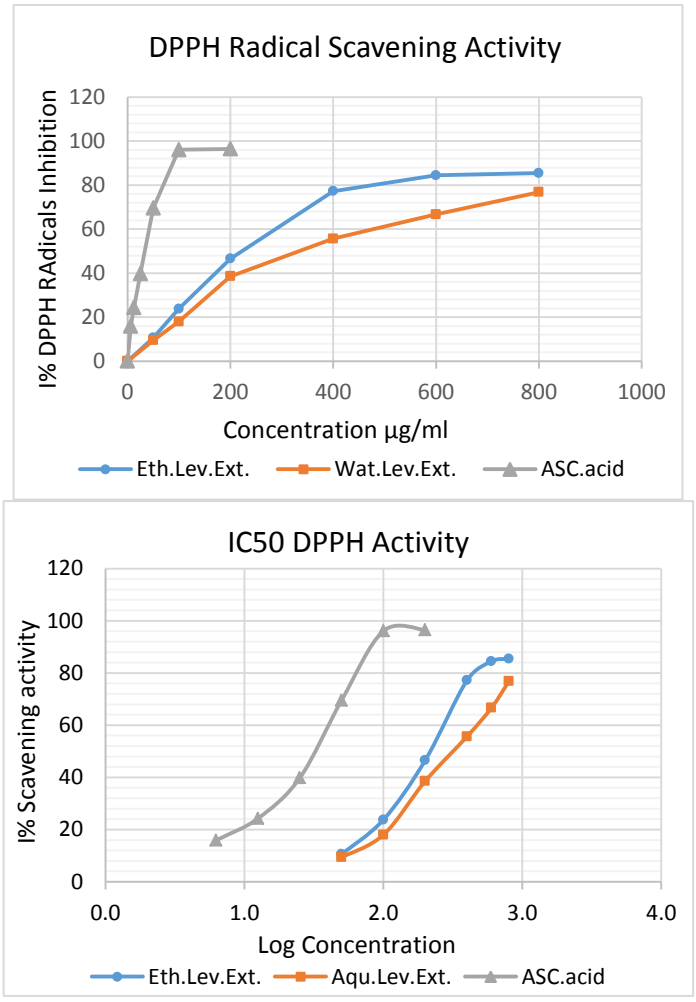

Fig. 4 (a) DPPH Antioxidant activities of extracts of *S. marianum* leaves

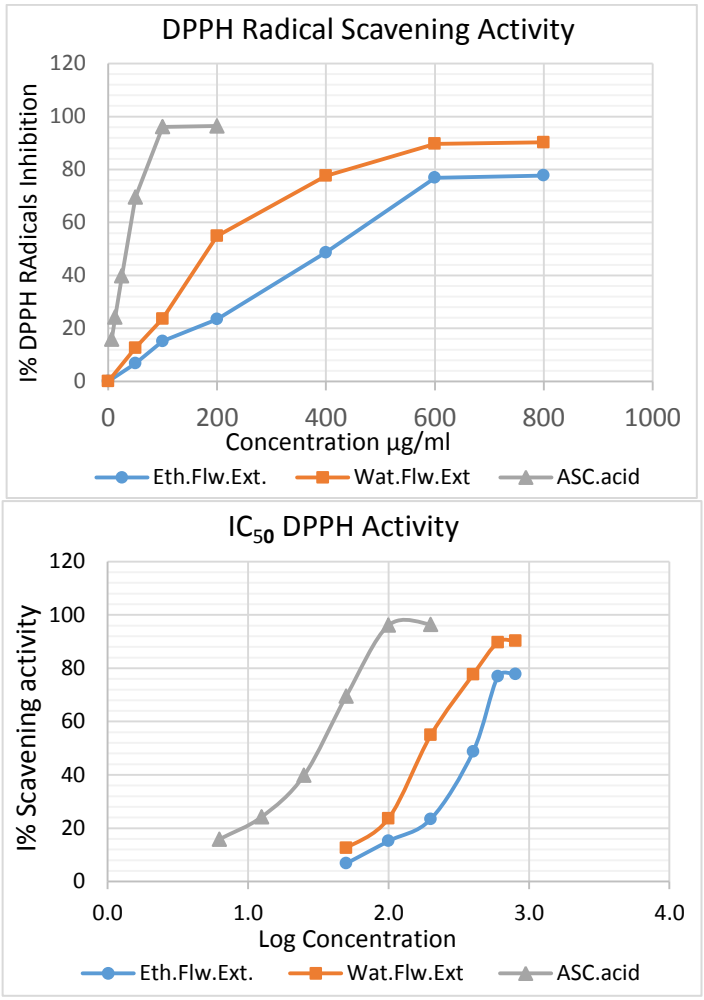

Fig. 4 (b) DPPH Antioxidant activities of extracts of *S. marianum* Flowers

When the concentrations were lower, the DPPH radical scavenging activity of leaves and flowers extracts were weak, when the concentration was higher their activity much enhanced. There is linear relationship of antioxidant activity of

extracts of *S. marianum* with the total phenolics contents [28]. Also, the antioxidant activity of 112 Chinese wild herbs is correlated with the total phenolic contents [29]. Present data are in line with the observation of many scientists who documented the relationship of antioxidant activity with total phenolic compounds [30].

Scavenging activity % and IC_{50} values of the ethanolic and aqueous extracts of leaves and flowers has been represented in the Table 2 which is comparable with standard ascorbic acid, IC_{50}: 37.98 µg\ml.

Reducing Power Activity

This assay compares antioxidants based on their ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion through the donation of an electron, with the resulting ferrous ion (Fe^{2+}) formation monitored spectrophotometrically at 700 nm. A given antioxidant donates electrons to reactive free radical's species, thus promoting the termination of free radical chain reactions. The ability of the antioxidant to reduce Fe^{3+} to its more active Fe^{2+} form might also be indicative of its ability to act as a pro oxidant in the system. The different extracts from *S. marianum* exhibited a dose dependent reducing power activity at various concentrations.

Table 3 and Fig. 5 showed the reducing capacity of ethanolic and aqueous extracts of leaves and flowers of *S. marianum* in comparison with ascorbic acid used as a standard. The ethanolic extract of leaves showed higher reducing power as compared to aqueous extract, While the flower extracts gave the opposite result to what we got in the leaves extracts, and reducing capability increases as the concentration increased. At concentration 1 mg/mL *S. marianum* extracts shows poor reducing ability, but we can get better reducing capability at higher concentrations. The reducing power activity of all leaves and flowers extracts is lower than that in Ascorbic acid, whereas the IC_{50} values were 318.90 and 433.74 µg/mL in Eth.Lev.Ext.

and Wat.Lev.Ext. respectively (Fig 5 a), while The IC_{50} values in ehanolic and aqueous flowers extract was 425.74 and 339.30 µg/mL respectively (Fig 5 b), but the ascorbic acid IC_{50} was 25.34 µg/mL.

Table 3 I % **Reducing Power Activity and IC_{50} values of the plant extracts and standard**

Plant Ext. Con. µg/ml	**I% Reducing Power Activity**				**Asc. Acid Con. µg/ml**	**I% RP. Activity**
	Eth.Lev.Ext.	**Aqu.Lev.Ext.**	**Eth.Flw.Ext.**	**Aqu.Flw.Ext.**		**Asc.Acid**
1000	72.687±1.75	66.341±0.93	58.648±2.01	69.856±0.77	250	89.064±0.21
500	58.249±0.54	50.360±0.67	40.647±1.09	56.55±0.88	125	79.957±0.17
250	40.385±0.49	35.514±1.29	25.339±0.93	36.041±0.22	62.5	67.077±0.67
125	27.059±0.65	23.757±0.87	7.821±0.41	20.755±1.31	31.25	52.778±0.37
62.5	12.676±1.27	9.804±79	6.250±0.65	16.556±1.27	15.625	30.996±0.92
31.25	9.859±1.87	7.383±0.68	2.941±1.17	10.638±1.02	7.863	17.621±0.84
IC_{50}	**318.90**	**433.74**	**425.74**	**339.30**	**IC_{50}**	**25.34**

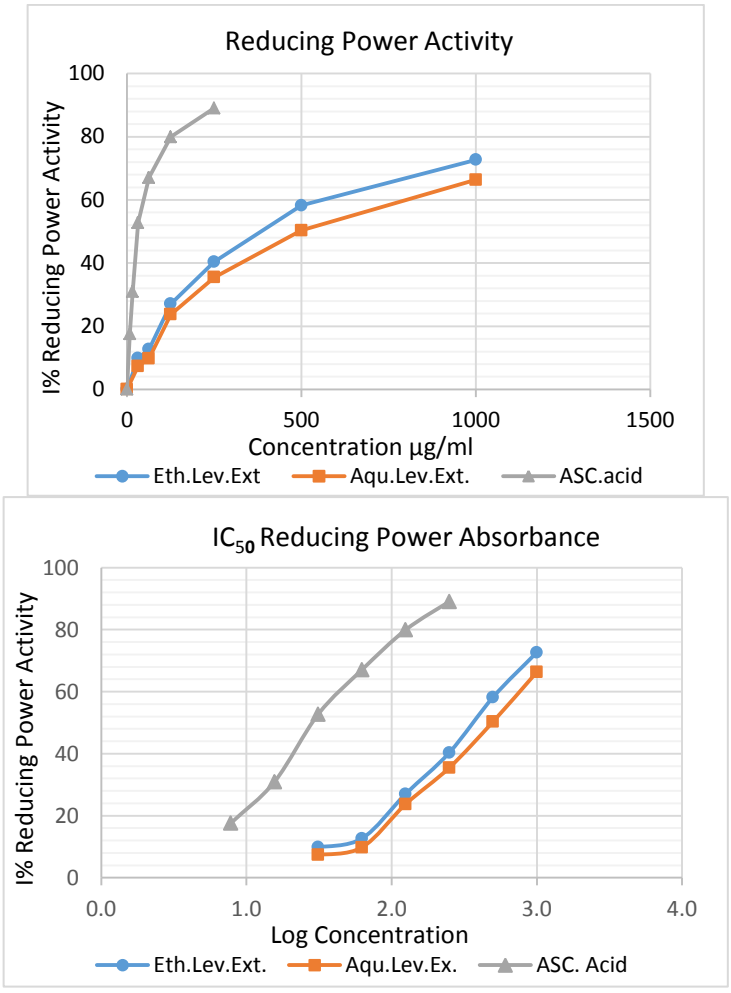

Fig. 5 a Reducing power abilities of extracts of *S. marianum* leaves

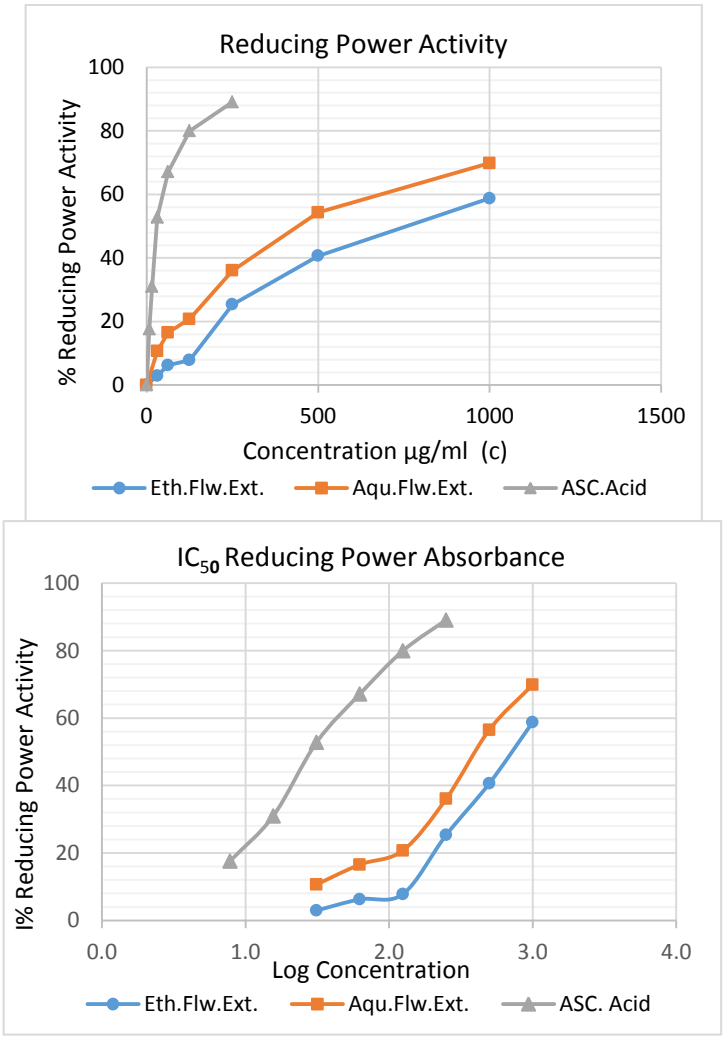

Fig. 5 b Reducing power abilities of extracts of *S. marianum* Flowers

The reducing powers of ethanolic and aqueous extracts of leaves of *S. marianum* were 0.454 and 0.410 at 1 mg/mL respectively (Fig. 6 a). At the same concentration, the ethanolic extract of

flowers was found to have the absorbance value 0.399 while the aqueous extract had value 0.418 (Fig. 6 b).

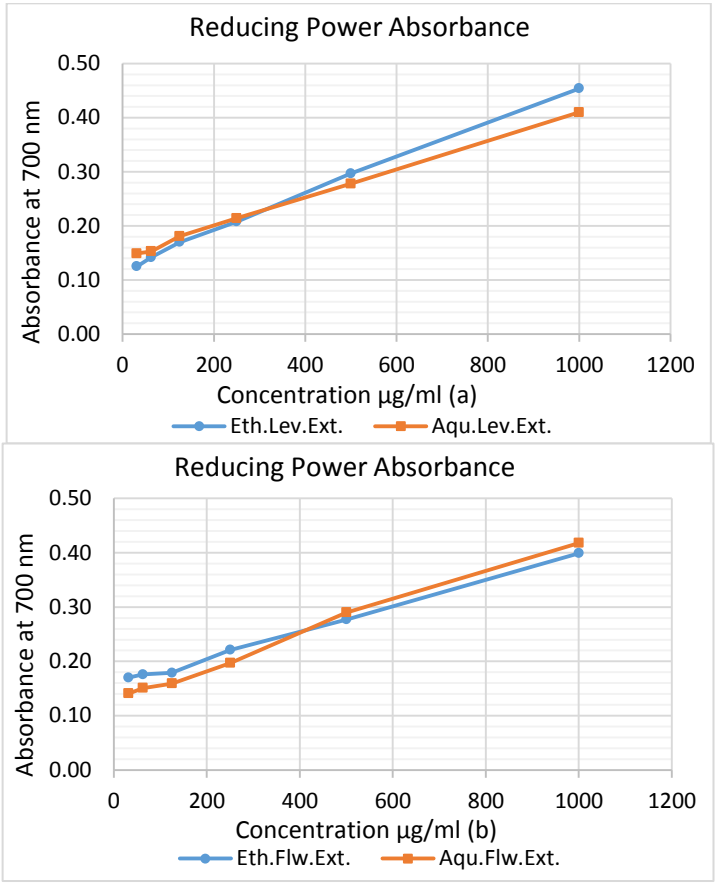

Fig.5 Reducing Power Absorbance of Ethanolic and Aqueous Extracts of Leaves and Flowers of *S. marianum*

Polyphenolic compounds, often present in plant extracts, have various biological activities among which are antioxidant activity. There is a direct relation between antioxidant activity and the reducing power. Furthermore, the direct relationship

between reducing power and antioxidant activity has also been correlated with extract concentration and extraction method [31]. There was no direct relationship between phenolic content and antioxidant activity [32,33]. However, for medicinal plant extracts, many previous studies as [34, 35] confirmed my own observations. Therefore, it is possible that the antioxidant properties of *S. marianum* L. plants were affected by many compounds besides flavonoids and phenolics, such as acids, sugars, and glycosides could be included in the ethanol extracts of medical plants. The synergistic effect of these compounds may be present in *S. marianum* L. plants [36]. These studies indicate that phenolics have varying antioxidant behavior which depends on the type of compounds.

Most of documented researches about *S. marianum* are about seeds extracts, as it is rich in ingredients that have proven important in treating liver diseases. Therefore, numerous experimental and clinical studies have documented that *S. marianum* with its antioxidant activity and other liver protective properties is a unique hepatoprotective agent. Silymarin is the major flavonoid component of *S. marianum* that has been documented to be highly hepatoprotective [37]. Various studies indicate that Silymarin exhibits strong antioxidant activity [38], and shows protective effects against hepatic toxicity induced by a wide variety of agents by inhibiting lipid peroxidation [39]. It has been used for the treatment of numerous liver disorders characterized by functional impairment or degenerative necrosis. Although its mechanisms of action are not fully understood, it seems that it acts in different ways, including antioxidant and anti-inflammatory activities. Higher total phenolic content has been known to contribute to the antioxidant activity of extracts, while antioxidant activity has also been linked to the hepatoprotective effect of some extracts [40].

Conclusion

In present study, various in vitro assays were carried out to determine the total phenolic, flavonoid contents, and antioxidant activity of ethanolic and aqueous extracts of *Silybum marianum*. The extract was found to possess well antioxidant activity which may be credited to the presence of phytoconstituents like flavonoids and phenols in the ethanolic and aqueous extracts. This study suggests that the extracts of *Silybum marianum* possess well free radical scavenging activity and phytochemical constituent which might be useful for further studies to unravel novel treatment strategies for diseases associated with free radical induced tissue damage. However, further research is needed to determine the study-specific phytochemicals with the mechanisms behind these activities.

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