Phenotypic, anatomical and phytochemical investigation of Iraqi *Silybum marianum*

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**Abstract.** Asteraceae (Compositae) famous as the aster, daisy or sunflower family is the biggest flowering family divided into thirteen subfamily that involve approximately two thousand genus, an important member of this family is *Silybum marianum* (Milk thistle). Management of hepatic diseases is the main use of milk thistle in traditional medicine also it has anticancer activity against different type of cancer in addition to antiviral, antioxidant, anti-inflammatory and anti-diabetic activity. This study was designed to investigate the pharmacognostical feature and the phytochemical ingredient of Iraqi *Silybum marianum*. The plant were examined macroscopically to investigate the morphological characters of plant and microscopically to determine the type of stomata and trachoma then flowers, leaves, stems and seeds of the plant were extracted by ethanol in a soxhlet apparatus individually and subjected to standard methods for active constituents identification and that total flaononid, total phenolic and total tannins content were determined using aluminum chloride colorimetric, folin-ciocaltue and acidified vanillin methods respectively. The results shows that *Silybum marianum* leave have a thick cell wall, anomocytic stomata, annual vessel, and unicellular unbranched trichomas. Also an important active constituents have been detected that terpenes, steroid and flavonoids were present in all plant parts, saponin appear only in the leaves and stems while alkalooids and coumarins are not detected in any part. Also the study referred to the seeds as the richest part of the plant with flavonoid and phenolic compounds followed by the flowers, leaves and stems which contain the less amount, while the higher content of tannin were observed in the leaves and stems.

**Keywords.** *Silybum marianum*, Milk thistle, Stomata, Trachoma, Flavonoid, Phenolic and tannin.

**1. Introduction**

The Asteraceae (Compositae) is famous as the aster, daisy or sunflower family; this biggest flowering family is divided into thirteen subfamily that involve approximately two thousand genus, *Silybum marianum* is an important member of this family [1]. The famous widespread name for *S. marianum* is...
milk thistle, various other names are also used such as Pig leaves, Royal thistle, Marian thistle, Lady’s thistle, Christ’s crown, Snake milk, Venus thistle, Heal thistle, Variegated thistle, Sow thistle and Wild artichoke [2]. The plant is a high biennial herb of about 5-10 feet, stiff, having greenish shine leaves with barbed margins and a whitish lines along the veins. The flower is purple containing a small seed inside [3]. Its fruits have stiff skin achenes of about six to eight mm long; have a brown color with a white silk at the top which is collected after flowering in May [4]. Management of hepatic disease was the main use of milk thistle in traditional medicine that at early 19th century it was advised and administered for the management of blood and liver problems as well as for intestinal cleansing [5], also it have anticancer activity against different type of cancer in addition to antiviral, antioxidant, anti-inflammatory and anti-diabetic activity [6]. This study was designed to investigate the phenotypic, anatomical and the phytochemical ingredients of Iraqi *Silybum marianum*, with the determination of the total flavonoid, tannin and phenolic content in the seeds, flowers, leaves and stems of the plant.

2. Materials and Methods

2.1. During Plant materials

All the parts of the plants were collected from the College of Pharmacy/ Al-Mustansiriyah University. The plant was authenticated by Dr. Sukaina Abbas/ College of Science/ Baghdad University/ Baghdad/ Iraq, and then it was identified microscopically in College of Pharmacy/ Al-Mustansiriyah University. Leaves, flowers, stems and seeds of the plant were washed thoroughly by tap water, dried in shade, at room temperature from 2 weeks for flowers and seeds up to 1 month for leaves and stems, then grinded to a powder and weight for further investigations.

2.2. Pharmacognostical (phenotypic and anatomical) study

2.2.1. Macroscopic examination

Fresh specimens of *S. marianum* were used to investigate the morphological characters of plant, such as shape, color, size, and margins of leaves, flowers, stems and seeds.

2.2.2. Microscopic examinations

Fresh and dried leaves powder is used for the microscopic examination. The type of stomata and trachoma were observed by taking the outer epidermal layer of fresh leaf on a slide and added few drops of chloral hydrate solution to obtain a clear section and observed under a microscope. Then the powder of the dried leaves were placed on slide and 2 drops of chloral hydrate were added and discarded (2 to 3 times) to bleach the color and clarify the picture then heated at a heater, finally examined under microscope after covering the slide [7]. The photographers were obtained by using digital camera and diagnosis the different cell components.

2.3. Extraction method

Each part of the plant was put in a thimble and extracted by absolute ethanol using Soxhlet apparatus for 3 days and then the extract were completely evaporated by a rotary evaporator, the total dried extract of each part was weight to subsequently determine the amount of the contents in the dry weight and then labeled for further investigation [8].
2.4. Phytochemical study

2.4.1. Preliminary phytochemical screening

The plant extract was phytochemically screened for the qualitative investigation of major classes of secondary metabolites.

2.4.1.1. Flavonoids

A positive result is recorded when a yellow color is observed after the addition of 2ml ethanolic KOH to 1ml alcoholic extract of plant [9].

2.4.1.2. Tannins

Few drops of alcoholic extract were diluted to 10 times its volume, filtered and mixed with 1% aqueous ferric chloride, formation of dark green-blue color ensure tannin presence [10].

2.4.1.3. Saponin

Froth assay is used for saponin identification that by shaking distilled water vigorously with few ml of plant extract for fifteen minute. A persistent froth is an indication for saponins presence [11].

2.4.1.4. Terpenoids

4ml of the plant extract was treated with 1ml of equal quantity of acetic anhydride and chloroform. Then concerted solution of sulphuric acid was added gradually and red violet color was seen for terpenoids [12].

2.4.1.5. Sterols and steroids

Liebermann reaction was used to indicate the presence of steroids. Dried ethanolic extract was diluted with 0.5ml of hot acetic anhydride and filtered, then treated with Liebermann burrachardt. The appearance of a blue-green ring at the interphase indicated sterol nucleus presence [13].

2.4.1.6. Coumarins

A few spot from a mixture of 0.5ml 10% NH4OH and 5ml of ethanolic extract was added on a filter paper and examined under U.V light. Coumarins give intense fluorescence under UV [14].

2.4.1.7. Alkaloids

Extracts were dissolved individually in 8ml of 1% hydrochloric acid and filtered. The filtrate was divided into two parts; the first one was reacted with Mayer's reagent, a positive result is indicated by white precipitate. The second part was reacted with Dragendroff's reagent. A red precipitate refers to alkaloid existence [15].

2.4.2. Estimation of total phenolic content

Total phenolic content was measured by adding 1ml of deionized water and 1ml of folin-ciocalteu reagent to 1ml of probably diluted extract sample, 5 minute later 1ml of 10% NaCO₃ was added and
the mixture was kept at room temperature for at least 90 min, the absorbance was measured at 760nm by UV spectrophotometer, the process was repeated three times for each sample and the average was recorded [16]. Calibration curve was done by measuring different concentration of gallic acid by the same procedure, and the result was expressed as mg gallic acid equivalent/g of dry plant material.

2.4.3. Estimation of total tannins content

0.05 ml of diluted alcoholic extract were reacted with 3ml of 4% methanolic vanillin solution and 1.5ml H2SO4, that condensed tannin will react with vanillin solution and form anthocyanidols in the presence of concentrated sulphuric acid, the absorbance was measured at 500nm after fifteen minute for three replicate of each sample, the result was expressed in mg equivalent of gallic acid/ g of dry plant material [17].

2.4.5. Estimation of total flavonoid content

0.5 ml of diluted extract was mixed with 0.15 ml of 7% NaNO2 solution, after 7 minute 0.3 ml of 10% AlCl3 solution was added, later after 6 minute 1 ml of NaOH was added to the mixture and the volume was completed to5 ml by distilled water, the absorbance was measured at 510 nm and the result was recorded as mg quercetin equivalent /g of dry weight [18].

3. Results and Discussion

3.1. Macroscopic identification

*Silybum marianum* range from 25 cm to 2 m in height and from 5 cm to 1.5 m base diameter, the stem is green, grooved, branched, rigid and contain multiple leaves, large stem have a hollow in the middle. The leaves are lanceolate, lobate, and pinnate with spinny margin, they are hairless, greenish with milk-white veins, growing only on the base of the plants; they are small at December when they start growing and reach their maximum size at the end of March and April. Flowers are 4 to 13 cm long and wide, pale yellow to white at summer and purple at the end of winter and during spring; they are round, solitary at the apex of the stem or its branches, surrounded by needle like bracts, The seeds (fruits) are about 1 cm long, smooth, shiny with a simple long very fine pappus, surrounded by a yellow basal ring, it have a white color surrounded by a hard brownish to black achenes. Figure1. These morphologicel characteristic are identical to the plant characteristic prescribed at world health organization monograph [19], a guide of medicinal plants in Africa [20] and many papers around the worlds [21, 22, 23, 24].
Figure 1. *Silybum marianum* picture. A- purple flower, B- white flower, C- seed, D- whole plant.

3.2. Microscopic examination

Powdered leaves of *Silybum* were diagnosed under microscope by a thick cell wall, anomocytic stomata, annual vessel, and unicellular unbranched trichomes as shown in Figure 2.
Figure 2. A-Thick cell wall, B- Anomocytic stomata, C- Annual vessel, D- Annual vessel 40X. E- fiber 10X, F- fiber 40X. G- Unicellular unbranched trichomas, H- Unicellular unbranched trichomas 40X.

3.3. Phytochemical results

3.3.1. Qualitative identification of Silybum marianum
Various qualitative phytochemical screening tests were done to establish the chemical composition of each extract; these tests provide important information regarding the type of secondary metabolites present in plant to establish a suitable procedure for isolation of these metabolites from different extracts. Primary chemical experiments for the extract of flowers, leaves, seeds and stems revealed the presence of terpenes, steroid and flavonoids while saponin appear only in the leaves and stems and the extract of flowers and seeds give a negative result. Alkaloids and coumarins are not detected in any part as shown in Table 1.

| Part of the plant | Alkaloid | Terpenoid | Steroid | Flavonoid | Saponin | Coumarin |
|-------------------|----------|-----------|---------|-----------|---------|----------|
| Leave             | -ve      | +ve       | +ve     | +ve       | +ve     | -ve      |
| Flower            | -ve      | +ve       | +ve     | +ve       | -ve     | -ve      |
| Seed              | -ve      | +ve       | +ve     | +ve       | -ve     | -ve      |
| Stem              | -ve      | +ve       | +ve     | +ve       | +ve     | -ve      |

3.4. Quantitative assessment of S. marianum

Different concentration of gallic acid, tannic acid and quercetin was used for calibration curve calculation of phenolic, tannins, and flavonoids respectively as shown in Figure 3, 4 and 5. Different phytochemical ingredients vary considerably among different parts of the plant and affected by different biological stress and environmental conditions [25], therefore flavonoid, phenolic, and tannin content were determined in different parts of the plant and each measurement were repeated three times to reduce error and the results are documented in table 2.

![Figure 3](image3.png)

**Figure 3.** Standard curve for the determination of total phenolic content.

![Figure 4](image4.png)

**Figure 4.** Standard curve for the determination of total tannin content.
Figure 5. Standard curve for the determination of total flavonoid content.

Table 2. Total phenolic, flavonoid and tannin content in different parts of Iraqi *Silybum marianum*.

| Part of the plant | Total phenolic content as μg gallic acid /mg dry plant | Total flavonoid content as μg quercetin /mg dry plant | Total tannin content as μg tannic acid /mg dry plant |
|-------------------|------------------------------------------------------|------------------------------------------------------|------------------------------------------------------|
| Flowers           | 22.64±0.07                                           | 9.08±0.3                                             | 17.7±0.05                                            |
| Seeds             | 67.03±0.56                                           | 12.32±0.45                                           | 15.6±0.43                                            |
| Leaves            | 12.6±0.5                                            | 10.02±0.09                                           | 74±0.63                                              |
| Stems             | 11.03±0.45                                           | 7.5±0.2                                              | 66.34±0.35                                           |

From the information above it is obvious that the seeds are the richest part of the plant with flavonoid and phenolic compounds followed by the flowers then the leaves, the stems contain the less amount, while the higher content of tannin were observed in the leaves and stems.

4. Conclusion

*Silybum marianum* which is distributed widely in Iraq is an important medicinal plant and contain highly valuable active constituents in all the plant parts such as terpenes, flavonoids, steroids and saponins, and this is the first study that describes the microscopic feature of Iraqi *Silybum* and
compare between the phytochemical components of the plants parts, further fractionation and isolation of this constituents are required.

5. Acknowledgement

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