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SHORT COMMUNICATION article

Chemical composition and amino acid analysis of the seeds of the milk thistle plant (*Silybum marianum Gaertn* L.) widespread in Tulkarem, Palestine

Khaled Taha Muhammad Abu Thaher

College of Law and Forensic Sciences - Department of Forensic Sciences, Al-Istiqlal University (Palestinian Academy for Security Sciences), Jericho, Palestine

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Abstract: In this study, the milk thistle (*Silybum marianum* Gaertn L.), widespread in Palestine, was selected to study the basic qualitative chemical composition of aqueous, ethanol, chloroform, and hexane extracts from its seeds, and to identify the active compounds present. The results of the phytochemical analysis showed that the seeds of the plant (*Silybum marianum* Gaertn L.) contain a full range of chemically active compounds in all its extracts, such as: flavonoids, amino acids, carbohydrates, sterols, saponins, anthraquinones, phenolic compounds, steroids, terpenoids, tannins, alkaloids, anthocyanins, quinones, and glycosides. For the first time in Palestine, seventeen amino acids were identified in *Silybum marianum* Gaertn L. plant seeds using phytochemical analysis, paper and thin-layer chromatography, and standard amino acid assays. As a result of the phytochemical analysis, a range of amino acids were identified in the plant seeds, including: arginine, leucine, valine, lysine, phenylalanine, isoleucine, threonine, histidine, glutamic acid, glycine, serine, proline, aspartic acid, alanine, tyrosine, methionine, cysteine.

Introduction

The use of medicinal plants dates back to the dawn of human history. In recent years, the use of medicinal plants containing phenolic compounds has increased due to their diverse medicinal effects, low cost, and lack of side effects compared to manufactured drugs [1, 2]. Especially for plants that are globally important in the fields of nutrition and the treatment of various diseases, the determination of the qualitative chemical composition of the active substances in their parts, especially local species that have not been thoroughly studied in the country, has attracted the attention of researchers and scientists [2].

Silybum marianum (L.) Gaertn, commonly known as Milk Thistle, belongs to the Asteraceae family. It is an annual or biennial herbaceous plant native to the Mediterranean basin and now widespread throughout the world [3]. Its seeds contain a range of active compounds, and the plant is used to treat liver problems and other ailments. The leaves are spiny and have lateral spots around the veins. Milk thistle is self-pollinating and has pink or purple flowers, blooming in May and June. It has been used in traditional folk medicine for over 2000 years, most commonly in the treatment of liver diseases such as liver toxicity, cirrhosis, and hepatitis [4]. **Figure 1**. Milk thistle is cultivated commercially as a medicinal plant in Europe, Egypt, China, and Argentina [5]. On the other hand, based on the results of studies and research, milk thistle is currently considered an interesting crop for bioenergy production in the Mediterranean environment [6] and a source for biodiesel production [7].

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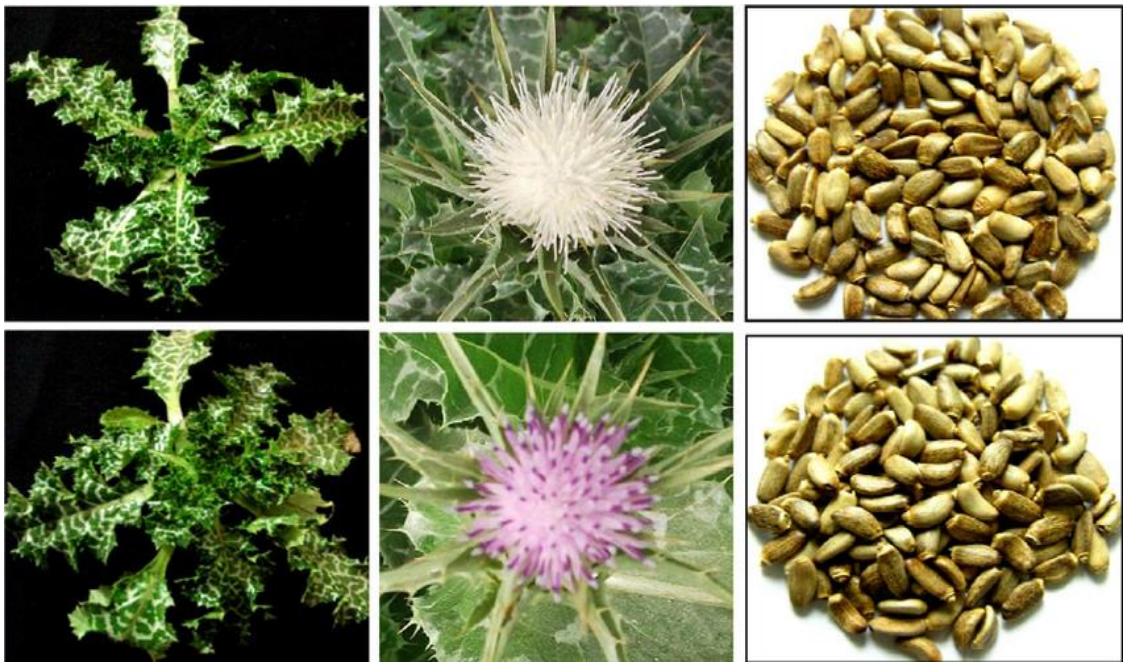

Figure 1: Plants, flowers and seeds of *Silybum marianum* L.

Extracts obtained from milk thistle are used for multiple medicinal purposes, such as anti-diabetic, liver and heart protection, cholesterol reduction, blood pressure reduction, anti-hemorrhagic, anti-inflammatory and antiviral, antispasmodic, nerve protection, anti-cancer, antioxidant, and having a protective effect against Alzheimer's [3, 8-12]. Milk thistle has therapeutic effects, which are closely related to the presence of a flavonoid compound called silymarin [13]. This compound stimulates liver regeneration and is recognized as an antioxidant and anti-inflammatory agent, and is therefore effective in treating food poisoning, hepatitis, and liver cirrhosis [14]. Recently, the hepatoprotective activity of silymarin, which is based on its antioxidant properties, has been documented, and it has become used in the manufacture of plant-based pharmaceuticals such as (Lagalo, Flavobion) used in the treatment of mycotoxicosis that affects the liver (Amanita sp.) [15]. Silymarin has been found in the highest concentration in the fruit of the plant, but it is also present in the leaves and seeds. At the beginning of 2014, silymarin extracted from milk thistle seeds was ranked sixth among the top 20 best-selling dietary supplements in the health and natural food market, and ranked twelfth among the top 40 herbal supplements in the plant-based products market in the United States [16, 17].

In different countries, the leaves, stems, flowers, and seeds of Milk thistle have been used for various purposes. Milk thistle seeds contain silymarin, bioflavonoids, flavonoids, amino acids, glutamic acid, fumaric acid, saponins, sterols, tocopherols, oils, starches, gum, minerals, tannins, fatty acids and polyphenolic components [18], and the oil extracted from these seeds can be used as a treatment for many diseases, including viral hepatitis and cirrhosis of the liver [19, 20]. Studies have shown that seeds contain many active substances beneficial to health, such as protein, with a valuable content of essential amino acids, and some active phytochemicals that possess pharmacological properties such as antioxidants or anti-cancer and anti-free radical effects [21]. In Palestine, only a few studies have been conducted on the uses of milk thistle in traditional medicine [22, 23]. Furthermore, adding milk thistle seed flour to food products and dietary supplements improves their absorption. Experts recommend consuming these products daily to help prevent diseases such as cardiovascular disease and some types of cancer. Therefore, studying the phytochemicals of the active ingredients in seeds is a crucial strategy [21]. The current study aimed to study the qualitative phytochemical composition of (*Silybum marianum* L.) seeds and to determine the qualitative chemical composition of the seeds in terms of amino acids. In addition, to the best of the researcher's knowledge, the qualitative phytochemical composition of the seeds of the wild *Silybum marianum* plant in Palestine has not been explored.

Materials and methods

Plant collection: Samples of milk thistle (*Silybum Marianum* L.) seeds were carefully collected at maturity from the area of Far'un village (32°17′10″N 35°01′23″E) in the city of Tulkarm in the West Bank of Palestine during October 2025. The seeds were then left to dry naturally at room temperature for 30 days. After that, the seeds were ground into a fine powder. We will use this powder for qualitative chemical analysis of the active ingredients and to detect amino acids. The plant was formally identified as (*Silybum Marianum* L.) by Dr. Rami Al-Qaisi from the National Agricultural Research Center - MOA.

Preparation of (Silybum marianum L.) seed extracts: 100 grams of pre-prepared *Silybum marianum* seed powder were extracted at room temperature by soaking in 500 ml of petroleum ether for 72 hours, stirring occasionally. The extract was then filtered through Whatman No. 1 filter paper, and the solid residue was extracted using two methods: The sequential method using a variety of solvents (water, ethanol, chloroform, and hexane). The ratio of seed material to extract was (1: 10), and the process was carried out in a Soxhlet apparatus for 10 hours at a temperature of 90°C. The extracted material was then collected in a separate conical flask for each liquid and concentrated under low pressure and temperature using a rotary evaporator [24]. The samples obtained in this way will be used to determine the specific phytochemical composition of the seeds of the *Silybum marianum* L. plant.

Second, add 150 ml of 97.0% ethanol to (40 g) of *Silybum marianum* L. seeds and then heat in a water bath for approximately half an hour. Then cool, filter, and place in a centrifuge. Next, we take the clear liquid from the top of the test tube and place it in a water bath at a temperature of 40 - 50°C. The sample obtained in this way will be used to qualitatively determine the amino acids [25]. Ethanol was deliberately chosen as an extract and solvent due to its polar properties, which enable it to efficiently dissolve a wide variety of active compounds found in plant seeds. Furthermore, it has a positive safety record and environmentally friendly characteristics compared to other polluting and hazardous polar solvents.

Qualitative tests for detecting the chemically active compounds: Qualitative phytochemical analysis of extracts derived from the seeds of the plant (*Silybum marianum* L.) was carried out according to the standard procedures and methods described in references [26-29].

Qualitative tests for amino acids: Ninhydrin reaction: In a test tube, 1.0 ml of *Silybum marianum* (L.) seed extract was taken, and 1.0 ml of ninhydrin reagent was added. A purple color was observed, indicating the presence of amino acids [25].

Qualitative determination of amino acids using two-dimensional paper chromatography: The amino acid content of the sample was estimated using a two-dimensional ascending paper chromatography method in which two liquid systems were used and Whatman No. 1 chromatography paper was used as the stationary phase for the paper chromatography. The first solvent regimen was A -n-butanol: acetic acid: water, at a ratio of 4: 1: 5 (v/v). The second solvent regimen was B- ethanol: toluene, at a ratio of 2: 1 (v/v). Ninhydrin reagent was used as a color developer [25]. The process is carried out in successive steps [30].

Amino acid separation using one-dimensional paper chromatography: The amino acids in the seeds of the plant *Silybum marianum* (L.) were separated using a one-dimensional paper chromatography method, using the solvent system: n-butanol: acetic acid: water, in a ratio of 4: 1: 5 (v/v) according to the method mentioned above, and were identified using standard amino acid models [25].

Separation and identification of amino acids by Thin-Layer Chromatography (TLC): The amino acids in the seeds of the plant Silybum marianum (L.) were separated by thin-layer chromatography (TLC) using an aluminum-coated silica gel plate (Merk Co. Inc., Kieselgel F256) in a solvent system: n-butanol: acetic acid: water, at a ratio of 4: 1: 5 (v/v), and identified using standard amino acids [25].

Ethical issues: No ethical approval is needed for this study.

Results and discussion

The phytochemicals extracted from the (*Silybum Marianum* L.) plant play an important role in treating many different diseases and are used in the manufacture of many medicinal preparations and nutritional supplements. This plant is still used in traditional and modern folk medicine practices [30]. The results of the phytochemical study conducted on the seeds of *Silybum marianum* L. showed that its seeds contain: flavonoids, amino acids, carbohydrates, sterols, saponins, anthraquinones, phenolic compounds, steroids, terpenoids, tannins, alkaloids, anthocyanins, quinones, and glycosides. The results are shown in **Table 1**.

The current study, which investigated the active compounds in (*Silybum Marianum* L.) seeds, showed good agreement with the results of other studies in Romania, Egypt, Turkey, and Kazakhstan [15, 31-33]. As a result of phytochemical examination (qualitative analysis - chromatographic techniques) and comparison with reference samples, seventeen free amino acids were found in (*Silybum marianum* L.) seeds. Data showed that the seeds of the studied plant contain a wide variety of amino acids: Arginine, leucine, valine, lysine, phenylalanine, isoleucine, threonine, histidine, glutamic acid, glycine, serine, proline, aspartic acid, alanine, tyrosine, methionine, cysteine. The results are shown in **Table 2**. Amino acids, which are found in many parts of plants, are of great importance to plants as they play a vital role in various physiological processes that occur within the plant, such as the synthesis of phenolic compounds and other processes of manufacturing active substances. In addition, those plants that have a large stock of their active components, amino acids, are considered to be an important source of amino acids and can be used in the manufacture of pharmaceutical products, food supplements, and other medical materials [34, 35].

The amino acids lysine and valine are essential for all muscle proteins; tyrosine is necessary for the synthesis of neurotransmitters such as dopamine, norepinephrine, and adrenaline. The amino acid isoleucine is crucial for the production of hemoglobin in red blood cells. Phenylalanine, however, is used in cases of depression and loss of appetite [30]. The results of our current study, which investigated the qualitative chemical analysis of amino acids in *Silybum marianum* L. seeds, showed good agreement with the results of other studies in Uzbekistan, Turkey, Romania, and Poland [21, 32, 36, 37]. The results of the phytochemical analysis of Silybum Marianum L. showed that the seeds contain a variety of active ingredients (**Table 1**), and a large and varied quantity of amino acids (**Table 2**). These results demonstrate the richness of this plant's seeds in active ingredients that play a vital role in bodily functions and possess diverse biological, therapeutic, and pharmacological properties, including antioxidant and antitumor activity, and can be used in the treatment of cancerous diseases [38, 39]. As we mentioned earlier, amino acids play an important and major role in plants through their function as an osmotic factor; they regulate stomatal penetration; they remove toxins, especially heavy metals; and they have a major role in the activity and synthesis of some enzymes [34].

Table 1: Qualitative chemical analysis of the active ingredients in the seeds of *Silybum Marianum* L.

Phytochemicals Compounds	Test	Finding
Flavonoids	Shibata's reaction (Shinoda reaction)	+
Amino acids	Biuret and Ninhydrin reaction	+
Carbohydrate	Molisch's test	+
Sterol	Liebermann's Burchard	+
Saponin	Foam (Froth) Test	+
Anthraquinones	Borntrager's reaction	+
Phenolic compound	Ferric chloride	+
Steroids and terpenoids	Salkowski	+
Tannin	1.0% Gelatine containing 10% sodium chloride	+
Coumarins	NaOH solution	-
Alkaloids	Dragendorff"s Test	+
Anthocyanin	HCl and ammonia	+
Quinone	NaOH test	+
Glycosides	Borntrager's test	+

Table 2: The qualitative composition of amino acids in the seeds of (*Silybum marianum* L.)

Amino acid	Rf values in mobile phase	
	A	B
Alanine	0.21	0.30
Arginine	0.05	0.24
Glutamic acid	0.18	0.15
Aspartic acid	0.16	0.04
Glycine	0.23	0.14
Leucine	0.65	0.63
Serine	0.18	0.11
Isoleucine	0.71	0.73
Methionine	0.40	0.44
Threonine	0.19	0.16
Cysteine	0.46	0.03
Valine	0.44	0.61
Lysine	0.04	0.12
Histidine	0.12	0.05
Proline	0.24	0.31
Phenylalanine	0.33	0.53
Tyrosine	0.55	0.37

***A-** n-butanol - acetic acid - water (4:1:2)- **B-** ethanol - toluene (2:1)*

The identification and isolation of flavonoid, phenol, and amino acid derivatives in the seeds of the plant (*Silybum Marianum* L.) is linked to the plant's therapeutic properties, and the researcher believes this may be the reason for the use of this plant in traditional Palestinian folk medicine and other countries. However, further studies are still needed on all parts of the plant to identify and isolate the compounds responsible for the plant's distinctive therapeutic properties, especially phenols and flavonoids, which opens the door to the possibility of using its seeds in treating diseases caused by free radicals, such as cancer.

Conclusions: After studying extracts of the *Silybum marianum* L. plant to detect the active ingredients, the results of the study showed that the extracts from the seeds of the plant contain biologically active components such as: flavonoids, amino acids, carbohydrates, sterols, saponin, anthraquinones, phenolic compounds, steroids, terpenoids, tannin, alkaloids, anthocyanin, quinone, and glycosides. For the first time in Palestine, a study conducted on extracts of the seeds of the Palestinian plant (*Silybum Marianum* L.) to detect seventeen amino acids using chemical chromatographic analysis showed that the seeds of this plant contain a variety of essential and non-essential amino acids: arginine, leucine, valine, lysine, phenylalanine, isoleucine, threonine, histidine, glutamic, glycine, serine, proline, aspartic, alanine, tyrosine, methionine, and cysteine.

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Conflict of interest: The author declares the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
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