RESEARCH ARTICLE

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The effect of sorbitol applications on total phenolic, flavonoid amount, and antioxidant activity in Safflower (*Carthamus tinctorius* L.)

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Abstract

It is already known that secondary metabolites in plants are affected by many parameters such as biotic and abiotic stress conditions and can vary in quantity. Sorbitol, which is used to increase osmolality in cells, is an important source of abiotic stress. The present study was conducted to determine the effects of sorbitol applications at different doses by foliar spraying on total phenolic, total flavonoid, and antioxidant activity in Safflower (*Carthamus tinctorius* L.) at different times. Olein variety was used as the material in the study and different sorbitol doses (0.5 g/L, 25 g/L, 50 g/L and 100 g/L) were applied by foliar spraying. Sorbitol doses were applied in three different growth periods (before, during, and after blooming). The total amount of phenolic and flavonoid substances and DPPH free radical scavenging activity of the drugs obtained from all samples were examined after the harvest was completed. Although it was determined that the effect of increasing sorbitol doses applied in different growth periods on the total amount of phenolic and flavonoid substances and on the DPPH free radical scavenging activity was statistically not significant, the interaction of sorbitol doses x different growth periods in the total phenolic substance content was significant. The highest total phenolic content was determined as 133.00 mg GAE/g in the before blooming period of the plant in safflower. The total amount of flavonoid substance was determined as 24.46 mg QE/g in the before blooming period of the highest plant. The highest DPPH free radical scavenging activity was found to be 92.86% in the after blooming period. As a result, when the study outcomes were evaluated according to different development periods in safflower, it is possible to rank the development periods of the total phenolic substance content and total flavonoid substance amounts as before blooming > blooming period > after blooming. We can list the antioxidant activity as after blooming > blooming period > before blooming.

Keywords: Asteraceae,Plant growth and development periods, Bioactive compounds, Osmotic stress

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is an important industrial plant of the Asteraceae or Compositaea family. Although safflower has been produced for different purposes in many countries in the world since ancient times, the most important among these purposes is medicinal production and use. Safflower (*Carthamus tinctorius* L), which is a member of the Compositaea family among the natural an-

tioxidant sources, is an important medicinal and aromatic plant. Safflower seeds are rich in unsaturated fatty acids and its flowers are rich in flavonoids, which are very beneficial for human health. Hydroxysafflor Yellow A (HYSA) is among the most important flavonoids in the petals of safflower flowers and plays important roles in the pharmacological effects of flavonoids (Feng et al., 2013; Wang et al., 2013). Flavonoids are important in the processes related to flowering and especially color change of the safflower plant (Tanaka et al., 2010). Flavonoids, which give yellow and red pigments, are the main active components of the safflower plant. The content and component of flavonoids affect the quality of safflower directly. For this reason, the number of studies conducted on the regulation of the biosynthesis of flavonoids is increasing with each passing day (Ren et al., 2019).

Stress is a condition limiting or hindering the regularly functioning biological and physiological systems and normal functions of plants (Mahajan and Tuteja, 2005). In plants, stress is divided into two groups, biotic and abiotic. Biotic stress is caused by polysaccharides (dextran, chitin, pectin and cellulose) and micro-organisms (yeast extract, fungi and bacteria) consisting of plant cell walls of biological origin. Abiotic stress, on the other hand, is physical, chemical and hormonal factors that do not have biological origin. Physical stress factors are light, ultraviolet light (UV, UV A, UV B and UV C), osmotic (sorbitol, proline and polyethylene glycol) stress, drought, salinity and thermal stress (Açıkgöz, 2017; Açıkgöz, 2021). Osmotics such as mannitol or sorbitol inhibit mineral uptake from cells. For this reason, the growth and development of plants slows down and is affected negatively (Dodds and Roberts, 1985; Thompson et al., 1986; Açıkgöz et al., 2019; Açıkgöz, 2020). There are many studies employing Polyethylene Glycol (PEG) or sorbitol to create artificial drought stress. Sorbitol solution is not metabolized by plants. Also, it reduces the osmotic potential of the nutrient medium and creates water stress that is not metabolized by plants (Rai et al., 2011; Bidabadi et al., 2012; Placide et al., 2012; Vanhove et al., 2012). Although the resistance of the plant to drought stress changes according to the duration of exposure to stress, it also changes according to the period of exposure to stress.

Secondary substances in the safflower plant are affected by many parameters such as variety, sowing time and frequency, fertilization, irrigation, development periods, harvest time, and environmental stress conditions. It was reported by previous studies that these parameters cause quantitative variations in secondary substances (Kizil et al., 2008; Mohammadi and Tavakoli., 2015; Caliskan and Caliskan., 2018). Also, there are studies reporting that different harvest times of safflower significantly change the secondary metabolite content of the plant (Salem et al., 2011). However, there are not many studies conducted on the effects of the stress factors created by foliar spraying on the chemical composition of the plant.

The present study was conducted to determine the total phenolic, total flavonoid substance amounts and DPPH free radical scavenging activity of sorbitol applications at different growth stages (before blooming, during blooming, after blooming) and by foliar spraying in Safflower (*C. tinctorius*).

MATERIALS AND METHODS

Material

The present study was conducted in the greenhouse area of the Plant and Animal Production Department of Amasya University Suluova Vocational School Campus in 2021 production period. As the study material, Olein variety obtained from a commercial company was used.

Equipment Used

The Thermo Scientific-Evolution 201 spectrophotometer was the main equipment used in the study. Also, Lab Companion-BS 06 water bath, Rotary Evaporator RE300, DENVER-S1234 analytical scale, IKA-KS 501 shaker, Memmert Inc. 153 med CO_2 oven, and ISOLAB micropipettes were the main equipment used in the analysis.

Method

Trial Design

The trial was performed according to the randomized plots trial design in different growth periods (pre-bloom, flowering period, post-bloom) in six repetitions and different sorbitol doses (0.5 g/L, 25 g/L, 50 g/L, 100 g/L) on 19.03.2021. Safflower seeds were sown in sterilized plastic pots (22.5 cm depth, 25 cm diameter) after filling them with a mixture of sand, field soil and burnt barn manure. Seeds were sown at a depth of 3-5 cm, 10 in each pot. It was determined on 06.04.2021 that seeds started to grow in all pots. The dilution process was made so that 5 plants with similar appearance remained after emergence. During the study, when the soil surface was dry in all of the pots, irrigation was performed to keep the soil moist. Before the flowering period of the plants, a calculation of 20 kg/da was made and ammonium sulphate fertilizer was given to the pots. Before flowering, sorbitol applications were made by foliar spraying method on 23.04.2021. The sorbitol applications were done as foliar spraying on 18.06.2021 and 02.07.2021, respectively during the flowering period and after the flowering. When the plants reached harvest maturity, they were harvested on 19.07.2021 before the seeding period.

Extraction method

The samples were crushed in a hand mill after the harvest was completed. The fragmented plant samples were taken into 50 mL falcons and stored at 25°C. In the extraction of plant samples in falcon tubes, 5 g of plants were weighed on analytical scale. The plant samples were then placed in lidded glass jars and 200 ml of methanol was added to and mixed with the help of a shaker.

The resulting mixtures were extracted by ultrasonic-assisted extraction method for 2 hours. Ultrasonic-assisted extraction method was preferred because it is an effective method for preserving the structural and molecular properties of bioactive compounds by preventing damage to the extracts because it is applied at high efficiency, fast and low temperatures. The supernatant obtained at the end of the extraction was then filtered. The final product (the supernatant) was passed through the Rotary Evaporator and the solvent was removed from the medium.

Total phenolic content method

The Folin-Ciocalteu Method was used to determine the total amount of phenolic substances (Folin and Ciocalteu, 1927). The extracts that were prepared at a concentration of 1mg/mL were taken into 0.1 mL tubes and 4.5 mL of distilled water was added. After adding 0.1 mL Folin-Ciocalteu reagent and 0.3 mL of 2% sodium carbonate solution, the tubes were mixed and kept in the dark for 2 hours. It was measured against blank (distilled water) at 760 nm (Slinkard and Singleton, 1997). The amount of phenolic substance was expressed as gallic acid. The comparison with gallic acid was made in the calibration curve. The results are given as mg gallic acid/g (mg GAE/g) in the dried sample.

Total flavonoid substance amount method

The total flavonoid substance content was determined with quercetin standard solution according to the method that was developed by Park et al. (2008). The plant extract was placed in 1 mL test tubes and 2 mL distilled water, 0.15 mL of 0.5 M NaNO2 and 0.15 mL of 0.3 M AlCl3 reagent were added. After waiting for 5 minutes, 1 mL NaOH was added and the absorption was measured at 510 nm with a spectrophotometer. The calibration curve was compared with quercetin. The total amount of flavonoid substance was defined as mg equivalents of quercetin (mg QE/g) per g dried sample.

DPPH free radical scavenging activity method

In the present study, antioxidant activity was determined by using the DPPH free radical scavenging method. Herbal solutions of different concentrations (25-400 µg/mL) were prepared and 0.5 mL was taken from these solutions and 3 mL ethanol and 300 mL 20 mg/L DPPH solution were added. After adding butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and trolox (100-500 µg/mL) to this solution, the mixture was shaken vigorously and absorbance was measured at 517 nm and 0.75 mL water was used as a control instead of the sample. The percent inhibition activity was calculated with the equation given below.

Free radical scavenging activity %= [(A0 - A1) / A0] x 100.

(A0 = control absorbance and A1 = sample solution absorbance)

Statiscal analysis

The experiments were done in six replications and the results are presented as mean ± SD. The data were subjected to analysis of variance (ANOVA) using statistical analysis system software (SPSS) version 22. Significant differences were calculated using the smallest significant difference (Tukey), with differences considered statistically significant at $P < 0.05$.

Total Phenolic Substance Amount

It was found in the present study that the highest total phenolic substance content was observed in the pre-flowering period with 133 mg GAE/g and 5 g/L sorbitol application. The lowest amount of total phenolic substance was obtained from 79.21 mg GAE/g and 50 g/L sorbitol applications in the post-flowering period. There were no statistically significant effects of increasing sorbitol doses and different growth periods on the total amount of phenolic substances separately (Table 1). The effect of different growth periods on the total phenolic content of the plant is very important. When the previous studies were evaluated, it was found that the amounts of total flavonoid, phenolic and antioxidant activities in plants vary according to different development periods and harvest times. It was understood that the reasons for these differences were factors such as the genetic difference of the cultivars or populations used, the development stage of the plant, the harvest hours, the organ from which the sample was taken, the location and environmental stress conditions. Yolci et al. (2021) harvested the safflower plant at different harvest periods and examined the changes in the total phenolic substance content of the plant during these periods. The highest total phenolic content (132.30 mg GA/100g) was obtained from the 2nd harvest time, which was two weeks after the beginning of flowering. In their study, Kuşoğlu (2015) reported that the total phenolic content of safflower plant extracts was in the range of 35.33-276 mg/ml as gallic acid equivalent. The total amount of phenolic substances in the leaves of the echinops (*Echinops orientalis* Trautv.) plant from the Asteraceae family was found to be 45 µg GAE/mg (Yılmaz, 2012). In a study that was conducted on *Vaccinium vitisidaea* L. plant, root, leaf and fruit were harvested at different times, and decreases were detected in total phenol and total antioxidant activity in leaf samples depending on the prolongation of the harvest time. However, it was also reported that there was no change in root and fruit (Bujor, Ginies, Popa, and Dufour, 2018). Again, in another study that was conducted on *Lycopus lucidus* plant, it was reported that total phenolic substance and antioxidant activity decreased depending on the delay in harvesting, but some values increased in terms of phenolic contents (Lu et al., 2015). It was reported in some studies that total phenol, flavonoid and antioxidant activity did not change with the prolongation or shortening of the harvest time, but they gave fluctuating results (Nemeth-Zamborine, Seidler-Łożyko-

wska and Szabo, 2019; Ribeiro et al., 2020). As seen in the present study, although the highest amount of phenolic substance was detected during the flowering period, it was found to be the lowest in the post-flowering period, which is the maturation period of the plant.

periods were effective on the amount of flavonoid substances. Although the highest amount of flavonoid substance was observed in the before blooming period, it was found that the amount of flavonoid substance decreased 1.5 times in the after blooming period. When compared with previous studies, it was found that the study was in agreement with the literature in terms of decreasing the amount of flavonoid substance as the development period progressed.

Table 1. The Total Phenolic Substance Amount (mg GAE/g) in Safflower (*C. tinctorius* L.) that Underwent Sorbitol Application at Different Periods and at Different Doses

Growth Periods	Sorbitol Doses (g/L)					
	0	5	25	50	100	Mean
Belofe blooming	123.69± 1.75abc	133.00 ±2.20a	92.42± 1.55de	127.39± 3.10ab	107.64± 0.95bd	116.82± 1.00
Blooming	92.03± 0.90de	96.79± 2.15de	100.31± 1.30cde	96.50± 1.80de	106.68± 1.08bd	98.46± 2.20
After blooming	108.00± 2.00bcd	98.06± 1.00de	102.41± 1.65cde	79.21± 0.78e	103.17± 1.70cd	98.17± 1.72
Mean	107.90± 2.34	109.28± 1.74	98.38± 0.85	101.03± 1.44	105.83± 1.60	

*There were no statistically significant differences between the mean values shown with the same letter.

Total Flavonoid Substance Amount

It was found that the highest total flavonoid substance amount in Safflower that underwent different growth stages (before blooming, flowering period, after blooming) and different doses was 24.46 mg QE/g and 5 g/L sorbitol application in the before blooming period (Table 2). However, the lowest total flavonoid substance content was obtained from 50 g/L sorbitol with 16.09 QE/g in the post-flowering period. It was seen in the study that the effect of different growth periods on the total amount of flavonoid substances of the plant was very important. In their study, Yolci et al. (2021) reported that the total amount of flavonoid substances in the safflower plant harvested at different periods was 19.15 mg QE/100g in the period one week after the beginning of flowering. Kuşoğlu (2015) reported that the total amount of flavonoid substance in safflower plant leaf extracts was 97.41 ± 2.13 µg catechin/mg). It was determined as 62.2 ± 1.9 mg QE/g (Yu et al., 2013) in a study investigating the effect of flower harvesting at different weeks on the flavonoid cartamidine substance in safflower and the amount of cartamidine increased gradually in the harvests made from the beginning of flowering until the third week, and gradually decreased in the harvests after the third week (Steberl, Hartung, Munz and Graeff-Hönninger, 2020). As seen in the literature, the amount of flavonoid substances in plants varied according to the harvest time. In the present study, it was found that different development

DPPH Free Radical Scavenging Activity

It was seen that the highest DPPH free radical scavenging activity in safflower with different doses of sorbitol applied in different growth periods to increase the antioxidant activity was 92.86% in the after-blooming period with 5 g/L sorbitol application (Table 3). However, the period with the lowest DPPH activity was obtained from 100 g/L sorbitol applications with 64.49% during the blooming period. Although the highest DPPH was seen in the after blooming period, this was 1.5 times less in the before blooming period. Kuşoğlu (2015) reported the highest activity in the flower extract as 96% as a result of the DPPH radical removal activity of the safflower plant. The DPPH activity of echinops (*Echinops orientalis* Trautv.) from the Asteraceae family was 65%, 70% and 20% in seeds, leaves and stems, respectively (Yılmaz, 2012). When these values weare compared with safflower, it was higher than the safflower plant (Ay et al., 2018). In a study conducted on Galanthus elwesii, the harvest was made from flower, leaf, root and bulb parts at the beginning of flowering and fruit ripening periods and determined that the highest antioxidant activity was in the leaf and fruit ripening period. It was also reported that growth periods were effective on antioxidant activity.

Table 2. The Amount of Total Flavonoid Substance (mg QE/g) in Safflower (*C. tinctorius* L.) that Underwent Sorbitol Application at Different Periods and at Different Doses

Growth periods	Sorbitol Doses (g/L)					
	0	5	25	50	100	Mean
Before blooming	17.48± 0.30	24.46± 0.30	17.55± 1.00	18.02± 0.45	22.92± 0.32	20.08± 0.78ab
Blooming period	20.06± 0.72	24.02± 0.62	21.63± 0.50	23.35± 1.12	23.73± 1.00	22.55± 1.10a
After blooming	18.33± 0.16	17.74± 0.78	20.87± 0.90	16.09± 0.48	19.16± 0.10	18.43± 0.60b
Mean	18.62± 0.20	22.07± 0.70	20.01± 0.30	19.15± 0.88	21.93± 0.15	

*There were no statistically significant differences between the mean values shown with the same letter.

Table 3. The DPPH Free Radical Scaveng,ng Activity (% inhibition) in Safflower (*C. tinctorius* L.) that Underwent Sorbitol Application at Different Periods and at Different Doses

Growth periods	Sorbitol Doses (g/L)					
	0	**5**	**25**	**50**	**100**	**Mean**
Before blooming	69.12± 2.00	72.00± 0.80	77.47± 1.30	78.09± 1.28	64.49± 0.87	72.23± 0.56b*
Blooming period	78.90± 0.90	71.93± 1.38	71.57± 2.35	86.56± 0.82	86.65± 2.00	79.12± 1.20b
After blooming	88.47± 1.75	92.86± 1.36	89.50± 1.42	85.01± 1.40	82.21± 1.16	87.61± 0.75a
Mean	78.83± 0.80	78.93± 0.96	79.51± 0.90	83.22± 1.10	77.78± 0.64	

*There were no statistically significant differences between the mean values shown with the same letter.

These studies and our results show that the highest antioxidant activity was detected during maturation periods, which is in agreement with the literature data. DPPH free radical scavenging activities in different plants were 75% in sorrel, 82% in dill, 40% in arugula, 23% in cress and 67.5% in the leaves of turnp radish (Isbilir, 2008; Akagun, 2009). When all these data were compared, it was seen that the DPPH radical scavenging activity of the safflower plant was considerably higher than the antioxidant activity of other plants.

CONCLUSION

Safflower, which is one of the natural antioxidant sources, is from the Asteraceae family, and its secondary metabolite components, amount and antioxidant activity change in different growth periods (before blooming, blooming period, after blooming). For this reason, significant variations may occur in plant samples to be collected at different times in plants. In this research, the effects of sorbitol applications at different periods (before blooming, blooming period, after blooming) and foliar spraying on total phenolic, total flavonoid and DPPH free radical scavenging activities were investigated in safflower.

When evaluated according to different growth periods in safflower, it was found that the amount of total flavonoid substance and total phenolic substance were the highest before the blooming period. It is possible to list the growth periods as before blooming> blooming period> after blooming for total flavonoid amount and total phenolic substance amount. Contrary to what is seen in total flavonoid and total phenolic substance amounts in DPPH free radical scavenging activity, the period when DPPH free radical scavenging activity is highest is after blooming period. It is possible to list the growth periods of DPPH free radical removal activity as after blooming> blooming period> before blooming.

As a conclusion, it was found that the antioxidant activity of safflower varies significantly according to the growth period of the plant. For this reason, it was understood that the development period of the plant can be optimized depending on the expected targets and stress factors can be applied in the periods where the highest yield and quality antioxidant activity will be obtained, considering variabilities.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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