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ORIGINAL ARTICLE

Effects of Potassium Sulfate [K_2SO_4] on The Element Contents, Polyphenol Content, Antioxidant and Antimicrobial Activities of Milk Thistle [Silybum Marianum]

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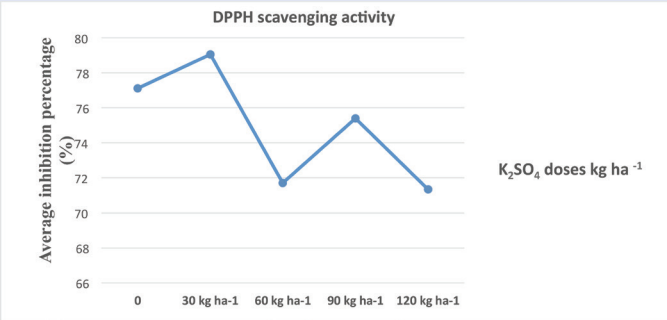
ABSTRACT

Background: *Silybum marianum* L. (Milk thistle) is native to the Mediterranean basin and is now widespread throughout the world. It's sprout is used as a herbal medicine for the treatment of liver disease for centuries. The seeds of milk thistle contain silymarin, an isomeric mixture of flavonolignans [silybin, silychristin, and silydianin]. Silymarin acts as a strong anti-hepatotoxic. **Objectives:** The objective of this study was to evaluate the influences of potassium sulfate [K_2SO_4] fertilizer doses on polyphenol content, some nutrient elements, antioxidant and antimicrobial activities of milk thistle at experimental fields of Ordu University in Turkey. **Methods:** The antimicrobial activities of seed ethanol extracts and seed oil were tested *in vitro* against Pseudomonas aeruginosa (P. aeruginosa), Escherichia coli, (E. coli) Staphylococcus aureus (S. aureus), Aspergillus niger (A. niger) and Candida albicans (C. albicans) using the disc diffusion method. Free radical scavenging activity of the ethanolic extracts of milk thistle was determined spectrophotometrically by monitoring the disappearance of 2, 2-diphenyl-1-picrylhydrazil (DPPH•) at 517 nm according to the method described by Brand-Williams *et al.*[17] The phenolic contents in the ethanolic extracts of milk thistle were determined according to the procedure described by Slinkard and Singleton[19] with a slight modification of using a Folin-Ciocalteu phenolic reagent. The amount of total flavonoid in the ethanolic extracts was measured by aluminum chloride [$AlCl_3$] colorimetric assay. The ions in aerosol samples were determined by using Dionex ICS 1100 Series ion chromatography. **Results:** Seed and seed oils obtained from obvious doses of potassium sulfate [0, 30, 60, 90 and 120 kg ha $^{-1}$] fertilizer applications showed antimicrobial activities against *E. coli, A. niger* and *P. aeruginosa*. The application of 90 kg ha^{-1} of K_2SO_4 on seed oil resulted in the highest antimicrobial activities. At 100 µg mL^{-1}and 200 µg mL^{-1}, except the highest potassium application [120 kg ha $^{-1}$] extract, all extracts showed high and similar DPPH scavenging activity. The highest phenolic compounds were obtained with 30 kg ha^{-1} of K_2SO_4, whereas the use of 60 kg ha^{-1} caused the highest total flavonoid content. This plant is a good source of K^+, Ca^{+2}, $PO4^{-3}$, and Cl^{-1}. **Conclusion**: In this study, increasing doses of potassium sulfate had significant effect on element, polyphenol content, antioxidant and antimicrobial activities of the milk thistle.

Key words: Antimicrobial activity, antioxidant activity, elements, potassium sulfate, Silybum marianum

SUMMARY

- All tested extracts were active against all tested microbial species.
- All extracts have shown high and similar DPPH scavenging activity.
- There was a gradual increase in the biological properties of the milk thistle seeds with rising levels of potassium sulfate.
- The milk thistle seeds are rather rich sources of K^+, Ca^{+2}, $PO4^{-3}$ and Cl^{-1} potentially bioavailable for human consumption.



Abbreviations used: AlCl3: aluminum chloride, Ca+2: calcium, Cl-: chloride, Cr: chromium CE: catechol equivalents, DPPH: 2,2-diphenylpicrylhydrazyl, ABTS: 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid, DAP: diamonyum fosfat, F-: fluoride, Fe: iron, K2SO4: potassium sulfate, K+ : potassium, Li+: lithium, Mg^{+2} : magnesium, NH_4^+ : amonyum, Na^+: sodium, NO_2^-: nitrite, NO_3^-: nitrate, Ni: nickel, $NaNO_2$: sodium nitrite, NaOH: sodium hidroksit. ND: Not detectable, $PO4^{-3}$: phosphorus, Zn: zinc

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INTRODUCTION

Silybum marianum L. (Milk thistle) belongs to Asteraceae family annual plant with toothed and prickly leaves, purple flowers, and brown seeds. [1] These leaves, stems, flowers and seeds of milk thistle were used for various purposes in different countries for centuries. In recent years, considerable attention has been laid on medicinal plants with antioxidant and antimicrobial activity. Main components of these seeds are rich in crude oil, starches, mucilage, minerals tannins, and flavonolignans.[2] Mineral elements are assumed to have immense value as each of these elements show a distinctive individual role in the structural and functional integrity of the organization of living systems.[3] Silymarin which is an active component of extract of milk thistle is a mixture of flavonolignans and strong antioxidant ,that has been proven to promote liver cell regeneration, reduce blood cholesterol, and help in preventing cancer. In addition, the antioxidative, anticarcinogenic and anti-inflammatory effects of silymarin were reported by earlier studies.[4-8]

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There is a significant demand on the production of this plant from European countries in recent years. It is noteworthy that milk thistle is an important pharmaceutical plant for pharmaceutical industries and it has gained interest in Turkey. In this context, some agricultural studies were conducted in order to determine the potential production area of this plant.[9,10,1,2] Fertilization is one of the significant agricultural practices used to improve yield and quality of the traditional crops. Potassium has an important place in fertilization due to its physiological and biochemical functions in plants, and it is one of the most up taken and accumulated elements for plant growth and development. Potassium increases enzyme activity (antioxidant enzymes) and neutralize negative effects of free radicals by antioxidant enzyme.[11,12] The number of fertilization studies conducted on agricultural parameters and quality of milk thistle is limited. In addition to yield and quality parameters, the effects of fertilizer doses on bioactivity of plant extracts were also very few. Hence, in the present study, the effects of different doses of potassium sulfate fertilizer on total phenolic and flavonoid contents, antioxidant, and antimicrobial activities of milk thistle were investigated. The current study was also designed to estimate the concentration of some nutrient elements of different doses of potassium sulfate fertilizer for milk thistle.

MATERIALS AND METHODS

Plant material and field experiments

In the present study, the dried seeds of milk thistle were obtained from Cumra Vocational School of Selcuk University, Turkey.. A voucher specimen was deposited at the University. The field experiments were performed during two successive seasons, 2012 and 2013, at the experimental farm (40°58'36" N,37°59'55" E), which is located at about 10 m altitude, belonging to the Ordu Universitiy. The soil properties of experimental fields were as follows: rich in phosphorus [10.3 ppm], potassium [235 ppm] and organic matter [4.7%], clay-loam and slightly alkaline [pH=7.8]. According to the climatic data collected during the vegetation period of the experimental years (April-July); the average temperature was 19.5°C, total rainfall was 269.95 mm, and average humidity was 69.19%, respectively.[13] The experiments were arranged in the Completely Randomized Blocks Design with four replicates. Each experimental plot consisted of five rows that were 67 m long with a row-to-row distance of 0.7 m and plant-to-plant distance of 0.4 m; the total number of plants in every plot was 75. The plants were sown in early spring and harvested 110-110 days later during the two seasons. As base fertilizer, 60 kg ha $^{-1}$ DAP and 40 kg ha $^{-1}$ ammonium nitrate were applied at sowing time in accordance with the soil analysis. As experimental factors, the different doses of potassium sulfate [0, 30, 60, 90 and 120 kg ha^{-1}] fertilizer were applied with sowing. After germination of the seeds, 40 kg ha^{-1} ammonium nitrate fertilizer was applied to plots, as upper fertilizer.

Extraction of fixed oil

The content of seed fatty oil was determined using the Soxhalet method. The seed samples were finely grounded by the coffee grinder (manufactured by Bran) and extracted with *n*-hexane within a Soxhalet apparatus for 8 hours at a constant temperature of 80 °C.[14]

Antibacterial assays

The disk diffusion method was used to determine the bactericidal activity of seed crude oil and seed extracts. For this purpose, Mueller Hinton Agar (Oxoid) for bacteria and Saboraud Dextrose Agar (Oxoid) media were used. Three bacterial and three fungal strains employed in the bioassay: *Pseudomonas aeruginosa* ATCC 27853[*P aeruginosa*], *Escherichia coli* ATCC 25922[*E. coli*], *Staphylococcus aureus* ATCC 25923 [*S. aureus*] for antibacterial assays, *Aspergillus niger* ATCC 9642 [*A. niger],* and *Candida albicans* ATCC 10231 [*C. albicans*] for fungal activity were used.

The extracts were prepared according to the method described by Holopainen *et al.*[15] The air-dried seed samples were stored in an air-tight glass container in dark at -20°C until being used. The extracts were prepared by stirring 30 g samples in 150 mL of 95% ethanol at room temperature, and the extracts were kept at 4°C for a week. The extracts were filtered through 45 µm membrane filter and then the solution was dried with an evaporator. The crude extracts were stored at 20°C until being used. The crude oil obtained from Soxhalet apparatus was also stored at 20°C until the analysis was performed.

The antibacterial activities of the extracts were determined according to the method proposed by Ronald[16] as follows:

- After sterilization by autoclaving [15 min, 1.5atm and 121 °C], 20 mL of agar was added to the petri dishes.
- 15 µL of extracts were inoculated. In addition, Ampicillin, Nystatin and Cephazol were used for positive control. Bacterial and fungal strains were incubated at 37 ± 0.1 °C for 24 h and at 25 ± 0.1 °C for 48 h.
- After incubation, the inhibition zones (mm) were measured and all the applications were repeated three times.

Antioxidant assay: DPPH assay method

The effects of seed extracts obtained from milk thistle seeds, which were subjected to different doses of potassium sulfate fertilizer, were examined for their antioxidant activities. Therefore, 25 grams from each seed samples were extracted with 300 mL ethanol in water-bath at 40°C for 18 h and then filtered. The detailed information concerned with extraction system is shown in Table 1. The filtrates were evaporated under vacuum using the rotary evaporator and then dissolved in 10 mL distilled water and were lyophilized. All extracts were stored at - 20°C prior to experiments. The plant materials, their designations, and extraction yields are presented in Table 1.

The free radical scavenging activity of the ethanolic extracts of milk thistle was determined spectrophotometrically by monitoring the disappearance of 2, 2-diphenyl-1-picrylhydrazil (DPPH•) at 517 nm, according to the method described by Brand-Williams *et al.*[17] Briefly,

- 0.15 mM solution of DPPH in ethanol was prepared.
- 1 mL of this solution was added to 3 ml of the extracts at different concentrations [25, 50, 100 and 200 µg mL^{-1}]. These solutions were incubated in a dark environment.
- The absorbance of these solutions was measured at 517 nm with Hitachi U-1900, UV-VIS Spectrophotometer 200V against blank samples. All analyses were repeated three times.
- The DPPH• scavenging capacity of the extracts was calculated using the following equation: *DPPH· Scavenging Effect (% inhibition) = [(A0–A1/A0) x 100]*[18], where *A0* is the absorbance of the control reaction and *A1* is the absorbance in the presence of tested extracts.

Table 1: The extraction system and extract yield by varying K_2SO_4 doses

Extraction Procedure	Solvents	Concentration of Potassium (kg ha^{-1})	Yield(%)*
Water Bath - 18 h	Ethanol	No potassium	4.4
		30 kg ha^{-1}	3.2
		60 kg ha^{-1}	3.5
		90 kg ha^{-1}	3.9
		120 kg da^{-1}	3.6

Yield (%)* = Weight of extract (g) / 20 g of powdered plant sample x 100

Total phenolic content

The phenolic contents in the ethanolic extracts of milk thistle were determined according to the procedure described by Slinkard and Singleton [19] with slight modification of using a Folin-Ciocalteu phenolic reagent. Gallic acid was used as a standard phenolic compound. Briefly:

- 2 mL of distilled water was added to 0.01 g seed extracts [5 mg ml^{-1}] of milk thistle.
- The prepared stock solution was then diluted to 1mg mL^{-1}.
- Gallic acid was prepared to make a calibration curve; 0, 25, 50, 100, 150 and 200 mg L^{-1}. 20 µL from each calibration solution, sample, or blank was placed into separate cuvettes.
- 1.58 mL water and 100 µL Folin-Ciocalteu reagent [Sigma®] was added to each cuvette and then mixed well. After 2 minutes, 300 µL Na_2CO_3 solution was added and shaken well.
- The solutions were incubated at 20°C for 2 h and absorbance of each solution was measured at 765 nm against the blank using the spectrophotometer. The amounts of total phenolic compounds in milk thistle seed extracts were determined as micrograms of gallic acid equivalent, using an equation that was obtained from a standard gallic acid graph [R^2: 0.9944]. All analyses were repeated three times.

Total flavonoid content

The amount of total flavonoid in the ethanolic extracts was measured by aluminum chloride [$AlCl_3$] colorimetric assay. Catechol was used as a reference flavonoid. 2500 mg mL^{-1} and 1250 mg mL^{-1} concentrations of the extracts were prepared in ethanol. The different concentrations of catechol [20, 40, 60, 80 and 100 mg mL^{-1}] were prepared to obtain standard calibration curve of catechol. Briefly:

- 500 µL of extract solution or standard solution of catechol was added to a 10 mL test tube containing 2 mL distilled water.
- 150 µL 5% $NaNO_2$ was added to the test tubes. After 5 min, 150 µL of 10% $AlCl_3$ was added.
- At 6 min, 1000 µL of 1M NaOH was added to the mixture. Immediately, the reaction tube was diluted to volume 5 mL with the addition of 1200 µL distilled water and thoroughly mixed.
- Absorbance of the mixture was determined at 510 nm versus a blank. The samples were analyzed three times.[20] The total flavonoid content of milk thistle seed extracts were expressed as mg catechol equivalents (CE) 100g^{-1} dried weight of extracts.

Estimation of Element Content

To prepare the samples for the element content determination, 5 g of samples were extracted with 50 mL deionized water, in ultrasonic water bath during 30 minutes. Then, extracts were filtered with 0.22 µm cellulose acetate filter and prepared for the analysis. Before sample analysis, the standard Dionex anion mix and Dionex cation mix were used for calibration. The ions in aerosol samples were determined by using Dionex ICS 1100 Series ion chromatography. The results were checked by using the ERM-CA408 simulated rainwater (low contents).

Statistical analysis

The Antibacterial activity parameters were analyzed by MSTAT-C statistical program, and the differences between individual averages were compared by LSD at $p<0.05$ and $p<0.01$ probability levels.

RESULTS

Antibacterial activity

The biological activities of ethanol extract of the seeds and their crude oil of milk thistle were investigated; the average data is given in Tables 2.

The result of antibacterial activity showed that all tested extracts were active against all tested microbial species, including *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Aspergillus niger* ATCC 9642, and *Candida albicans* ATCC 10231.

The difference between antibacterial effect of the ethanol extract and their crude oil was found to be statistically significant. The seed crude oil extract of milk thistle had significant antibacterial effects on investigated bacterial strains except for *S. typhi* and *C. albicans*.

The antibacterial effect of the seeds and their crude oil obtained from different potassium sulfate doses applications on investigated bacterial trains had statistically significant differences on other bacteria investigated except for *S. typhi* and *A.niger* [Table 2]. The highest antibacterial activity against all tested microbial species was observed in 90 kg ha^{-1} fertilizer applications. It could be deduced that antibacterial potent metabolites of the seeds and its crude oil composition were affected positively by increasing potassium doses [Table 3].

When seed and seed crude oil extracts were evaluated together, the highest interaction values were obtained from 90 kg ha^{-1} potassium application for *S. aureus*. For *P. aureus,* the highest values were obtained from 90 kg ha^{-1} potassium application as well [Figure 1].

Table 2: Average values of antibacterial activity of *Silybum marianum* L by used parts (mm)

Used Part	E. coli	P. aureus	S. saurus	S. typhi	A. niger	C. albicans
Fatty oil	14.13 a	14.07 a	16.67 a	8.20	13.53 a	9.00
Seed	12.07 b	11.93 b	15.47 b	5.13	12.60 b	6.80
LSD (5 %)	1.250	1.322	0.8605	NS	0.5730	NS

Table 3: Average values of antibacterial activity of *Silybum marianum* L by varying K_2SO_4 doses

K_2SO_4 Doses (kg ha^{-1})	*E.coli*	*P.aurus*	*S.aurus*	*S.typhi*	*A.niger*	*C.albicans*
0	12.67 bc	12.33 b	14.67 c	5.50	12.50	7.00 ab
30	13.50 ab	13.00 b	15.17 bc	7.00	13.00	9.00 a
60	12.00 c	12.33 b	16.33 b	5.83	13.17	8.83 a
90	14.33 a	14.83 a	18.17 a	7.50	14.17	6.00 b
120	13.00 bc	12.50 b	16.00 bc	7.50	1.50	8.67 a
Ampicillin	15	28	10.0	28	-	-
Cephazolin	15	24	-	22	-	-
Nystatin	-	-	-	NT	16	15
Etanol	-	-	-	-	-	-
LSD (5 %)	1.162	0.7541	1.501	NS	NS	2.611

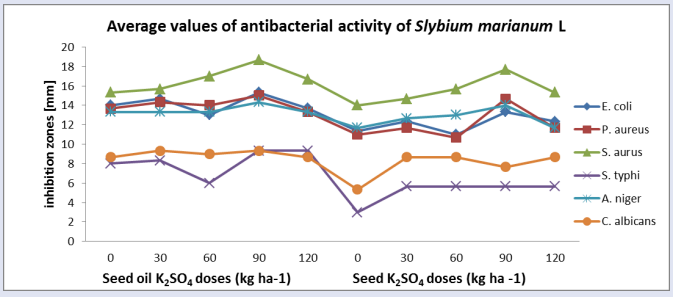

Figure 1: Average values of antibacterial activity of *Silybum marianum* L by varying K_2SO_4 doses and used parts (mm)

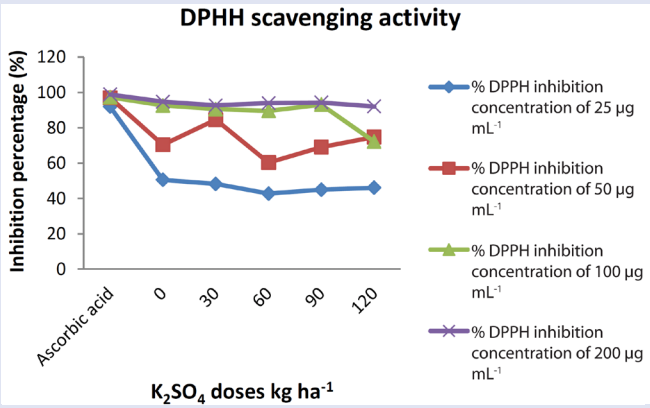

Figure 2: DPPH scavenging activity of *Silybum marianum* L by varying K_2SO_4 doses

Antioxidant activity

The DPPH• scavenging activity of the ethanolic extracts from different potassium sulfate doses of milk thistle is summarized in Figure 2. It appeared that at the concentration of 200 µg mL^{-1}, the extract from seeds of this plant possess the significantly higher DPPH• scavenging activity [93.71%] compared to other extracts. At minimum concentration [25 µg mL^{-1}], all extracts showed low and similar DPPH radical scavenging activity. When extracts were used at 50 µg/mL, the highest radical scavenging activity was observed when the smallest amount of potassium [30 kg ha $^{-1}$] was applied [84.62 %]. At 100 µg mL^{-1} and over, except the highest potassium application [120 kg ha $^{-1}$] extract, all extracts have shown high and similar DPPH scavenging activity [Figure 2].

Phenolic and Flavonoid Content

The data related to total phenolic and flavonoid contents of the different K_2SO_4 doses extracts are reported in Figure 3. The total phenolic content of milk thistle extracts range from 59.67 to 125.30 GA g-**1** dry extract seeds. The lowest values were recorded in the 90 kg ha^{-1} doses of K_2SO_4 [Figure 3]. The lowest doses of potassium [30 kg ha $^{-1}$] demonstrated highest phenolic compounds [125.30 ± 0.00 GA g-**1** dry extract] compared to other potassium and control seed extracts applied. After this concentration [30 kg ha $^{-1}$], the total phenolic contents of other applied potassium extracts were found to be lower than control seed extract [96.55 ± 0.00 GA g -**1**dry extract]. The results for total flavonoid content indicated that 60 kg ha^{-1} potassium extracts contained the highest flavonoids compared to other potassium and control seed extracts applied. Among all extracts included in the study, 90 kg ha^{-1} potassium seed extracts have the lowest phenol and flavonoid contents. However, there is no correlation between total phenol and flavonoid contents of other tested extracts [Figure 3].

Element content

The present study was conducted for the evaluation of elements such as Calcium [Ca^{+2}], Magnesium [Mg^{+2}], Lithium [Li^{+}], Amonyum [NH_4^{+}], Potassium [K^{+}], Sodium [Na^{+}], Fluoride[F^{-}], Chloride [Cl^{-}], Nitrite [NO_2^{-}], Nitrate[NO_3^{-}], Sülfat [SO_4^{-2}], and Phosphorus [PO_4^{-3}] in the seeds of milk thistle. The results indicated that the plant seeds contain highest concentration of $PO4^{-3}$and K^{+} , 3.23 and 2.75 mg g^{-1} and lowest concentration of Cl^{-} [1.29 mg g^{-1}] and Ca^{+2} [0.55 mg g^{-1}] and Mg^{+2} [0.07 mg g^{-1}], respectively [Figure 4]. All tested extracts did not include Na^{+}, NH_4^{+}, F^{-} , NO_2^{-} , NO_3^{-}elements.

In this study, the concentration of Mg ranged from 0.07 to 0.17 mg g^{-1}. The highest value was determined from 120 kg ha^{-1} doses of potassium sulfate application, and the lowest value was determined from 30 kg ha^{-1} doses of potassium sulfate application, respectively [Figure 4]. Mg has got prime role in the maintenance of normal physiology in all living organisms. Mg prevents cardiac arrhythmia disorders, high blood pressure.[21,22] K concentrations of milk thistle varied between 1.81 and 2.75 mg g^{-1} [Figure 4]. The highest doses of potassium [120 kg ha^{-1}] application demonstrated highest K concentration [2.75 mg g^{-1}] compared to other potassium and control seed extracts applied. The importance of K is speculated from its participation in large number of biological processes, such as acid base balance, movement of muscles, nerve impulse conduction, and regulation of osmotic pressure. There is no international limit for K, which reflects the content of potassium in plants, however, the average intake of potassium is 2300 mg/day for adult women and 3100 mg/day for adult men.[23] Ca concentrations of milk thistle varied between 0.42 and 0.55 mg g^{-1} [Figure 4]. While the highest values were obtained from 90 kg ha^{-1} doses of potassium application, the lowest values were obtained from 60 kg ha^{-1} doses of potassium application. Calcium is an extremely important element in human body. Ca plays a significant role in building strong bones teeth and heart functions.[24] Ca may result in tetany and convulsions due to impetuous discharges of nerve impulses. The recommended daily Ca intake required for normal biochemical activities of the body is 1500 mg.[25] Cl was present in the range of 1.0-1.29 mg^{-1}g. The highest concentration was present in 90 kg ha $^{-1}$ doses of potassium application followed by 120 kg ha^{-1} [1.16 mg g^{-1}], 30 kg ha^{-1}[1.14 mg g^{-1}], and 60 kg ha^{-1} [1.13 mg g^{-1}]. $SO4^{-2}$ concentrations of milk thistle ranged from 0.83 to 0.97 mg g^{-1} while its maximum content [0.97 mg g^{-1}] was presented in 90 kg ha^{-1} potassium extracts, and its minimum content [0.07 mg g^{-1}]

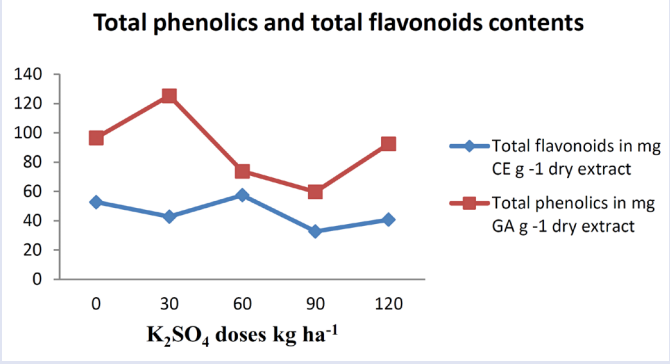

Figure 3: Total phenolics [mg GA g $^{-1}$] and total flavonoids contents [mg CE g $^{-1}$] of *Silybum marianum* L. by varying K_2SO_4 doses

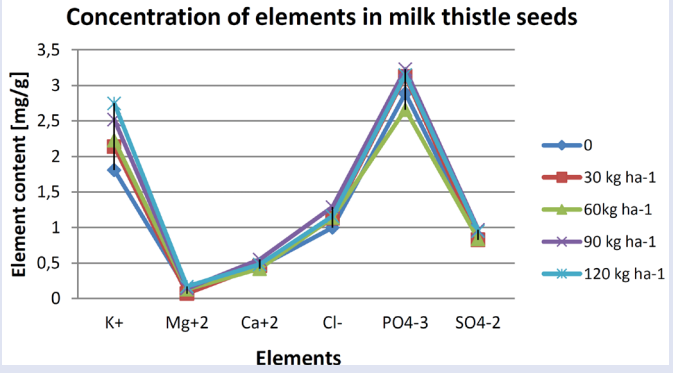

Figure 4: Concentration of elements in milk thistle seeds (mg/g) by varying K_2SO_4 doses

was presented in 30 kg ha^{-1} potassium extracts. In the present study, the concentration range of PO_4-**3** was 2.66-3.23 mg g^{-1}, as shown in Figure 4. The highest level of that form of PO_4-**3** was found in 90 kg ha^{-1} doses of potassium application followed by 120 kg ha^{-1} doses of potassium application [3.15 mg g^{-1}].

DISCUSSION

The antibacterial activity of aqueous and ethanol extracts of *S. marianum* seeds against *B. subtilis*, *E. coli*, *K. pneumonia*, *P. aeruginosa*, *P. vulgaris*, *S. aureus,* and *S. typhi* ,with methicillin and oxacillin antibiotic as a control was studied by Hassan *et al.*[26] The extracts were found to be active against all bacteria. Puri *et al.*[27] reported that the ethanol extracts have shown activity at a higher dose [200µl] against all the four bacterial strains under 11 mm, 7 mm, 5 mm, and 2 mm zones of inhibitions for *S. aureus* [ATCC 25923], *P. aeruginosa* [ATCC 27853], *E. coli* [ATCC 25922] and *E. faecalis* [MTCC 439], respectively. Juodeikiene *et al.*[28] reported that essential oil extracts obtained from milk thistle seeds show quite low antimicrobial activity against only *B. subtilis*, whereas the extracts prepared from fermented seeds with *Pediococcus acidilactici* KTU05-7 show inhibition radius up to 3 mm against *E. coli* and *P. gladioli* and lower inhibition against *B. subtilis*, *P. cepacia,* and *P. aureofaciens*. Kumar and Mishra[29] indicated that seed extracts have antimicrobial effect on Gram positive bacteria, *Bacillus cereus*, *Bacillus licheniformis,* and *Staphylococcus aures*. The same researches evaluate antibacterial activities of ethanolic, methanolic, petroleum ether, and acetone seed extracts of milk thistle with total phenolic and flavonoids contents and DPPH radical scavenging activities. It was found that petroleum ether and ethanolic extracts have high total phenolic and flavonoids contents, DPPH radical scavenging activities, and antimicrobial activity.

According to the findings of this study, the antibacterial activity of milk thistle [2.67-18.67 mm] is higher than those reported in earlier studies. The differences between our results and earlier studies may be due to the use of different extracts for analysis, different environmental and genetic factors, different chemo-types and the nutritional status of the plants as well as other factors that can influence the antibacterial activity.[30]

In this study, the DPPH• scavenging activity of the ethanolic extracts, from different potassium sulfate doses of milk thistle, ranged from 42.80% to 94.79% [Figure 2]. The highest values were recorded in control and 90 kg ha^{-1} doses of K_2SO_4. The total phenolic content of milk thistle extracts ranged from 59.67 to 125.30 mg GA g-**1** dry extract seeds; the highest values were recorded in the 30 kg ha^{-1} doses of K_2SO_4. The total flavonoid content milk thistle extracts ranged from 32.68 to 57.44 mg CE g^{-1}dry extract seeds; the highest values were recorded in the 60 kg ha^{-1} doses of K_2SO_4[Figure 3].

In the earlier studies in the literature, Shah *et al.*[31] found that flavonoids are in high quantity (21%) in blue flowering and less (19%) in the white flowering plant. Also, the concentration of phenol recorded in blue flowering was 0.43 % and 0.42% in the white flowering plant. Cagdas *et al.*[32] used DPPH method and reported that the values varied between 30-60 %. They found that the antioxidant activity increased by increasing extraction time intervals for ultrasound assisted extraction and the highest effect was observed at soxhlet extraction. Wojdyło *et al.*[33] estimated the total phenolic contents spectrophotometrically and reported that the values ranged from 4.77 ± 0.09 to 65.7 ± 0.02g µM trolox/100 g dw for milk thistle seed extracts. The researchers employed the free radical scavenging activity and reported the order of diphenylpicrylhydrazine with IC_{50} as 92.45 ± 1.91%. Luccini *et al.*[34] found the total phenolic content of milk thistle genotypes ranged from 206 to 360 mg gallic acid equivalent per 100 g achenes. Sulas *et al.* [35] antioxidant capacity detected in milk thistle achenes by means of ABTS and by DPPH methods and reported that antioxidant capacity values ranged from 3.45 to 5.42 and 3.83 to 6.32 mmol/100 g dry weight of Trolox eguivalent, respectively. Morales *et al.*[36] found in milk thistle a content of polyphenols and flavonoids of 3.72 g GAE kg^{-1} and 1.13 g CE kg^{-1}, respectively. Ahmad *et al.*[37] evaluated the antioxidant activity in different parts of milk thistle by DPPH method and found that the tested plant materials had significant free radical scavenging activity, suggesting that such plant materials can be used as a source of antioxidant for different diseases.

Although present findings are somehow similar to the results of earlier studies, differences in extraction and antioxidant activity methods, climate, soil, environmental factors, plant genetics, and cultural practices and plant parts used in analyses may significantly affect the antioxidant activity of plants.[38,39] Plant genetics and cultural practices may significantly affect phenolic contents, and thus, they play significant roles in nutritional values of the food stuff.[40,41]

According to the results obtained from this study, the highest mineral values were found in the potassium sulfate application at 90 kg ha^{-1} [Figure 4]. According to the earlier scientific studies conducted on nutritive composition of wild plants, high quantities of minerals can be found especially in K, Na, Ca, P and Mg.[42,43] The metal ions including Fe^{3+}, Zn^{2+}, Mg^{2+}, K^{+}, Ca^{2+} and some other micronutrients are cofactor for nearly 100 enzymes, which are involved in cell division, nucleic acid metabolism and protein synthesis.[44] The researches have shown that application of micronutrients reduces the effects of environmental stresses.[45] Bachheti *et al.*[46] reported that in the ash of selected plants the max. P [15100 ppm] and K [8300 ppm] was in *Zingiber officinalis* rhizome, Cl [103.0 Mg L^{-1}] in *Terminalia arjun* bark, SO4 [152.0 Mg L^{-1}] in *Asparagus racemosus* root, Ca [20900 ppm] and Mg [20500 ppm] in *Adhatoda vasica* root, respectively. In the similar studies, Hassan *et al.*[25] reported the major minerals of *Silybum marianum* seeds as Na [4 mg kg^{-1}], Ca [2 mg kg^{-1}],Cr [48.80 mg kg^{-1}], Ni [33.75 mg kg^{-1}], Fe[360 mg kg^{-1}], K [2 mg kg^{-1}], Zn [99.50 mg kg^{-1}]. Ibrar *et al.*[47] indicated that *Silybum marianum* Gaerth contented K [7007 ± 0.00], Na [1983 ± 2.63] [µg g^{-1}]. Andrzejewska and Skinder[48] reported that the content of potassium in milk thistle dries vegetative mass, depending on fertilization dose [0, 70,140 kg ha^{-1} K_2O] ranged from 2.3 to 3.5%. The same researches reported that the potassium content was fixed in hulled achenes and amounted 0.6%. Cwalina-Ambroziak *et al*,[49] found that phosphorus has a significant effect on the yields of achenes and the content of silymarin

The contents of Ca and K obtained from the present study are higher compared to the results of other researches. These high levels could be caused by different doses of potassium sulfate fertilizer used in the growing area.

CONCLUSION

In this study, increasing doses of potassium sulfate had significant effect on element, polyphenol content, antioxidant and antimicrobial activities of milk thistle. There was a gradual increase in the biological properties of the plant with rising levels of potassium sulfate. The seed and seed fatty oil extracts demonstrated significant effect against *P. aureus, E. coli, A. niger* and *S. aurus*. In addition, the seed crude oil extracts were more effective than seed extracts against microorganisms included to be investigated. The free radical scavenging activity varied depending on the extract concentration and different doses of potassium sulfate fertilizer and fertilization did not elicit a significant variation in scavenging potency. It was also shown that milk thistle seeds are rather rich sources of K^{+}, Ca^{+2}, $PO4^{-3,}$ and Cl^{-1} and potentially bioavailable for human consumption. Finally, potassium sulfate application had positive effects on chemical and biological activities of the extracts and optimum potassium fertilizer dose for all.

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Conflicts of interest

There are no conflicts of interest.

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