Journal of Pharmacy & Allied Medicine

JPAM, 1(2), 106-112, 2023
https://doi.org/10.58985/jpam.2023.v01i02.14
ISSN (Print): 3005-2726

Research Article

The bio-constituents extracted from *Silybum marianum* L plant and their effects as antimicrobial agent.

Fatima Mustafa Melad[1], Hawa Alsadi[1] and Salem Mohamed Edrah[2]*

1. Chemistry Department, Education College, El-Mergib University, Khums Libya
2. Chemistry Department, Science College Al-Khums, El-Mergib University, Khums Libya

Article Information
Received: 06 September 2023
Revised: 04 December 2023
Accepted: 07 December 2023
Published: 17 December 2023

Academic Editor
Prof. Dr. Christian Celia

Corresponding Author
Prof. Dr. Salem Mohamed Edrah
E-mail: drsalemedrah@gmail.com
Tell: +218 94 415 7827.

Keywords
Silybum marianum L, phytoco -nstituents, bio-constituents, antimicrobial activities, natural products, traditional drugs.

Abstract

Medicinal plants contain various phytochemicals that provide their beneficial properties. These phytochemicals contribute to the healing and therapeutic effects of the plants. The aqueous, ethanolic, and methanolic extracts of *Silybum marianum* L were thoroughly examined to determine the presence of various metabolites. The analysis results revealed various components, including triterpenoids, steroids, tannins, proteins, glycosides, reducing sugars, alkaloids, flavonoids, carbohydrates, sugars, fats, fixed oils, and saponins. The yields obtained from leaves, seeds, and flowers were as follows: 58.71, 66.32, 63.34, 47.23, 51.39, 60.22, 42.37, 41.23, and 39.71%, respectively. The results of the quantitative evaluation demonstrated an impressive presence of tannins, saponins, alkaloids, and flavonoids, with percentages of 48.21, 53.67, 72.38, and 68.41%, respectively. In addition, these extractions were carried out in order to evaluate their effectiveness against harmful bacteria such as *Escherichia coli, Salmonella Typhi, Staphylococcus aureus,* and *Staphylococcus epidermis,* as well as the fungal strain *Candida albicans*. The findings demonstrated significant potential for effectively inhibiting the proliferation of pathogenic bacteria.

1. Introduction

Plants are vital in medicine, especially in developing nations. People have used plants for healing and some traditional medicines are still in use. In Libya, camel thistle (*Silybum marianum* L) is a medicinal herb. It belongs to the "Asteraceae" family and grows in various regions of Libya and the Mediterranean. *Silybum marianum* L, commonly referred to as milk thistle, is a biennial herb that belongs to the Asteraceae family. It is indigenous to the Mediterranean and North African regions but is now present globally [1]. The plant has a tall, erect stem with a solitary, sizable, purple flower that terminates in pointed spines. The seeds are black to shiny brown and possess a white, silky pappus [2].

The *Silybum marianum* L plant has strong therapeutic effects against diseases, especially cancer and liver conditions. Studies show its potential in treating different liver diseases, including liver cancer, by reducing inflammation and damage. Likewise, *Silybum marianum* L eliminates toxins produced by certain mushrooms and enhances the effectiveness of chemotherapy for various types of cancer, reducing its adverse effects, in addition, *Silybum marianum* L used since ancient times to treat diverse ailments, and more recently liver damage due to toxins, including *Amanita phaloides* poisoning and others [3-6]. Plants produce vital secondary metabolites to defend themselves against different pathogens [7]. The active

components found in *S. marianum* seeds include silybonol, apigenin, betaine, proteins, and free fatty acids [8].

2. Materials and methods

2.1 Processing of plant sample

The medicinal plant *Silybum marianum* L was carefully harvested from the pristine wilderness surrounding the city of Alkhums, Libya. The plant material undergoes a meticulous procedure of washing, drying, and grinding until it transforms into a delicate powder using the help of an electric mill. Following this, the powder is meticulously sifted through a 75-micron sieve. Each part of the plant was meticulously powdered and stored in a light-resistant, airtight bottle for optimum preservation. These precious powders were kept in a cool and dry location until they were ready to be used.

2.2 Extraction processes

Silybum marianum L. plant extracts are prepared from leaves, seeds, and flowers using maceration. Fine powders were combined with solvent, left for 72 hours, filtered, and centrifuged. Supernatants were collected, filtered, weighed, and stored for analysis at 4 ^{O}C.

2.3 Yields calculations

$$\text{Extraction yield (\%)} = \text{Weight of dried extract (g)} / \text{Weight of powdered plant material (g)} \times 100 \quad (1)$$

2.4 Phytochemical study

The purpose of this thorough phytochemical study is to analyze and quantify the various components found in different parts of the medicinal plant *Silybum marianum* L. Specifically, the study will concentrate on examining the leaves, seeds, and flowers of this plant. Furthermore, we will determine the most effective extract for each part by conducting experiments using different solvent mediums.

2.5 Preliminary screening

To obtain a comprehensive understanding of the main categories of secondary metabolites, a preliminary phytochemical screening was performed on the plant extract. This screening of chemical analysis enables us to detect the existence of various compounds in plants, including flavonoids, tannins, alkaloids, phenols, anthraquinones, saponins, steroids, terpenoids, gums, and mucilage, as well as carbohydrates like reducing sugars and polysaccharides [9–19].

2.6 Phytochemical screening (quantitively)

Consistent with the results of the qualitative analysis of the secondary metabolites products obtained for each of the saponins, alkaloids and flavonoids, these components were quantitively evaluated as the following [16, 19-20].

2.7 Determination of total flavonoids

10g of the plant's parts powders undergo multiple extractions using 100 mL of 80% aqueous methanol each time, separately. The resulting mixture is meticulously filtered using Whatman no.1 filter paper into a 250 mL beaker, which has been pre-weighed. Subsequently, the filtrate is transferred to a water bath and left to completely evaporate. Finally, the remaining sample is weighed, yielding the final weight.

$$\text{Percentage of total flavonoid (\%)} = \text{Weight of residue} / \text{Weight of the taken sample} \times 100 \quad (2)$$

2.8 Determination of total saponins

A 10-gram of the plant's parts powders was meticulously combined with 200 ml of 20% ethanol, separately. The mixture was continuously stirred while being heated over a water bath at a temperature of 55 °C for 4 hours. After this, the resulting mixture underwent filtration. The residue obtained from the filtration was then re-extracted in the same manner as before. The extracted substances from both sources were combined and condensed to a volume of approximately 20 ml using a water bath set at 90 °C. This concentrated solution was then poured into a 250 ml separator funnel. To this, 10 ml of diethyl ether was added, and the entire mixture was vigorously shaken to ensure thorough mixing. The ether layer was discarded, while the aqueous layer was preserved. Subsequently, the purification process was repeated, this time with the addition of 25 ml of 1-butanol. The combined 1-butanol extracts were washed twice with 10 ml of a 5% aqueous sodium chloride solution. The remaining solution was then heated in a water bath, and after evaporation, the samples were dried and weighed. The content was calculated accordingly.

$$\text{Percentage of total saponins (\%)} = \text{Weight of residue} / \text{Weight of the taken sample} \times 100 \quad (3)$$

2.9 Determination of total alkaloid

10g of the plant's parts powders was mixed with 200 ml of a mixture of ethanol acetic acid (10%) (Ethanol 180 ml and 20 ml of acetic acid), separately. The mixture was left for 4 hours at room temperature. The

https://doi.org/10.58985/jpam.2023.v01i02.14

Table 1. The organoleptic properties of crude extracts of *Silybum marianum* L with pH and percentage yields

Organoleptic Properties	Types of *Silybum marianum* L. Crude Extracts								
	Leaves			Seeds			Flowers		
	AQS	EtOH	MeOH	AQS	EtOH	MeOH	AQS	EtOH	MeOH
pH	6.36	-	-	6.8	-	-	6.94	-	-
Yield (%)	58.71	66.32	63.34	47.23	51.39	60.22	42.37	41.23	39.71
Colour	Dark Brown	Oily Dark Green	Oily Dark Green	Dark Brown	Oily Dark Green	Oily Dark Green	Dark Brown	Oily Dark Green	Oily Dark Green
Odour	Aromatically			Moderately aromatic			Intensely aromatic		
Taste	Distinctive flavor			Distinctive flavor			Distinctive flavor		
Shape	Thick and sticky texture.			Thick and sticky texture.			Thick and sticky texture.		

AQS = Aqueous Extract, EtOH = Ethanol Extract, and MeOH = Methanol Extract.

mixture was then filtered using Whatman No. 42 - 125 mm filter paper. The filtrate was concentrated on a quarter of its initial volume utilizing a water bath. Then a 5mL of concentrated ammonium hydroxide solution (NH_4OH) was added to the reduced mixture drop-wise until precipitation occurred. After filtration and drying in an oven at 40 °C, the precipitate was collected and weighed. The percentage of the total alkaloid content was calculated as follows:

$$Percentage\ of\ the\ total\ alkaloid\ (\%) = Weight\ of\ residue\ Weight\ of\ the\ taken\ sample \times 100 \quad (4)$$

2.10 Determination of total tannin

8 grams of powdered plant parts were dissolved in 8 ml of distilled water. The mixture was thoroughly combined and vigorously shaken for one hour. Subsequently, it was filtered to remove any impurities. Next, 8 ml of the filtered solution was mixed with 7 ml of 0.1 mM $FeCl_3$ in 0.1 N HCl and 0.008M potassium ferro cyanide in a test tube. Within 10 minutes, the absorbance of the mixture was measured using a spectrophotometer set at 120 nm. To establish a baseline, a blank sample was also prepared, and colour was developed and read at the precise wavelength. A standardized solution was developed using tannic acid to achieve a concentration of 100 ppm, enabling accurate estimation of tannins.

2.11 Antimicrobial assessing

Plants have been used for prevention and treatment since ancient times. Early humans noticed that adding plants to their meals during illness improved their health without side effects. This method has become a standard practice and continues to be acknowledged by the World Health Organization as a critical aspect of primary healthcare that benefits a staggering 80% of the world's population.

2.12 Antimicrobial activities (disk diffusion method)

The plant extracts were tested for antimicrobial properties using bacterial and fungal strains. Solutions were made with dry plant extracts dissolved in DMSO and applied to agar plates. Zones of inhibition were measured after incubation [20-22].

3. Results and discussion

Table 1 presents the organoleptic properties of crude extracts from *Silybum marianum* L. The results contain important details about pH levels and the percentage yields obtained. Where each of the pH of aqueous crude extracts of leaves is 6.36, Seeds 6.8 and Flowers 6.94. The percentage of productivity of leaves, seeds and flowers was as follows 58.71, 66.32, 63.34, 47.23, 51.39, 60.22, 42.37, 41.23 and 39.71%, respectively. The color of leaves, seeds, and flowers in the crude extracts appeared to have distinct organoleptic properties. The aqueous extracts revealed a rich, deep brown color, while the alcoholic extracts oily dark green brand. The extract's odors of leaves, seeds, and flower had a pleasant, mildly fragrant, and highly fragrant aroma. Although the flavors of all extracts had unique tastes, the consistency was consistently thick and sticky for all extracts as well. The choice of the extraction method and solvent greatly influences both the quality and quantity of components derived from the plant, seeds are rich in a variety of valuable components, such as crude oil, starches, mucilage, minerals, tannins, and flavonolignans [23].

3.1 Phytochemical constituents

3.1.1 Qualitative phytochemical

Table 2 shows the results of the phytochemical

https://doi.org/10.58985/jpam.2023.v01i02.14

Table 2. Results of phytochemical screening of aqueous and ethanolic *Silybum marianum* L. crude extracts

Phytochemicals		Plant parts and type of extracts								
		Leaves			Seeds			Flowers		
		AQS	EtOH	MeOH	AQS	EtOH	MeOH	AQS	EtOH	MeOH
Flavonoids		+++	+++	+++	+++	+++	+++	+++	+++	+++
		+++	+++	+++	+++	+++	+++	+++	+++	+++
Tannins		+++	+++	+++	+++	+++	+++	+++	+++	+++
		+++	+++	+++	+++	+++	+++	+++	+++	+++
Alkaloids		+++	+++	+++	+++	+++	+++	+++	+++	+++
Phenols		++	++	++	++	++	++	++	++	++
Anthraquinones		-	-	-	-	-	-	-	-	-
Saponins		+++	+++	++	+++	+++	++	+++	+++	++
Steroids		+	++	+	++	+	++	+	++	+
Terpenoids		+++	+++	++	++	++	++	++	++	++
Gums and Mucilage		+	+	+	+	+	+	+	+	+
Carbohydrates	Reducing Sugars	++	++	++	++	++	++	+	+	+
	Polysaccharides	++	++	++	++	++	++	+	+	+

AQS stands for the aqueous extract, EtOH represents the ethanol extract, and MeOH represents the methanol extract. "+" denotes a weak or minimal amount, "++" indicates a moderate or considerable amount, and "+++" signifies the strongest or significant amount. "-" indicates that the amount is nonexistent.

Table 3. Results of the quantitative evaluation of the *Silybum marianum* L

Plant Name and Associated Parts		Yields (%)			
		Tannins	Saponins	Alkaloids	Flavonoids
Silybum marianum L	Leaves	48. 21	53.67	72.38	68.41
	Seeds	12. 61	44.83	22.78	46.78
	Flowers	10.39	50.12	29.62	67.15

screening of the metabolites contained in the crude aqueous, ethanolic and methanolic extracts of *Silybum marianum* L, which are triterpenoids, steroids, tannins, protein, glycosides, reducing sugars, alkaloids, flavonoids, carbohydrates sugars, fats, fixed oils, saponins, where it was found that all the detected components were between medium and rich in presence in both extracts. The presence of vital and active constituents in the plant's leaves, seeds and flowers especially secondary metabolites makes it play a very significant role in the prevention, resistance and elimination of many pathogenic microbes that are harmful to human health. From the facts provided previously, it is evident that the presence of both primary and secondary metabolite ingredients plays a vital role in ensuring the overall health and vitality of leaves. The seeds and flowers of *Silybum marianum* L have numerous health benefits and can be used as both preventative measures and treatment for various diseases.

3.1.2 Quantitative phytochemical

Table 3 presents the quantitative evaluation results of *Silybum marianum* L. The percentage yields of tannins, saponins, alkaloids, and flavonoids were determined for leaves, seeds, and flowers. The leaf extract exhibited impressive percentages, with tannins, saponins, alkaloids, and flavonoids at 48.21, 53.67, 72.38, and 68.41%, respectively. Meanwhile, the seed extract demonstrated significant results, with percentages of 12.61, 44.83, 22.78, and 46.78%, respectively. Remarkably, the flower extract exhibited important levels of tannins (10.39%), saponins (50.12%), alkaloids (29.62%), and flavonoids (67.15%). Tannins, saponins, alkaloids, and flavonoids are crucial elements in fighting diseases and eliminating harmful microbes [24-26]. Perhaps because these compounds have great antioxidant properties, they also show enhanced efficacy due to the presence of functional groups. Traditional herbs are popular due to their affordability and availability. Concerns about synthetic medications have increased. The method of extraction and the selection of solvent greatly affect both the quantity and quality of plant components [27-29]. Secondary metabolites are essential for regulating vital pathways. To regulate important pathways in a highly effective manner. Plants have

https://doi.org/10.58985/jpam.2023.v01i02.14

Table 4. Results of the biological activity assessment of extracts of leaves, seeds and flowers of *Silybum marianum* L against different types of bacterial and fungal

Microbial	Types of Crude Extracts and the zones of inhibition (mm)										
	Leaves			Seeds			Flowers			Antibiotics	
	AQS	EtOH	MeOH	AQS	EtOH	MeOH	AQS	EtOH	MeOH	Gent	Cont.
Escherichia coli(-)	14	15	18	13	15	18	13	17	19	25	-
Salmonella spp.(-)	16	17	19	17	16	17	15	18	16	24	-
Staphylococcus aureus(+)	19	14	13	18	14	16	17	14	14	24	-
Staphylococcus epidermis(+)	18	17	15	11	19	17	13	16	13	22	-
*Candida albicans*y	10	15	16	17	12	14	14	16	13	-	20

AQS = Aqueous Extract, EtOH = Ethanol Extract, and MeOH = Methanol Extract. Gentamycin = Gent; Contamazole = Cont. (+) = Gram-positive bacteria include *Staphylococcus aureus* and *Staphylococcus epidermidis;* (+) = Gram-negative bacteria include *Escherichia coli and Salmonella spp.;* y = *Candida albicans* is an opportunistic pathogenic yeast in the fungus kingdom.

valuable secondary metabolites with pharmacological properties. They serve as defense mechanisms and have specialized functions [30-32]. Compounds like flavonoids, saponins, terpenoids, and alkaloids have anti-diabetic properties. The compounds mentioned possess remarkable characteristics and hold great promise as medicinal agents for treating diabetes [33-36].

3.2 Antibacterial activity

Table 4 presents the results of the Antibacterial evaluation on the crude extracts of various plant parts, including leaves, seeds, and flowers of the medicinal plant *Silybum marianum* L evaluated the effectiveness of these extracts against both pathogenic bacteria and Candida albicans.

The aqueous extracts exhibited significant antibacterial activities. *Escherichia Coli* displayed impressive values of 14, 13, and 13 mm. When tested against *Salmonella SPP*., their activity increased even further with values of 16, 17, and 15 mm. For *Staphylococcus aureus*, the activities were 19, 18, and 17 mm, and for *Staphylococcus epidermidis*, they were 18, 11, and 13 mm. *Candida albicans* showed activities of 10, 17, and 14 mm with the aqueous extracts. Ethanolic extracts also demonstrated distinguished antibacterial properties. Against *Escherichia Coli*, the activities were 15, 15, and 17 mm, and against *Salmonella SPP*., they were 17, 16, and 18 mm. The activities against *Staphylococcus aureus* were 14 mm, while for *Staphylococcus epidermidis* they were 17, 19, and 16 mm. *Candida albicans* showed activities of 15, 12, and 16 mm with the ethanolic extracts. Methanol-based extracts from the leaves, seeds, and flowers of *Silybum marianum* L not only exhibited noteworthy antibacterial properties but also demonstrated impressive performance. Against *Escherichia Coli*, the measured activities were 18, 18, and 19 mm, respectively. The measures taken to combat *Salmonella* bacteria. Were measured at 19, 17, and 16 mm. For *Staphylococcus aureus*, the observed activities were 13, 16, and 14 mm, and for *Staphylococcus epidermidis*, they were 15, 17, and 13 mm. Lastly, *Candida albicans* displayed activities of 16, 14, and 13 mm with the methanol-based extracts. It is worth noting that antibiotics showed the highest level of effectiveness against bacteria. Gentamicin and Contamazole, used as positive controls, exhibited superior activity, likely due to their pure form.

In Libya, the use of therapeutic plants, particularly in traditional medicine, is widely encouraged. This is a response to the growing concern surrounding the resistance of synthetic antimicrobial agents. Secondary metabolites, such as saponins, tannins, alkaloids, coumarins, glycosides, essential oils, and flavonoids, are a wide range of biologically active substances. The extracts derived from this plant have shown considerable potential in reducing the risk of bacterial infections [34]. Moreover, *Silybum marianum* L has also demonstrated antiparasitic activity against cutaneous leishmaniasis [34-36].

4. Conclusions

The therapeutic properties of *Silybum marianum* L, a medicinal plant, have been discovered in its leaves, seeds, and flowers, revealing its exceptional healing potential. The plant's abundance of secondary metabolites, including alkaloids, phenols, and flavonoids, contribute to these remarkable properties. Additionally, the research has revealed a treasure trove of tannins, saponins, alkaloids, and flavonoids

https://doi.org/10.58985/jpam.2023.v01i02.14

within the plant. Not only do these powerful compounds help in combating infections, but they also play a vital role in the prevention and treatment of numerous diseases. Furthermore, the plant's impressive ability to combat both bacteria and fungi highlights its immense potential. This study has not only revealed the plant's potent biological components, but also its remarkable impact on microbial activity. The comparison between the plant extracts and antibiotics demonstrates the plant's potential. Especially given the exceptional results obtained in this research, which showcased the significant efficacy of plant extracts when compared to the presence of antibiotics. Harnessing the power of these beneficial compounds can yield countless advantages, including disease prevention, particularly in terms of cancer, and potentially even completely replacing chemically synthesized antibiotics in the coming years.

Authors' contributions

Conducted valuable scientific research and collected scientific literature, S.E. and F.M.; Collaborated to materialize and type the review, S.E., F.M. and H.A.

Acknowledgements

We deeply appreciate the Chemistry Departments of the Faculty of Science and Faculty of Education at the University of Elmergib Alkhums in Libya for their incredible support and assistance. The success of our research article owes a great deal to their generous support and the provision of a fully-equipped laboratory.

Funding

We inform you that our research project has not secured any funding.

Availability of data and materials

The paper includes all the necessary data, along with its corresponding supporting information files. As per the Journal's request, we will provide the required supplementary data.

Conflicts of interest

There are no potential conflicts of interest to disclose in relation to the research, authorship, and/or publication of this article.

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