

Antioxidant, Antibacterial and Antifungal Activities of Different Extracts of *Silybum marianum* Collected from Duhok (Iraq)

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Abstract: In this study, antioxidant, antibacterial and antifungal activities of *Silybum marianum* (L.) Gaertn. collected from Duhok (Iraq) were determined. Ethanol, methanol and dichloromethane extracts of the fruit part of plant were obtained. Antioxidant potential was determined with TAS and TOS kits using ethanol extracts. Antibacterial and antifungal activity were determined using agar dilution method. Antibacterial activity was determined against 6 bacterial strains (*Staphylococcus aureus*, *S. aureus* MRSA, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*). Antifungal activity was determined against *Candida albicans*, *C. krusei* and *C. glabrata*. As a result of the studies, it was determined that plant extracts have high antioxidant activity. It was also found to be effective against bacteria at 25-400 µg/mL concentrations. Plant extracts were found to be more effective against gram negative bacteria. It was found to be effective against *Candida* species at 400-800 µg/mL concentrations. As a result, it was determined that the fruit parts of plant could be a natural antioxidant and antibacterial source.

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1. INTRODUCTION

Traditional medicine practices are common in many parts of the world. It is particularly common in China, India, Japan, Pakistan, Sri Lanka and Thailand. In China, about 40% of medical consumption comes from traditional medicines [1]. Iraq, where the study material is collected, hosts many natural plants due to its geographical diversity and climatic conditions. Iraq is located north and east of the mountainous Turkey and Iran regions and has a similar geography. In Iraq, 363 medicinal plant species belonging to approximately 98 families and 270 genera have a natural distribution [2]. In this study, *S. marianum* plant collected from Duhok region of Iraq was selected as the material. *S. marianum*, a member of Asteraceae, is a one-year herbaceous plant. The plant spreads naturally in Europe, Africa and Asia [3]. In previous studies, antioxidant, hepatoprotective, protective effect against DNA damage, anti-aging, anti-aflatoxin, antibacterial and immunomodulatory activities of *S. marianum* were

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reported [4-8]. In this study, it was aimed to determine the antioxidant, oxidant, antibacterial and antifungal activities of *S. marianum* fruit parts. *S. marianum* is consumed extensively by local people. Therefore, it was aimed to determine the antioxidant activity and activity of the plant. In addition, by determining oxidant level and oxidative stress index, it was aimed to determine the oxidative risk status in consumption.

2. MATERIAL and METHODS

Samples of *S. marianum* were collected from Iraq's Duhok-Bamarne region. Flora of Iraq was used to identify the plant [9]. Herbarium specimens were collected at Zakho University, Faculty of Arts and Sciences, Department of Biology herbarium.

2.1. Laboratory studies

Plant samples collected from the field were brought to the laboratory under appropriate conditions. The samples were allowed to dry in a shade and breathing environment. Dried marianum samples were cut and pulverized. 30 g of the fruit parts of the plant were extracted with soxhlet extractor with ethanol (EtOH), methanol (MeOH) and dichloromethane (DCM) at 50 ° C for about 6 hours. The extracts obtained were concentrated by rotary evaporator.

2.2. Antioxidant, Oxidant and Oxidative stress Tests

The antioxidant and oxidant values of EtOH extracts of *S. marianum* were determined using Rel Assay TAS and TOS kits [10,11]. Trolox was used as a calibrator for TAS tests. Hydrogen peroxide was used as a calibrator for TOS tests. Oxidative stress index (OSI) (Arbitrary Unit = AU) value was determined according to the following formula.,

$$\text{OSI (AU)} = \frac{\text{TOS, } \mu\text{mol H}_2\text{O}_2 \text{ equiv./L}}{\text{TAS, mmol Trolox equiv./L} \times 10}$$

2.3. Antibacterial and Antifungal Activity Tests

Antibacterial and antifungal activity capacities of EtOH, MeOH and DCM extracts of *S. marianum* were determined using agar dilution method. Minimal inhibitor concentrations (MICs) of the extracts were tested against standard bacterial and fungus strains. *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Acinetobacter baumannii* ATCC 19606 were used for antibacterial activity. *Candida albicans* ATCC 10231, *C. krusei* ATCC 34135 ATCC 13803 and *C. glabrata* ATCC 90030 strains were used for antifungal activity. Bacterial strains were pre-cultured on Muller Hinton Broth medium. *Candida* strains were pre-cultured on RPMI 1640 Broth medium. Turbidity of bacteria and fungi was prepared according to McFarland 0.5 scale to obtain a standard inoculum. Plant extracts were tested at 12.5-800 µg/mL concentrations. Dilutions were made with distilled water. Fluconazole and Amphotericin B were used as reference drugs for *Candida* strains. Amikacin, Ampicillin and Ciprofloxacin were used as reference drugs for bacterial strains. The lowest concentration that prevents the growth of bacteria and fungi were determined as MIC [12-17].

3. RESULTS and DISCUSSION

3.1. Antioxidant and Oxidant Potential

Antioxidant-based drug formulations are used in the prevention and treatment of serious health problems such as atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [18,19] Therefore, it is very important to determine the natural materials that can be used in

antioxidant drug formulations. In this context, antioxidant and oxidant potentials of *S. marianum* were determined. In addition, oxidative stress status was determined due to TAS and TOS values. The values obtained are shown in Table 1.

Table 1. Antioxidant, Oxidant status and Oxidative stress index of *S. marianum*

	TAS (mmol/L)	TOS ($\mu\text{mol/L}$)	OSI (TOS/(TASx10))
<i>S. marianum</i>	5.767 \pm 0.128	12.144 \pm 0.060	0.211 \pm 0.003

Values are presented as mean \pm SD; Experiments were made in 5 parallels

Different researchers in different parts of the world have reported that *S. marianum* has antioxidant activity. In a study conducted in Egypt, ethanol extracts of the air-dried plant material of *S. marianum* were used and reported to have high antioxidant activity [20]. Methanol and hexane extracts of *S. marianum* seeds were used in the study conducted in Greece. Seed extracts of *S. marianum* collected from 30 different regions of Greece have been reported to have antioxidant potential [21]. It was reported that ethanol and petroleum ether extract of the seeds of *S. marianum* collected from Turkey were the antioxidant potential [22]. In China, ethanol extracts of leaf, man stem, root, fruit receptacle and pappus of *S. marianum* were used. Main stem showed the highest antioxidant activity. The lowest antioxidant activity was determined in extracts of root and pappus parts. In addition, *S. marianum* has been reported to have strong antioxidant activity [23]. In a study conducted in Russia, ethanol extracts of fruit parts of *S. marianum* were reported to have antioxidant activity [24]. In our study, fruit parts of *S. marianum* were used and it was found to have antioxidant potential. TAS, TOS and OSI values of *S. marianum* were determined for the first time. In studies on different plant species, TAS value of *Rosa canina* collected from Turkey was reported 4.602 mmol/L, TOS value was 6.294 $\mu\text{mol/L}$ and OSI value 0.138 [25]. TAS value of *Salvia multicaulis* collected from Turkey was reported 6.434 mmol/L, TOS value was 22.441 $\mu\text{mol/L}$ and OSI value 0.349 [26]. TAS value of *Rhus coriaria* var. *zebaria* collected from Iraq (Duhok) was reported 7.342 mmol/L, TOS value was 5.170 $\mu\text{mol/L}$ and OSI value 0.071 [27]. TAS value of *Mentha longifolia* subsp. *longifolia* collected from Turkey was reported 3.628 mmol/L, TOS value was 4.046 $\mu\text{mol/L}$ and OSI value 0.112 [28]. Also, TAS values of *Thermopsis turcica*, *Brassica rapa* and *Calendula officinalis* were reported as 2.06, 1.25 and 5.55 mmol/L, respectively [29-31]. The TAS value indicates the level of antioxidant compounds produced in the plant. The TOS value indicates the levels of oxidant compounds produced in the plant. OSI value indicates how much antioxidant compounds produced in plants suppress oxidant compounds. Compared to these studies, TAS value of *S. marianum* used in our study was higher than *M. longifolia* subsp. *longifolia*, *R. canina*, *T. turcica*, *B. rapa* and *C. officinalis*. It is lower than *R. coriaria* var. *zebaria* and *S. multicaulis*. TOS and OSI values of *S. marianum* was lower than *S. multicaulis*, *R. canina* and *R. coriaria* var. *zebaria*. Also TOS and OSI values of *S. marianum* was higher than *M. longifolia* subsp. *longifolia*. As a result, *S. marianum* has a high TAS value. In this context, it was determined that the fruit parts of *S. marianum* could be used as a natural antioxidant source.

3.2. Antibacterial and Antifungal Activity

Since ancient times, many plant species have been a source of medicine for humans. Evidence has been found that the Neanderthals who lived in Iraq 60,000 years ago used plants such as hollyhock. To date, plant species containing a lot of information on ethnobotany have been widely used. Today, almost half of the plant species used as medicines in the United States are used for antimicrobial purposes. Unconscious use of antibiotics in humans has led to the formation of resistant forms of microorganisms. For this reason, the antibiotics used in the market are insufficient in the fight against microorganisms [32,33]. In this context,

determination of antimicrobial potential of plant species is very important. In this study, the lowest extract concentrations of *S. marianum* that prevent the growth of bacteria and fungi were determined. The findings are shown in Table 2.

Table 2. Antibacterial and Antifungal Activity of *S. marianum*

	A	B	C	D	E	F	G	H	J
EtOH	100	100	400	25	50	100	400	400	400
MeOH	100	200	400	50	50	400	800	800	800
DCM	200	200	400	50	50	400	800	800	800
Ampicillin	1.56	3.12	1.56	3.12	3.12	-	-	-	-
Amikacin	-	-	-	1.56	3.12	3.12	-	-	-
Ciprofloksasin	1.56	3.12	1.56	1.56	3.12	3.12	-	-	-
Flukanazol	-	-	-	-	-	-	3.12	3.12	-
Amfoterisin B	-	-	-	-	-	-	3.12	3.12	3.12

*(A) *S. aureus*, (B) *S. aureus* MRSA, (C) *E. faecalis*, (D) *E. coli*, (E) *P. aeruginosa*, (F) *A. baumannii*, (G) *C. glabrata*, (H) *C. albicans*, (J) *C. krusei*

*800, 400, 200, 100, 50 and 25 µg/mL extract concentrations

Previously, n-hexane extract of seeds of *S. marianum* collected from Turkey was reported to be effective against *P. aeruginosa*, *E. coli*, *S. aureus*, *Salmonella typhi*, *Aspergillus niger* and *C. albicans* at different concentrations [34]. Methanol, n-hexane and chloroform extracts of *S. marianum* collected from 10 different regions of Pakistan were reported to be effective against *E. coli*, *Salmonella* spp., *Shigella* spp., *S. aureus* and *V. cholerae* at different concentrations [35]. Aqueous and methanol extracts of *S. marianum* collected from Algeria were reported to be effective against *S. aureus*, *E. coli*, *Klebsiella pneumoniae*, *P. aeruginosa*, *Enterobacter aerogenes* and *C. albicans* at different concentrations [36]. In our study, EtOH, MeOH and DCM extracts of fruit parts of *S. marianum* were used. EtOH extracts of plant showed the highest activity. Plant extracts were generally found to be more effective against gram negative bacteria (*E. coli*, *P. aeruginosa* and *A. baumannii*). Antifungal activity of plant extracts was found to be low. Plant extracts were found to be effective against bacterial species at 25-400 µg/mL concentrations. It was found to be effective against *Candida* species at 400-800 µg/mL concentrations. As a result, it was determined that the fruit parts of *S. marianum* could be a natural antibacterial agent against the tested microorganisms.

4. CONCLUSION

In this study, antioxidant, oxidant, antibacterial and antifungal activities of fruits parts of *S. marianum* collected from Duhok (Iraq) region were determined. Fruit parts of the plant have been found to have high antioxidant activity. In addition, plant extracts were found to be more effective against gram negative bacteria. The antifungal activity of the plant extracts were found to be low. As a result, it was determined that the fruit parts of *S. marianum* could be natural antioxidant and antibacterial agent.

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