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ONTOGENETIC VARIABILITY IN PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF AN EDIBLE HALOPHYTE, *SALSOLA SODA* L.

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Abstract

Halophytes are valuable plants for their ability to live in extreme habitats on the earth where water sources deplete continuously. We investigated ontogenetic variability in phenolic composition and antioxidant activity of *Salsola soda* L., (Chenopodiaceae) a halophyte, distributed in coastal regions of Lake Burdur which is a saline lake in the Lakes District in southwest Turkey. *In vitro* radical scavenging activity of collected plants between June and October was detected using the 1,1-diphenyl,2-picryl hydrazyl (DPPH), and superoxide (SO) radical scavenging activity (RSA) and cupric reducing antioxidant capacity (CUPRAC). Total phenolic content (TPC) and total flavonoid content (TFC) were detected by specific qualitative tests. Caffeic acid, chlorogenic acid, ferulic acid, p-coumaric acid, naringin, quercetin, and, rutin were detected by HPLC-DAD. TFC, DPPH and SO RSA and, CUPRAC were maximum in the last developmental stage, fruiting, (October), (p<0.05). TPC was higher in the vegetative stage than that in the generative stage. Caffeic acid, naringin, rutin and ferulic acid were higher than the other phenolic components in *S. soda* extracts. *S. soda* is a potential plant that has some bioactivities thanks to its valuable phytochemicals like flavonoids.

Key words: Ontogenetic variability, *Salsola soda* L., Phenolic content, Antioxidant activity.

Introduction

Extremophiles are a special group of organisms that can tolerate broad temperature ranges, crushing pressure, extreme pH, high salt concentrations, metal toxicity, or radiations. Extremophiles are an important multidisciplinary research area varying across studies investigating the origin of life, and biological adaptation under extreme constraints. Furthermore, extremophiles synthesize bioactive components like extremozymes, and these compounds offer great commercial interest in agriculture, industries, medicine, etc. because of their biotechnological applications (Rao *et al*., 2022).

One of the extremophile plants, halophytes is a group that has some salinity tolerance, and some are edible and some are used as forage plants, medicine, fuel, or ornamentals. Habitat diversity of halophytes stretches widely from coastal salt dunes, mudflats, and salt marshes to steppes, inland deserts, and salt flats (El Shaer, 2003). High physiological compliance to extremes is a major trait of these plants both in salt tolerance limits and climatic zones from which they originate. Although over 2500 halophyte plants are known worldwide for their bioactive properties and high salt tolerance, poor relevance has been given to them (Abdelly, 2006). Halophytes overcome salinity-induced oxidative stress by various biochemical mechanisms such as enabling water acquisition/retention, sustaining ion homeostasis, and retaining chloroplast functioning with essential traits including osmolyte synthesis and production of specific proteins and antioxidants. Using halophytes as traditional medicines and edible plants makes sense given these abilities (Ksouri *et al*., 2008). In spite that modern medicine making progress in recent decades, plants are still valuable contributors to health care as they provide variable and bioactive compounds which lead to the formulation of thousands of pharmaceutical drugs. Advantages lately in food science and technology pulled to produce new and healthy food products. Innovative processing techniques on wild plants made possible a substantial part of the food basket in both developing and developed countries (Bharucha & Pretty, 2010).

The Mediterranean basin is rich in wild plant species that are edible and useful for medicinal purposes for centuries. Lots of these species can grow under salinity and live by coasts while others adapt to highly saline conditions (Petropoulos *et al*., 2018). *Salsola soda* L. (Chenopodiaceae) is an erect, glabrous, shrubby, annual halophyte distributed mostly in marginal areas near the costs (Iannuzi *et al*., 2020). Genus *Salsola* consists of some 120 herbaceous or shrubby species distributed particularly in saline soils of the temperate and subtropical regions of Europe, Asia, Africa, and North America (Tundis *et al*., 2009). Italians call the edible plant buds of *S. soda* "aggretti" or "barba di frate" which are commonly consumed from the cultivated plant in spring in Italy. In the former times, *S. soda* with the name "soda" was also used as a sodium carbonate source. Members of the *Salsola* genus have been reported to contain alkaloids, flavonoids, coumarins, saponins, and sterols, together with their antioxidant, antimicrobial, anti-hypertensive, anti-cancer, anti-inflammatory, anti-cholinesterase, anti-ulcer, diuretic, emollient, purgative, and hypoglycaemic properties and as folk medicines (Tundis *et al*., 2009; Hamed *et al*., 2011; Rasheed *et al*., 2013). On the other hand, some researchers suggested that the potential components of *S. soda* conveniently mediate pathological conditions associated with diabetic complications, inflammatory mechanisms, and cancer (Iannuzzi *et al*., 2020).

Bioactive compounds have a crucial role in governing plants in their natural environment and capacity to adapt. The phytochemical content is influenced by the level of abiotic stress conditions, like radiation, temperature, and seasonality (de Sousa Araújo *et al*., 2015; Ouerghemmi *et al*., 2016). Phenolic compounds are widespread secondary metabolites of the plant kingdom and present as a class of molecules with valuable biological activities, especially

related to their antioxidant effects (Ribeiro *et al.*, 2020). Salinity is an example of biotic/abiotic stress which in response, stimulates the phenolic synthesis and accumulation in plants (Naczk & Shahidi, 2004). Knowledge about factors that specify the chemical changes and yields of each species is important not only to understand the physiological and biochemical nature of these substances but also in particular for medicinal plants, to optimize the collection time and to achieve higher yields of high-quality phytochemicals. This study was carried out to investigate variability in phenolic content and antioxidant activity of halophyte *S. soda* in its developmental stages. Although there're some studies on the chemical content and bioactivity of this edible species, this is the first report on the changes in chemical composition and antioxidant activity of it as we know so far.

Material and Methods

Chemicals and reagents: Analytical grade solvents and chemicals were used in the study. Methanol, dimethylsulphoxide (DMSO), Folin-Ciocalteu phenol reagent, copper chloride ($CuCl_2$), Neocuproin (2,9-dimethyl-1,10-phenanthroline), and sodium hydroxide (NaOH) were purchased from Merck (Germany), aluminum chloride ($AlCl_3$), sodium nitrite ($NaNO_3$), catechin, sodium carbonate (Na_2CO_3), ammonia acetate buffer, nitroblue tetrazolium (NBT), L-ascorbic acid, Trolox (6-hydroxy-2,5,7,8-tetrametylchroman-2-carboxylic acid), gallic acid and DPPH (2,2-diphenyl-1-picril hydrazyl) from Sigma-Aldrich (Sigma-Aldrich Co.St. Louis).

Plant material: *S. soda* aerial parts were collected in the salt marsh of Lake Burdur (in an approximation of 50 km to Lake Salda) in June, July, August, September, and October 2020. Plants were authenticated and deposited in the Medicinal Plants Laboratory of Mehmet Akif Ersoy University as herbarium material. Voucher specimens were coded as MPL202001. The growth stages of *S. soda* were as follows: vegetative (June-July), floral budding (August), full flowering (September), and fruiting (October).

Collected plants were dried in a shade and cool place for about 10 days and reserved at room temperature by protecting light and humidity until chemical analysis. A 10 cm long part of the shoot tip -except ones collected in June- from the representative individuals was used for the detection of phenolic and flavonoid content and antioxidant activity.

Extraction: 10 mL methanol or ethanol was added to 50 mg plant powder and extracted in an ultrasonic bath for 30 min. Extracts were filtered (Whatman no:1), then the extraction solvent was evaporated using a rotary evaporator. Extract yields calculated are given in (Table 1).

Total phenolic content (TPC): 150 µL of diluted Folin-Ciocalteu reagent (4:1 water/reagent) was added to 10 µL of sample or standard. After the addition of saturated sodium carbonate (7.5%) and incubation for 2 h at room temperature, the absorbance was measured at 725 nm (Singleton & Rossi, 1965). TPC was expressed as gallic acid equivalent (10-500 µg GAE /mL).

Table 1. Extract yields of methanol and ethanol extracts of *S. soda.*

	Yield (%)	
Months	**MeOH extract**	**EtOH extract**
Jun	10.6	2
Jul	9	1.5
Aug	11.8	3.6
Sep	15.4	7.75
Oct	15	8.12

Total flavonoid content (TFC): 10 µL 5% sodium nitrite, 10 µL 10% aluminum chloride, 150 µL 1 M sodium hydroxide, and 50 µL water were added respectively onto the 10 µL sample or standard in the 96-well-plate. The plate was stirred well and absorbance was read at 510 nm (Zhishen *et al.*, 1999). TFC was expressed as catechin equivalent (10-500 µg CE/ mL).

DPPH radical scavenging activity assay: DPPH RSA potentials of the methanol extracts of the plants were detected according to Blois's (1958) method. Each sample or control of 200 µL was added DPPH, a stable free radical, (50 µL, 1 mM) at certain concentrations (0.8-500 µg/mL) and then mixed well. DPPH RSA was expressed as percent inhibition and the ascorbic acid was used as the positive control. The absorbance was measured at 517 nm. The calculations of DPPH RSA were determined by using the following formula:

$$\text{Inhibition percentage} = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \times 100$$

An IC_{50} value was also expressed as the corresponding concentration value to the 50% inhibition on a concentration vs. percent inhibition plot.

Superoxide radical scavenging activity with alkaline DMSO method: The alkaline DMSO method according to Kunchandy and Rao (1990), with some modifications, was used to determine superoxide (SO) RSA. SO radical was generated in alkaline DMSO as a non-enzymatic system. 10 µL of 1 mg/mL NBT (in DMSO) was added to 30 µL of the extract or standard compounds (0.8-500 µg/mL) then 100 µL of alkaline DMSO (1 mL DMSO plus 0.1 mL 5 mM NaOH) was added. Absorbance was read at 560 nm in a microplate reader. The positive control was ascorbic acid. The calculations of SO RSA were determined by using the following formula:

$$\text{Inhibition percentage} = \frac{\text{Abs (sample)} - \text{Abs (control)}}{\text{Abs (sample)}} \times 100$$

An IC_{50} value was also expressed as the corresponding concentration value to the 50% inhibition on a concentration vs. percent inhibition plot.

Cupric (II) reducing antioxidant capacity assay (CUPRAC): CUPRAC assay was conducted by the method of Apak *et al.*, (2004), with some modifications. 0.01 M copper chloride, 0.75 M neocuproine, and 1 M ammonia acetate buffer (pH=7.0), 73 µL each, were mixed. 50 µL antioxidant or standard solution and 30 µL water were added

to the initial mixture. 1.5 h later the absorbance was measured at 450 nm. Trolox was the reference standard (0.8-500 µg/mL) and the results were expressed as µg TE/mL.

HPLC-DAD detection of phenolic compounds: HPLC-DAD detection of phenolic compounds (phenolic acids; caffeic acid, chlorogenic acid, ferulic acid, p-coumaric acid, flavonoids; naringin, quercetin, rutin) were done at Mehmet Akif Ersoy University, Scientific and Technology Application and Research Center. System: Shimadzu Prominence, CBM: 20ACBM, Detector: DAD (SPD-M20A), Pomp: LC20 AT, Autosampler: SIL 20ACHT, Column Oven: CTO-10ASVp, Computer Programme: LC Solution, Mobile Phase A: 3% Formic acid, Mobile Phase B: Methanol. The elution gradient was applied at a flow rate of 1 mL/min.: 95%A/5%B for 3 min., 80%A/20%B for 2 min., 60%A/40%B for 10 min., 50%A/50%B for 10 min., 100%B for 10 min. until the end of the run. 10 µL methanol samples were injected into the column (Caponio *et al*., 1999). Limit of detections, wavelengths, and retention times are given in (Table 2).

Statistical analysis

One-way variance analysis (ANOVA) before Tukey's HSD test was done to evaluate differences among groups. The Pearson correlation was conducted using R studio software (R Core Team, 2021). The level of significance was set at $p<0.05$. IBM SPSS Statistics software version 25 was used for statistical analysis.

Results and Discussion

Total phenolic and total flavonoid content: TPC and TFC which are determined spectroscopically as gallic acid and catechin equivalents, respectively, are seen in (Table 3). TPCs were ranged from June to October between 32.04±1.52 and 38.3±1.51 µg GAE/mL and higher in the vegetative stage (June-July) than in the generative stage whereas TFC was the highest in the fruit set (October) and the lowest at the beginning of blooming (August). TFC values ranged between 11.14±1.4 and 49.23±1.65 µg CE/mL. Some studies are reporting that phenolic content is higher in the vegetative stage, (Adegbaju *et al*., 2020; Farhadi *et al*., 2020) and decreases during maturation (Xu *et al*., 2010). In those studies, changing trends in TFC generally run parallel with changing trends of phenolic content. (Ksouri *et al*., 2008; Mandim *et al*., 2020) There are a few studies on the ontogenetic variations of the phenolic contents in halophyte plants. For example, Ksouri *et al*., (2008) determined the changes in TPC and TFC in vegetative and generative stages of some halophyte plants in Tunisia including another species of genus *Salsola*, *S. kali*. They harvested plants in May and July, as vegetative and reproductive stages, respectively, and found both phenolics and flavonoids were higher in the vegetative stage than in the generative stage. But we found that most of the phenolic compounds and radical scavenging activity of the extracts were at the maximum level at the end of the generative stage (fruiting). Similarly, yields of both methanol and ethanol extracts were higher in the generative stage than in the vegetative stage and there was an increase in the extract yields until the end of the development (Table 4). Plenitude of total phenolic/flavonoid contents can be associated with the extract yields, because of the increasing biochemical changes and differences in a plant against stresses in different developmental stages (Vlaisavljević *et al*., 2017).

Antioxidant activity: DPPH is one of the few stable radicals with a deep purple color. The principle of the DPPH RSA assay is measuring the absorbance of reduced DPPH by antioxidants in the extracts (Brand-Williams *et al*., 1995). SO RSA assay tests whether the extracts scavenge SO radicals. Alkaline DMSO is used as a SO radical generating system, and it reacts with nitroblue tetrazolium (NBT) to give colored diformazan. H_2O_2, OH, peroxynitrite, or singlet oxygen occurrence or initiating lipid peroxidation by SO anions damage biomolecules directly or indirectly during aging and in the course of some pathological events like ischemic reperfusion injury (Moncada & Higgs, 2006). Our results indicate that (Table 3), DPPH and SO RSA (IC_{50} values) ranged between 253.88±0.89 and 566.50±50.72 µg/mL and between 279.4±11.67 and >1000 µg/mL, respectively. The highest DPPH RSA and SO RSA were observed in the samples collected in October like the TPC and TFC. DPPH RSA of the extracts was higher than their SO RSA while IC_{50} for DPPH RSA of the positive control (ascorbic acid) was 51.61±4.63 µg/mL and 35.66±2.69 µg/mL for SO RSA. It means ascorbic acid can scavenge superoxide radicals better than DPPH radicals contrary to the extracts. When compared to the positive control, the RSA of the extracts was lower levels.

Many researchers measured the reducing power of antioxidant compounds (Cai *et al*., 2004) via CUPRAC assay. Metal ions such as Cu^{2+} can reduce free radical oxidation. Antioxidants reducing Cu^{2+} to Cu^{+} in neocuproine presence will cause a decline in free radical oxidation. A chromogen of Cu(1)-neocuproine is produced in the CUPRAC redox reaction as hydroxile groups of phenolics are converted to the corresponding quinones (Apak *et al*., 2004). Cupric ion reducing capacity of the extracts from June to October was ranged between 17.75±1.11 and 40.72±2.7 µg/mL as Trolox equivalent (a water-soluble analog of vitamin E) and the highest CUPRAC was observed in the samples collected in October like in the DPPH and SO RSA tests (Table 3).

Even though we could not find the antioxidant activity of the *S. soda* extracts by the scavenge radicals as much as the positive control has, it has antidiabetic and anti-inflammatory properties because of its flavonoids (Iannuzzi *et al*., 2020).

HPLC-DAD of phenolic compounds: Caffeic, ferulic, chlorogenic, and p-coumaric acids, and naringin, quercetin, and rutin were detected by HPLC-DAD. These phenolic compounds were found in different levels in the *S. soda* extracts from June to October ($p<0.05$) and some of them decreased while some of them increased (Fig. 1). HPLC analyses show that phenolic acid and flavonoid contents ranged between 0.001±0 and 2.99±0.03 µg/mL and the most abundant phenolic acid was caffeic acid (2.99±0.03 µg/mL) in the extracts (Table 4). Caffeic acid is one of the ample

phenolic acids in plant extracts as many researchers found (Ozkan *et al*., 2010; Xie *et al*., 2017; Mekinić *et al*., 2019). We found that gallic acid equivalent TPC was highest in the vegetative stage (June-July). HPLC analyses also show that caffeic acid, as one of the main phenolic acids in the extracts, was higher in the vegetative stage than that in the generative stage. Caffeic acid, naringin, rutin, and ferulic acid were the main components in *S. soda* extracts. Sokolowska-Krzaczek *et al*., (2009), similarly found that ferulic acid is one of the most abundant phenolic acids in another *Salsola* species, *S. kali*. Phenolic acids like ferulic acid and caffeic acid are ubiquitous phenolic acids that have many physiological functions like antioxidant, anti-inflammatory, antimicrobial, antithrombosis, and anti-cancer properties (Ou & Kwok, 2004). Ferulic acid was highly correlated with naringin, SO RSA, p-coumaric acid, TFC and CUPRAC with the Pearson correlation coefficients, 0.718, 0.862, 0.868, 0.921 and 0.972, respectively (p<0.01) (Fig. 2). An antioxidant and reactive oxygen species (ROS) scavenger, naringin is a flavonoid compound that leads to various bioactivities on human health (Özyürek *et al*., 2014). Correlations between variables were shown in the correlogram (Fig. 2). Principle Component Analysis (PCA) on the bioactivity and phenolic contents of *S. soda* in its developmental stages was shown in (Fig. 3). According to PCA, October samples clustered away from June and July samples and from August and September samples through the first principal component (Dim 1: 50.3%). June and July samples are clearly separated from August and September samples through the second component (Dim 2: 28.3%).

Overall, TFC, RSA, CUPRAC, and, naringin, ferulic acid, and at the least amount of p-coumaric acid were the highest levels in the last developmental stage, fruiting, (October) (p<0.05). After October, in the last vegetative month, July, CUPRAC and ferulic acid levels were significantly higher than those in the other months and there was some increase in DPPH RSA and TFC but non-significant (Tables 3 and 4). At the beginning of the blooming stage (August) TFC, RSA, and quercetin were at the minimum level (p<0.05). These results indicate that DPPH and SO RSA and CUPRAC can arise from naringin, ferulic acid, and at the least amount of p-coumaric acid. Antioxidant activity via the radical scavenging ability of the phenolic compounds is well known (Ou & Kwok, 2004). Plant extracts that have various phenolic groups scavenge radicals in different ways since the radical scavenging activity is in relation to the positions and numbers of the hydroxyles of the phenolic compounds (Awah *et al*., 2012). Also, antioxidant activity is not only implemented by phenolic compounds alone. Ascorbates, carotenoids, reducing carbohydrates, pigments, terpenes, tocopherols, and some other components as well as their synergistic effect could play a role in total antioxidant activity (Babbar *et al*., 2011). In general, the antioxidant activity can be related to the major compounds present in the extracts, but some minor components with a synergistic effect may also play a significant role in antioxidant activity (Aazza *et al*., 2014). During the field studies, we observed that *S. soda* plants in the area have become red gradually. This indicates the presence of pigment, probably belonging to betalains (a group of water-soluble pigments that the members of the Chenopodiaceae contain. The most intense red color was in the last developmental stage (October). There are some studies on the changing of color during the development associable with the phenol content (Xie *et al*., 2017).

Table 2. Limit of detection, wavelength, and retention times of the phenolic acids and flavonoids.

	Chlorogenic acid	Caffeic acid	p-Coumaric acid	Ferulic acid	Rutin	Naringin	Quercetin
LOD (ppm)	0.01	0.01	0.01	0.01	0.57	0.4	0.57
WL (nm)	320	280	320	320	360	280	360
RT (min)	18.2	22.7	26.1	30.1	45.6	49.7	70.4

Table 3. Changes in total phenolic and flavonoid contents and the antioxidant activity of *S. soda*.

Months	TPC (µg GAE/mL)	TFC (µg CE/mL)	DPPH RSA (IC50) (µg/mL)	SO RSA (IC50) (µg/mL)
Jun	38.15 ± 0.64b*	20.66 ± 4.36b	300.53 ± 10.31c	824.43 ± 62.74cd
Jul	38.3 ± 1.51b	24.47 ± 1.65b	282.35 ± 3.82bc	493.83 ± 83.19bc
Aug	32.04 ± 1.52a	11.14 ± 1.4a	566 ± 50.72e	1196.34 ± 366.99d
Sep	32.44 ± 0.46a	19.71 ± 1.42b	375.73 ± 9.43d	775.13 ± 73.53cd
Oct	33.85 ± 3.4a	49.23 ± 1.65c	253.88 ± 0.89b	279.4 ± 11.67ab
AA			51.61 ± 4.63a	35.66 ± 2.69a

*: Means ± SD (n=3), different letters within a row refer significant differences (p<0.05), AA: Ascorbic acid

Table 4. HPLC analysis of changes in some phenolic acids, flavonoid contents and the antioxidant activity of *S. soda*.

Months	Phenolic acids and flavonoids (µg/mL)						
	Chlorogenic acid	Caffeic acid	p-coumaric acid	Ferulic acid	Naringin	Rutin	Quercetin
Jun	0,015 ± 0b	2,99 ± 0,03d	0,008 ± 0c	0,035 ± 0a	0,52 ± 0a	0,247 ± 0,01b	0,07 ± 0b
Jul	0,003 ± 0a	2,97 ± 0,02d	0,006 ± 0b	0,301 ± 0d	0,65 ± 0b	0,55 ± 0c	0,08 ± 0c
Aug	0,015 ± 0b	1,16 ± 0,01c	0,016 ± 0d	0,087 ± 0b	1,035 ± 0c	1,35 ± 0d	0,021 ± 0a
Sep	0,003 ± 0a	1,034 ± 0,0a	0,001 ± 0a	0,23 ± 0c	1,36 ± 0,02d	2,08 ± 0e	0,155 ± 0d
Oct	0,002 ± 0a	1,08 ± 0,01b	0,103 ± 0e	0,67 ± 0,01e	1,63 ± 0,01e	0,16 ± 0a	0,08 ± 0c
Jun	0,015 ± 0b	2,99 ± 0,03d	0,008 ± 0c	0,035 ± 0a	0,52 ± 0a	0,247 ± 0,01b	0,07 ± 0b

*: Means ± SD (n=3), different letters within a row refer significant differences (p<0.05), AA: Ascorbic acid

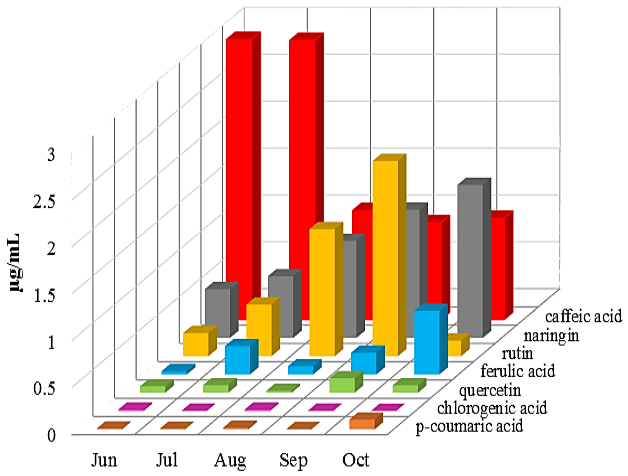

Fig. 1. HPLC-DAD detection of phenolic acid and flavonoid content of *S. soda*.

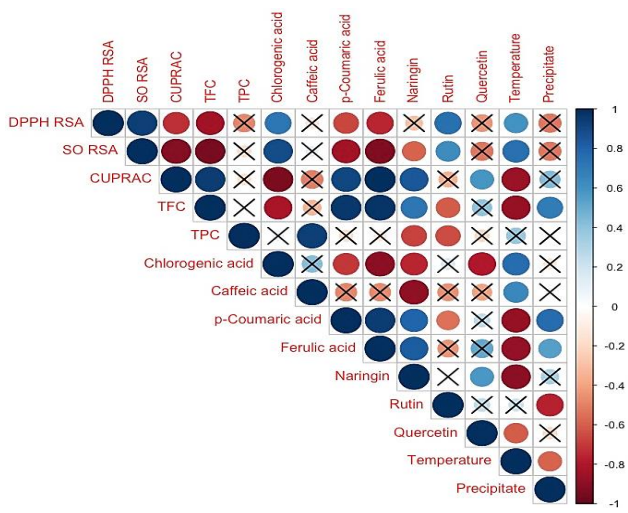

Fig. 2. Correlogram of the bioactivity and phenolic content of *S. soda*. Blue rounds represent positive correlations and red ones negative correlations. Color intencity and the size of the circle are in proportion with the correlation coefficients. Rounds with crossmarks represent insignificant correlations. The legend color display the correlation coefficients and the corresponding colors on the right side.

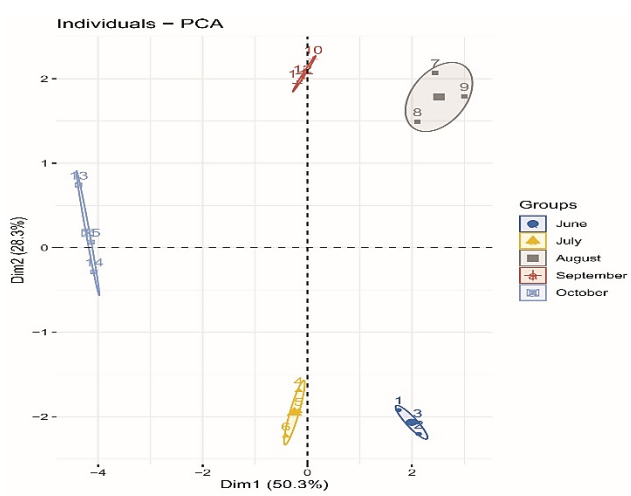

Fig. 3. Principle component analysis on the bioactivity and phenolic contents of *S. soda* in its developmental stages.

Flavonoids are a group of phenolic compounds that occur especially in fruits and flowers in various colors and are responsible for some physiological plant functions such as fruit coloration, ultra-violet protection, pigmentation, and aroma in flower production (Adegbaju *et al*., 2020; Wang *et al*., 2021). Flavonoids increasing at the flowering stage could be related to the major role they play in these physiological events (Adegbaju *et al*., 2020). Possible interactions between the pigments and phenolic content of this species would be better for further investigation. One of our findings is rutin level increased in the vegetative stage (June-July) and it reached the maximum level in September then it decreased to the minimum level in October, and the naringin level increased until the end of the fruiting stage. Inevitably, different phenolic compounds reach high levels and participate in certain metabolic reactions in different stages during plant development individually or with synergies.

Phytochemical content and associated biological activity of plants mostly depend on a variety and numerous abiotic factors, with temperature and rainfall being the most important. According to the Turkish State Meteorological Service (2022), the annual average temperature in Lake Burdur's surroundings are; 20.3°C, 26.9°C, 25.4°C, 17°C, and 14.4°C, and precipitation is; 30.2 mm, 3.4 mm, 14.6 mm, 5.6 mm and 33.0 mm in June, July, August, September and October 2020, respectively. Temperature is an important factor that affects plant development and the production of secondary metabolites (Farhadi *et al*., 2020). Increasing TPC in the vegetative stage (July) i.e. caffeic acid which is one of the main components in the plant extracts, can be explained by decreasing precipitation and increasing temperature. But most of the phenolic components were highest in October (fruiting), in which the temperature decreased. This can be attributed to the role of phenolics and flavonoids in fruit ripening, given that those compounds tend to accumulate during ripening (Tlili *et al*., 2011). CUPRAC, TFC, p-coumaric acid, ferulic acid, and naringin levels were negatively correlated with the temperature (p<0.01). However, DPPH and SO RSA, chlorogenic acid, and caffeic acid were positively correlated with temperature which means, when temperature increases, that phenolic content and RSA increase too (Fig. 2). There are a few studies on the variations of the phenolic contents in halophyte plants during their developmental stages. We highlighted that *S. soda* has the highest phenolic content and antioxidant activity in its fruiting stage on contrary to most of the other plant species (Ksouri *et al*., 2008; Farhadi *et al*., 2020, Mandim *et al*., 2020; Xu *et al*., 2010).

Conclusions

The plant growth stage is an important factor that affected the changing of phenolic content as well as antioxidant activity. As discussed above, the fruiting stage (October) is the richest in most of the phenolic compounds detected and the antioxidant activity of the extracts of *S. soda*. Although TPC was higher in the vegetative stage, TFC, RSA and most of the phenolic compounds were maximum at the end of the growth. Not only the abiotic factors, but physiological conditions of the plants play a

crucial role in phytochemical changing. *S. soda* is a potential plant that has some bioactivities such as anti-inflammatory and antidiabetic thanks to its valuable phytochemicals like flavonoids. Detailed studies are needed to highlight both the physiology and biochemistry of this potential functional food plant.

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