Ayaz Tilkat E *et al.* (2020)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca 48(4):1885-1900
DOI:10.15835/48412016
Research Article

Antioxidant and antimicrobial responses associated with *in vitro* salt stress of *in vitro* and *in vivo* grown *Pistacia khinjuk* stocks

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Abstract

P. khinjuk Stocks, known as Bıttım or Buttum in Turkey, is a member of the Anacardiaceae family. The essential oil of khinjuk pistachio has been used to treat various illnesses because of their anti-inflammatory, anticancer, antipyretic, antibacterial, anthelmintic, antiviral effects in various folk medicines. At the same time, fruits of khinjuk pistachio are used as edible wild fruits. In this study, it was aimed to determine and compare the antibacterial, antioxidant activities and total phenolic and flavonoid amounts of different parts (root, stem and leaf explants) of *in vivo* (grown naturally) and *in vitro* derived khinjuk pistachio plants under salt (NaCl) stress. Ethanol extracted explants were used for performing biological and chemical parameters. According to the results, generally, *in vivo* samples shows higher antioxidant and antimicrobial activity besides the higher number of phenolic compounds than their counterparts *in vitro*. We have also determined that the biological activity of *in vitro* salt elicited explants was higher than *in vitro* control explants. Generally, both female and male *in vivo* samples have higher antioxidants (DPPH, ABTS, CUPRAC) and antimicrobial activities than *in vitro* samples. The various plant parts (root, stem, leaf) belonging to both *in vivo* and *in vitro* samples have different biological activity level. In terms of antimicrobial activity, female plant extracts are more active than all other tested extracts. As a result, although increased salinity values significantly reduced antimicrobial activity, it is determined that 100 mM NaCl applications to *in vitro* leaf extracts exhibited moderate antimicrobial activity against *S. aureus* and *C. albicans*.

Keywords: antimicrobial; antioxidant; *P. khinjuk* Stocks; salt stress

Introduction

Pistacia, a genus of flowering plants from the family Anacardiaceae, contains about twenty species; among them, five are more well-known, including *P. vera*, *P. atlantica*, *P. terebinthus*, *P. khinjuk*, and *P. lentiscus*. The species of the genus *Pistacia* are evergreen or deciduous resin-bearing shrubs and trees, which are characterized as xerophytic trees and growing to 8-10 m tall. The leaves are alternate, pinnately compounds, and can be either evergreen or deciduous, depending on species. The genus *Pistacia* has been used by many

Received: 03 Sep 2020. Received in revised form: 19 Nov 2020. Accepted: 02 dec 2020. Published online: 22 Dec 2020.

civilizations from prehistoric times to the present day for various purposes due to their resin, fruit, leaves, and chemical components. The potential of antioxidant, anthelmintic, antimicrobial, anti-inflammatory, and cytotoxic effects of *Pistacia* species, primarily due to flavonoids and other secondary metabolites, have always attracted the attention of researchers. So far, there are many studies on the antimicrobial, antioxidant, anti-inflammatory, cytotoxicity antiangiogenic, etc. properties of this genus in the literature.

For example, under the light of literature, a flavone showed high antioxidant activity was identified as well as apigenin, luteolin, and other flavonoids from the extracts of *P. terebinthus* fruits (Topçu, 2007). In another study, antimicrobial and antioxidant characteristics of the extracts of the resin of *P. lentiscus* has also been reported (Giaginis and Theocharis, 2011). Also, Gallotannin, a kind of tannin, has more antioxidant potential isolated from the leaf extract of *P. weinmannifolia*, called "Pistafolia A" (Wei *et al.*, 2002). Furthermore, it was shown that the ethanol extracts of fruits and leaves of *P. vera* L. have a higher antioxidant effect than the resin, according to a study by Hosseinzadeh *et al.* (2012). It was also determined by the MIC test that *P. terebinthus* L. volatile components significantly inhibited the growth of different organisms such as *Shigella dysenteriae*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* (Holley and Patel, 2005; Mohagheghzadeh *et al.*, 2010). These studies mentioned above show that *Pistacia* species can use as an essential natural antioxidant and antimicrobial source. At the same time, it was reported that not only the seeds but also other parts of naturally grown *P. khinjuk* Stocks could be a source of phenolics and flavonoids compounds (Hacibekiroglu *et al.*, 2015, Hatamnia *et al.*, 2016; Ahmed *et al.*, 2017; Hazrati *et al.*, 2020). However, many of chemical and biological activity studies were found in the literature on extracted explants for other economically important plant species (Esmat *et al.*, 2012; Mirian *et al.*, 2014; Tahvilian *et al.*, 2016; Ahmed *et al.*, 2017; Taghizadeh *et al.*, 2018) there is no study about chemical and biological activity on *in vitro* grown plants under salt stress conditions. Although many studies confirm the negative effect of salinity on growth, it could be led to an increase in the production of secondary plant metabolites, antioxidant and antimicrobial effects.

Moreover, the enhanced synthesis of these secondary metabolites under salt stress conditions is believed to protect the cellular structures from oxidative effects. To avoid oxidative damage resulting from salt stress, higher plants have developed different adaptive mechanisms through the biosynthesis of a cascade of antioxidants. Indeed, polyphenolic compounds participate in the defence against reactive oxygen species (ROS), which are inevitably produced when environmental stresses impair aerobic or photosynthetic metabolism. It has been proven that the amount of antioxidant and antimicrobial components increases in adverse environmental conditions in plant tissues (Salem *et al.*, 2013).

In the light of the literature review about the effect of salt stress on *Pistacia* species, it has been found that the articles are mostly related to physiological studies, and generally, medium and high salt applications negatively affect growth and increase soluble sugar and proline content (Rahneshan *et al.,* 2018).

Considering the studies investigating the physiological responses of *in vitro* salt stress on the *Pistacia* genus, in a study on two different species (*P. vera* L. and *P. atlantica* Desf.), the seeds were cultured for 25 days at different NaCl concentrations (0, 40, 60, 80, 131, 158.5 and 240 mM). It was noted that low NaCl applications did not cause plant death in any sample, but under high salt conditions (158.5 and 240 mM), 20-25% mortality was observed. Besides, regarding salinity effects 60 and 80 mM NaCl levels caused significant reductions in stem growth and leaf number in *P. vera* species. However, salinity between 40 and 80 mM NaCl caused a reduction in the number of roots of both species. After 45 days of culture, fresh weights also decreased significantly, and it was observed that high NaCl applications (131-240 mM NaCl) caused a significant increase in proline content in both *Pistacia* species (Chelli-Chaabouni *et al.,* 2010).

In another study, the salinity tolerance of pistachio (*Pistacia vera* L.), embryos were investigated. The embryos developed from mature seeds were isolated and cultured *in vitro* and subjected to different NaCl concentrations (0, 42.8, 85.5, 171.1 and 256.6 mM) for 30 days. According to the results *in vitro* germination of embryonic axes was not affected by the salt concentration. However, the germinated embryo survival rates

decreased from 100% for the control to 62.9% for the highest salt concentration (256.6 mM) (Benmahioul *et al.,* 2009).

Another study (Ayaz Tilkat *et al.,* 2019) reported that the effect of different sodium chloride (NaCl) concentrations (0, 50, 100, 150, 200, 250 mM) on growth and physiological parameters of *Pistacia lentiscus* L. seedlings raised in *in vitro* condition for four weeks was investigated. According to the study, the morphological, physiological and biochemical changes that occurred in the seedlings were measured and recorded after exposure to salt stress. The results indicate that the visible leaf damage of Lentisk seedlings is affected by high salt concentrations. High salinity concentrations significantly reduce root and stem lengths, relative water content (RWC), total chlorophyll, Chl a, Chl b and carotenoid values after the culture periods. At 250 mM salt concentration, root and stem growth were found to be stopped entirely. Consequently, the parameters that over the 50 mM salt concentrations are caused in a decrease in the activity of the antioxidant enzyme peroxidase (POD).

There is only one study in the literature investigating the physiological effects of salt stress applied *in vitro* in khinjuk pistachio (Ayaz Tilkat *et al*., 2017). In this study, mature khinjuk pistachio seeds cultured in *in-vitro* conditions were exposed to different salt (NaCl) parameters (0, 50, 100, 150, 200, 250 mM). The seeds germinated in PGR-free MS (Murashige and Skoog, 1962) medium containing different concentrations of NaCl were exposed to salt stress for four weeks. As a result, it was determined that there is a positive correlation between soluble carbohydrate values and POD activities due to the increased NaCl concentration (Ayaz Tilkat *et al*., 2017).

All these studies aside, in this context, the current study aims to investigate and compare the antioxidant and antimicrobial properties of the root, stem, and leaf extracts obtained from the plantlets germinated under different salt concentrations *in vitro*, and the same parts extract from *in vivo* grown plants. Antimicrobial activity screening was determined using the disc diffusion method, and minimal inhibitory concentration (MIC) values were defined. The antioxidant activity potential of the extracts was evaluated by different methods, namely, Folin-Ciocalteu (FCR), 1,1-diphenyl-2-picrylhydrazyl (DPPH) 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging capacity tests and cupric ion reducing antioxidant capacity (CUPRAC) method.

Since there is no study in the literature about antimicrobial and antioxidant activities of *in vivo* and *in vitro* samples of khinjuk pistachio, the topic is highly original, and the results will be reported for the first time.

Materials and Methods

Biological material

In this study, leaves, roots, and stems of mature male and female *P. khinjuk* Stocks trees raised in Gaziantep Pistachio Research Institute were used for obtaining *in vivo* extracts, and mature seeds of this trees were used as *in-vitro* salt stress experiments for antioxidant and antimicrobial activity studies. The protocols developed by Tilkat *et al.* (2005) were modified for *in vitro* micropropagation of seeds. The diagram of all the processes in the study was shown in Figure 1.

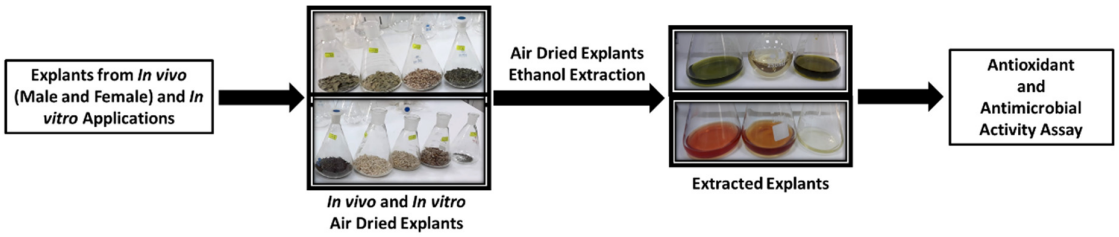

Figure 1. Diagram of the plant extraction process

Harvesting in-vivo explants

The root, stem, and leaves originated mature female, and male khinjuk pistachio was harvested from the orchards of Gaziantep Pistachio Research Institute in July 2019, then dried at room temperature in the dark, pounded using pestles, and wooden mortars separately for using in the extraction procedures.

Obtaining of in-vitro explants under salt stress

For all the stages of surface sterilization of seeds, culture initiation, and proliferation of seedlings, the protocol developed by Tilkat *et al.* (2005) was modified and used. The seeds were surface sterilized by immersion in a 20% (w/v) commercial bleach solution (NaOCl) for 20 min. In this context, the seed coats were then removed, and the kernels were washed three times with sterile distilled water before inoculating onto the MS basal medium. The plant growth regulator (PGR)-free MS basal medium was supplemented with 100 mg/l l-ascorbic acid, 3% sucrose (w/v), and solidified with agar (0.7%, w/v). The media were adjusted to pH 5.7 by using hydrochloric acid (HCl) at 0.1 N and sodium hydroxide (NaOH) at 0.5 N before autoclaving (120 °C for 20 min). Cultures were maintained at 25 ± 2 °C with a 16 h photoperiod (40 µmol $m^{-2}s^{-1}$). Almost 1 cm long shoots of *in vitro* cultivated *P. khinjuk* Stocks, were taken from axenic stock cultures and transferred into MS medium containing different NaCl concentrations (0, 50, 100, 200 mM) with a control group for a culture period (28 days). Leaf, root, and stem parts of seedlings obtained *in vitro* were isolated separately after four weeks to use in the extraction procedures.

Plant extraction

In vivo and *in vitro,* plant samples were dried in the dark at room temperature and powdered. The material was macerated with ethanol a few times, and then the solvent was evaporated, and the crude extract was obtained.

Antioxidant activity assays

The antioxidant activity of the extracts was determined by three different methods, including DPPH free radical (Blois, 1958; Ongphimai, 2013), ABTS cation radical scavenging activity (Re *et al.*, 1999) and cupric reducing antioxidant capacity (CUPRAC) (Apak *et al.*, 2004). Ascorbic Acid (AA), Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluen (BHT) and were used as positive controls.

DPPH method

In DPPH method different concentrations (10, 25, 50, 100 µg/ml) of extracts prepared. 4 ml of 0.004% DPPH/ethanol solution and 1 ml of extract was mixed in the test tube and were kept at room temperature for 30 min. After the incubation period, the absorbance (A) was measured at 517 nm. The inhibition % (I%) was calculated by the following equation:

Inhibition % = $[A_{control}-A_{sample}/A_{control}] \times 100$

ABTS method

In ABTS method, different concentrations (10, 25, 50, 100 µg/ml) of extracts prepared. 7 mM ABTS solution was adjusted to 0.7 absorbances at 734 nm. 4 ml of ABTS solution and 1 ml of the extract have mixed in the test tube and were kept at room temperature for 30 min. After the incubation period, the absorbance (A) was measured at 734 nm. The inhibition (I%) was calculated by the following equation:

Inhibition % = $[A_{control}-A_{sample}/A_{control}] \times 100$

CUPRAC method

In CUPRAC method, different concentrations (10, 25, 50, 100 µg/ml) of extracts prepared. 1 ml of $CuCl_2$ solution, 1 ml of neocuproine solution and 1 ml of $NH_4CH_3CO_2$ solution were mixed in the test tube. 1 ml of extract was added to tube content. After the 60 minutes of the incubation period, the absorbance was measured at 450 nm. The absorbance values of the samples were evaluated against the control. The increased absorbance value indicates increased activity.

The total phenolic and flavonoid content

The total phenolic and flavonoid content of the extracts was determined using the Folin-Ciocalteu reagent (FCR) (Slinkard and Singleton, 1977) and by the aluminium nitrate method (Moreno *et al.*, 2000), respectively. Total phenolic and flavonoid contents in the crude extracts expressing as Gallic acid and quercetin equivalents.

4.6 ml of different concentrations of gallic acid was incubated with 0.1 ml FCR for 3 min. 0.3 ml of 2% Na_2CO_3 solution was added to a test tube and incubated for 2 hours. After then the absorbance was measured at 760 nm. A standard curve was plotted, and the amount of total phenolic content was calculated according to the following equations:

Absorbance = 0.0356 gallic acid (µg) - 0.0047 (R2 = 0.9970)

4.8 ml of different concentrations of quercetin was incubated with 0.1 ml of 1 M CH_3CO_2K for 60 min. 0.1 ml of 10% Al $(NO_3)_3$ solution was added to the test tube and incubated for 40 min. After then the absorbance was measured at 415 nm. A standard curve was plotted, and the amount of total phenolic content was calculated according to the following equations:

Absorbance = 0.0619 quercetin (µg) - 0.0043 (R2 = 0.9991)

Antimicrobial activity

The antimicrobial activity of the extracts was evaluated by the disc diffusion method (NCCLS, 1997) and minimum inhibitory concentrations (MIC) (NCCLS, 2009). Five microorganisms (*Escherichia coli* ATCC25922, *Pseudomonas aeroginosa* ATCC27853, *Staphylococcus aureus* ATCC25923, *Streptococcus pyogenes* ATCC19615 and *Candida albicans* ATCC10231) were used for antimicrobial assays. Ampicillin and nystatin were used as positive controls. The nutrient broth medium was inoculated with each microorganism and incubated for 12-16 hours at 37 °C. 100 µl of the test microorganisms prepared overnight culture was spread into plates containing nutrient agar. Then 15 µl of 100 mg/ml concentrations of all extracts were prepared and impregnated on sterile paper discs placed in plates. Inhibition zone diameters were measured after 24 hours' incubation at 37 °C for bacteria and 48 hours' incubation at 30 °C for yeast. The active extracts were then processed to determine the MIC value. 100 µl serial dilutions of extracts, 90 µl broth, and 10 µl microorganism overnight cultures (turbidity equal to 0.5 McFarland) were pipetted into 96 well sterile plates. After 24 hours incubation at the appropriate temperature, the wells were evaluated. 100 µl was taken from the lowest concentration well without visible growth and spread on solid medium. After incubation at the appropriate temperature and time mentioned above, MIC values were determined according to the number of colonies on the plate.

Statistical analysis

All the experiments were conducted using a completely randomized block design, and they were performed in triplicate. Statistical analysis was performed using SPSS 25.0 Statistical program. One-way ANOVA was used for the analysis and mean values were compared by employing Tukey's test at $p \leq 0.05$ probability level.

Results

The modified *in vitro* micropropagation protocol developed by Tilkat *et al.* (2005) was applied to *P. khinjuk* Stocks seeds to initiate shoot cultures, and successful results were obtained (Figure 2).

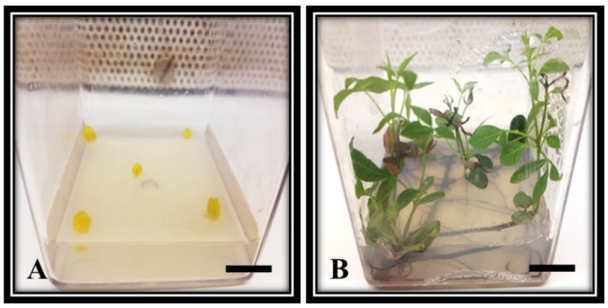

Figure 2. Propagation of *P. khinjuk* Stocks from seeds
A) Mature seeds inoculated on PGR-free MS culture medium, B) The seedlings, four weeks after culturing *in vitro* (Bars: 0.9 cm)

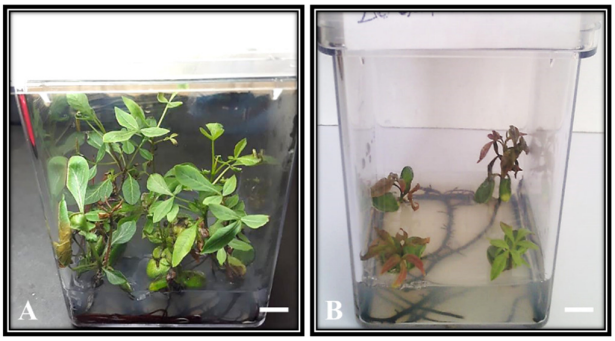

Figure 3. The seedlings germinated *in vitro* under different NaCl applications
A) 50 mM NaCl application, B) 200 mM NaCl application

In general, it was seen that healthy shoot growth was obtained from seeds belonging to the control group, whereas seeds elicited by different salt concentrations show weaker shoot growth. The observations indicate that the shoot growth of seeds was reduced by salt application (50, 100, and 200 mM). Parallel to increasing salt concentrations, stem number, and root-stem-leaf dry weights were decreased; especially chlorosis was detected in shoots. Antioxidant and antimicrobial activity result of *in vitro* seedlings, which are elicited in 3 different salt concentrations and *in vivo* leaves, roots, and stems of male and female *P. khinjuk* Stocks were separately investigated and presented in tables below.

We observed a strong positive correlation between TPC in the plant and its antioxidant activity, which suggests that phenolic compounds significantly contribute to this antioxidant activity.

Assessment of DPPH activity, generally *in vivo* male and female samples were showed higher activity than *in vitro* elicited seedling explants. Applied minimum salt concentration to *in vitro* male root was showed higher activity (56.80 ± 1.50/10 µg) compare from other genotype samples. Between *in vitro* elicited seedling samples was observed highest activity in 50 mM NaCl leaf sample (39.67 ± 0.51/10 µg) (Table 1). When evaluated in terms of antioxidant activity in the root part, there is a significant increase compared to the control together with salt concentration increases.

Table 1. Results of antioxidant activity by DPPH method (I %)

Explant Type			10 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml
ROOT	*In vivo*	Male	56.80 ± 1.50 b C	78.30 ± 1.67 b B	91.05 ± 1.31 b A	92.29 ± 0.50 c A
		Female	48.81 ± 1.14 c D	78.30 ± 1.29 b C	90.29 ± 0.90 b B	92.29 ± 0.48 c A
	In vitro	Control	17.72 ± 0.87 d D	21.68 ± 0.73 e C	23.09 ± 0.51 e B	25.15 ± 0.29 f A
		50 NaCl	22.93 ± 1.02 d B	24.64 ± 0.18 de A	25.02 ± 0.65 e A	26.35 ± 0.68 f A
		100 NaCl	22.83 ± 0.94 d D	32.06 ± 1.07 c C	43.76 ± 1.05 d B	50.33 ± 0.99 e A
		200 NaCl	19.98 ± 0.53 d D	28.54 ± 0.62 cd C	54.32 ± 0.70 c B	60.89 ± 0.95 d A
BHT			2.40 ± 0.12 e D	4.90 ± 0.19 f C	12.91 ± 0.18 f B	26.42 ± 0.25 f A
BHA			70.28 ± 2.18 a C	82.64 ± 1.57 b B	94.35 ± 1.24 b A	95.40 ± 0.42 b A
AA			68.19 ± 1.69 a C	96.13 ± 0.68 a B	99.78 ± 0.52 a A	99.82 ± 0.24 a A
STEM	*In vivo*	Male	35.01 ± 1.30 b D	54.42 ± 1.00 d C	75.73 ± 0.63 d B	92.10 ± 0.76 cd A
		Female	30.06 ± 1.04 bc D	43.86 ± 0.59 e C	74.21 ± 1.07 d B	91.24 ± 0.64 d A
	In vitro	Control	21.02 ± 0.65 d D	45.67 ± 1.29 e C	74.02 ± 0.77 d B	88.01 ± 0.26 e A
		50 NaCl	27.13 ± 0.88 cd D	63.28 ± 0.92 c C	89.12 ± 1.13 c B	94.25 ± 0.58 bc A
		100 NaCl	25.68 ± 1.11 cd D	37.96 ± 0.66 f C	41.86 ± 0.85 e B	66.79 ± 0.40 f A
		200 NaCl	23.88 ± 1.08 d D	31.01 ± 0.87 g C	35.01 ± 0.49 f B	52.80 ± 0.28 g A
BHT			2.40 ± 0.12 e D	4.90 ± 0.19 h C	12.91 ± 0.18 g B	26.42 ± 025 h A
BHA			70.28 ± 2.18 a C	82.64 ± 1.57 b B	94.35 ± 1.24 b A	95.40 ± 0.42 b A
AA			68.19 ± 1.69 a C	96.13 ± 0.68 a B	99.78 ± 0.05 a A	99.82 ± 0.04 a A
LEAF	*In vivo*	Male	39.20 ± 1.49 b C	62.03 ± 1.78 d C	86.96 ± 1.06 c B	92.38 ± 0.56 c A
		Female	45.57 ± 1.76 b D	76.21 ± 1.66 c C	85.63 ± 0.85 c B	93.05 ± 0.40 c A
	In vitro	Control	32.82 ± 0.50 bc D	44.33 ± 1.06 f C	77.09 ± 0.87 d B	87.91 ± 0.81 d A
		50 NaCl	39.67 ± 0.51 b D	53.66 ± 0.98 e C	88.27 ± 1.06 c B	96.25 ± 0.62 b A
		100 NaCl	32.15 ± 0.96 d D	38.15 ± 0.38 g C	63.14 ± 0.70 e B	86.48 ± 0.48 d A
		200 NaCl	29.40 ± 1.11 d D	36.72 ± 0.58 g C	43.57 ± 0.39 f B	68.50 ± 1.22 e A
BHT			2.40 ± 0.12 e D	4.90 ± 0.19 h C	12.91 ± 0.18 g B	26.42 ± 0.25 f A
BHA			70.28 ± 2.18 a C	82.64 ± 1.57 b B	94.35±1.24 b A	95.40 ± 0.42 bc A
AA			68.19 ± 1.69 a C	96.13 ± 0.68 a B	99.78 ± 0.51 a A	99.82 ± 0.04 a A

Each value is the means ± SE of 3 replicates measurement.

Means followed by different lower-case letters in the columns and upper-case letters in the rows showed significant differences, by the Tukey's test, at $p \leq 0.05$

In vivo samples showed very high activity in the ABTS test system, which is far above both *in vivo* samples and positive controls. In both female and male plants (Table 2.), *in vivo* derived stem extracts showed relatively low activity compared to leaves and roots. Most of the *in vitro* samples were exhibited activity close to positive controls. Besides this, *in vitro* root extract has a deficient activity compared to stem and leaf extracts (Table 2.) In terms of *in vitro* plants compared to control, the highest values stand out as 99.26 ± 0.13 (control 55.88 ± 1.39) at 100 µg/ml 200 mM NaCl in root; 87.26 ± 1.07 (control 71.77 ± 0.65) at 10 µg/ml 50 mM NaCl in stem and 98.70 ± 0.07 (control 69.00 ± 0.73) at 10 µg/ml 50 mM NaCl in leaf.

Table 2. Results of antioxidant activity by ABTS method (I%)

Explant type			10 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml
ROOT	*In vivo*	Male	99.26 ± 0.05 a A	99.26 ± 0.14 aA	-	-
		Female	99.44 ± 0.09 a A	99.26 ± 0.06 aA	-	-
	In vitro	Control	8.76 ± 0.23 fg D	13.25 ± 0.27 fC	20.74 ± 0.47 f B	55.88 ± 1.39 e A
		50 NaCl	10.33 ± 0.46 f D	20.84 ± 0.81 eC	29.33 ± 0.55 e B	65.12 ± 0.98 d A
		100 NaCl	28.22 ± 0.61 e D	64.02 ± 0.71 d C	92.61 ± 0.47 b B	96.67 ± 0.44 bc A
		200 NaCl	33.94 ± 0.70 d D	66.42 ± 0.42 d C	98.33 ± 0.36 a B	99.26 ± 0.13 ab A
BHT			74.69 ± 0.79 c D	86.34 ± 0.84 cC	90.47 ± 0.62 c B	95.32 ± 0.62 c A
BHA			7.23 ± 0.27 g D	15.14 ± 0.51 fC	51.70 ± 0.45 d B	95.72 ± 0.42 c A
AA			95.12 ± 0.54 b B	95.23 ± 0.17 bB	99.30 ± 0.08 a A	99.87 ± 0.01 a A
STEM	*In vivo*	Male	69.46 ± 0.59 d B	99.07 ± 0.15 aA	-	-
		Female	72.87 ± 0.47 c B	98.89 ± 0.10 aA	-	-
	In vitro	Control	71.77 ± 0.65 cd C	98.70 ± 0.14 aB	99.12 ± 0.16 ab A	-
		50 NaCl	87.26 ± 1.07 b B	99.29 ± 0.16 aA	99.81± 0.05 a A	-
		100 NaCl	40.77 ± 0.44 e C	79.70 ± 0.97 dB	98.15 ± 0.23 b A	-
		200 NaCl	34.31 ± 0.63 f C	64.39 ± 1.48 eB	97.97 ± 0.39 b A	-
BHT			74.69 ± 0.79 c D	86.34 ± 0.84 cC	90.47 ± 0.62 c B	95.32 ± 0.07 b A
BHA			7.23 ± 0.27 g D	15.14 ± 0.51 fC	51.70 ± 0.45 d B	95.72 ± 0.09 b A
AA			95.12 ± 0.54 a C	95.23 ± 0.17 bB	99.30 ± 0.08 ab A	99.87 ± 0.77 a A
LEAF	*In vivo*	Male	99.26 ± 0.06 a A	99.26 ± 0.17 aA	-	-
		Female	99.26 ± 0.17 a A	99.07 ± 0.09 aA	-	-
	In vitro	Control	69.00 ± 0.73 d B	98.89 ± 0.27 aA	98.52±0.29 a A	-
		50 NaCl	98.70 ± 0.07 a A	99.26 ± 0.13 aA	-	-
		100 NaCl	66.23 ± 0.82 d B	99.26 ± 0.07 aA	-	-
		200 NaCl	40.95 ± 1.17 f C	84.87 ± 0.44 cB	97.04 ± 0.29 b A	-
BHT			74.69 ± 0.79 c D	86.34 ± 0.84 cC	90.47 ± 0.62 c B	95.32 ± 0.07 b A
BHA			7.23 ± 0.27 g D	15.14 ± 0.51 d C	51.70 ± 0.45 d B	95.72 ± 0.09 b A
AA			95.12 ± 0.54 b B	95.23 ± 0.17 bB	99.30 ± 0.08 a A	99.87 ± 0.77 a A

Each value is the means ± SE of 3 replicates measurement.
Means followed by different lower-case letters in the columns and upper-case letters in the rows showed significant differences, by the Tukey's test, at p≤0.05

Table 3 shows the results of antioxidant activity by the CUPRAC method of *in vivo* and *in vitro* samples as absorbance value. Increased absorbance refers to increased activity. Comparing *in vivo* and *in vitro* samples, we generally see that *in vivo* samples exhibit higher activity. Furthermore, *in vivo* samples showed higher activity than positive controls. In terms of *in vitro* plants compared to control, the highest values stand out as 1.189 ± 0.01 (control 0.143 ± 0.01) at 100 µg/ml 200mM NaCl in root; 3.117 ± 0.05 (control 1.199 ± 0.02) at 100 µg/ml 50 mM NaCl in stem and 1.706 ± 0.01 (control 1.029 ± 0.02) at 50 µg/ml 50 mM NaCl in leaf. The activity order of the *in vitro* samples was found to be leaf> stem> root in parallel with DPPH method findings.

Table 3. Results of antioxidant activity by CUPRAC method (Absorbance)

Explant type			10 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml
ROOT	*In vivo*	Male	0.835 ± 0.02 b D	2.058 ± 0.03 ab C	3.542 ± 0.02 a B	>4 a A
		Female	0.835 ± 0.01 b D	1.907 ± 0.03 bc C	3.394 ± 0.04 ab B	>4 a A
	In vitro	Control	0.048 ± 0.01 d D	0.062 ± 0.01 e C	0.100 ± 0.01 g B	0.143 ± 0.01 f A
		50 NaCl	0.064 ± 0.01 d D	0.084 ± 0.01 e C	0.125 ± 0.01 g B	0.171 ± 0.01 f A
		100 NaCl	0.129 ± 0.02 d D	0.260 ± 0.01 d C	0.424 ± 0.01 f B	0.520 ± 0.03 e A
		200 NaCl	0.124 ± 0.01 d D	0.309 ± 0.01 d C	0.604 ± 0.01 d B	1.189 ± 0.01 d A
BHT			0.90 ± 0.02 ab D	1.75 ± 0.07 c C	2.33 ± 0.05 c B	2.6 ± 0.02 c A
BHA			0.96 ± 0.04 a D	2.20 ± 0.05 a C	3.30 ± 0.03 b B	3.65 ± 0.05 b A
AA			0.32 ± 0.02 c D	1.78 ± 0.03 c C	3.52 ± 0.05 a B	3.70 ± 0.04 b A
STEM	*In vivo*	Male	0.407 ± 0.01 b D	0.846 ± 0.01 c C	1.714 ± 0.01 d B	3.773 ± 0.02 a A
		Female	0.301 ± 0.01 d D	0.610 ± 0.01 de C	1.197 ± 0.01 f B	2.353 ± 0.02 d A
	In vitro	Control	0.261 ± 0.01 d D	0.566 ± 0.01 e C	1.075 ± 0.01 f B	1.199 ± 0.02 e A
		50 NaCl	0.396 ± 0.02 bc D	0.742 ± 0.01 cd C	1.438 ± 0.01 e B	3.117 ± 0.05 b A
		100 NaCl	0.152 ± 0.01 e D	0.284 ± 0.01 f C	0.525 ± 0.01 g B	1.126 ± 0.03 e A
		200 NaCl	0.142 ± 0.01 e D	0.278 ± 0.01 f C	0.472 ± 0.01 g B	0.498 ± 0.01 f A
BHT			0.90 ± 0.02 a D	1.75 ± 0.07 b C	2.33 ± 0.05 c B	2.6 ± 0.02 c A
BHA			0.96 ± 0.04 a D	2.20 ± 0.05 a C	3.30 ± 0.03 b B	3.65 ± 0.05 a A
AA			0.32 ± 0.02 cd D	1.78 ± 0.03 b C	3.52 ± 0.05 a B	3.70 ± 0.04 a A
LEAF	*In vivo*	Male	0.517 ± 0.01 c D	1.195 ± 0.01 d C	2.351 ± 0.01 d B	>4 a A
		Female	0.648 ± 0.01 b D	1.472 ± 0.01 c C	2.837 ± 0.02 c B	>4 a A
	In vitro	Control	0.224 ± 0.01 f D	0.505 ± 0.01 f C	1.029 ± 0.02 f B	2.00 ± 0.03 d A
		50 NaCl	0.387 ± 0.01 d D	0.85 ± 0.01 e C	1.706 ± 0.01 e B	>4 a A
		100 NaCl	0.293 ± 0.01 ef D	0.542 ± 0.01 f C	1.021 ± 0.01 f B	2.045 ± 0.02 d A
		200 NaCl	0.123 ± 0.01 g D	0.255 ± 0.01 g C	0.476 ± 0.01 g B	1.062 ± 0.01 e A
BHT			0.90 ± 0.02 a D	1.75 ± 0.07 b C	2.33 ± 0.05 d B	2.6 ± 0.02 c A
BHA			0.96 ± 0.04 a D	2.20 ± 0.05 a C	3.30 ± 0.03 b B	3.65 ± 0.05 b A
AA			0.32 ± 0.02 de D	1.78 ± 0.03 b C	3.52 ± 0.05 a B	3.70 ± 0.04 b A

Each value is the means ± SE of 3 replicates measurement.

Means followed by different lower-case letters in the columns and uppercase letters in the rows showed significant differences by the Tukey's test, at $p \leq 0.05$

As we have seen in Table 4, total phenolic and flavonoid content results of all samples are given as equivalent to gallic acid and quercetin, respectively. According to Table 4, *in vivo* samples showed higher phenolic content than *in vitro* samples, whereas all samples showed similar values in terms of flavonoids. The activity and total phenolic content order in the *in vivo* samples was found to be as root> leaf> stem; and in the *in vitro* samples, leaf> stem> root. Regarding the total phenolic and flavonoid content, our results showed significantly higher values in *in vivo* samples as compared to *in vitro* samples.

In vitro samples were found to respond differently depending on the concentration of salt applied. Increased activity and increased phenolic content were determined depending on increased salt concentration in root samples. This phenomenon is not obtained for stem and leaf samples. While the activity and phenolic content increased at 50 mM NaCl concentration compared to the control, then a decrease in activity was detected according to increasing salt concentration. Many studies are indicating the changes in phenolic compounds amount of plants under salt stress (Yuan *et al.*, 2010; Falleh *et al.*, 2012; Arzani and Ashraf, 2016; Golkar and Taghizadeh, 2018; Golkar *et al.*, 2019).

To evaluate the results of the chemical analysis as a summary; *in vivo* plant extracts were determined to have higher chemical activity compared to extracts from different parts of plants germinated in MS medium with low or no salt content. However, it has been determined that when the amount of extract and salt concentration is increased, *in vitro* extracts may exhibit higher chemical activity than the control extracts.

Table 4. Total phenolic and flavonoid content[a]

Explant type			Total phenolic content (μgGAs/mg extract) [a]	Total flavonoid content (μgQEs/mg extract) [b]
ROOT	*In vivo*	Male	492.55 ± 1.02 a	45.47 ± 0.45 a
		Female	471.48 ± 0.79 b	42.24 ± 0.47 b
	In vitro	Control	57.06 ± 0.48 f	32.40 ± 0.35 c
		50 NaCl	69.80 ± 0.73 e	34.16 ± 0.57 c
		100 NaCl	92.27 ± 1.13 d	33.36 ± 0.57 c
		200 NaCl	99.29 ± 0.49 c	34.16 ± 0.51 c
STEM	*In vivo*	Male	236.93 ± 1.00 a	41.43 ± 0.73 b
		Female	175.14 ± 1.28 c	36.59 ± 0.41 c
	In vitro	Control	145.64 ± 0.85 d	42.24 ± 0.82 b
		50 NaCl	181.72 ± 0.81 b	45.18 ± 0.68 a
		100 NaCl	103.51 ± 0.36 e	42.24 ± 0.35 b
		200 NaCl	97.89 ± 0.58 f	34.97 ± 0.33 c
LEAF	*In vivo*	Male	284.69 ± 0.95 b	40.63 ± 0.82 bc
		Female	342.27 ± 1.88 a	54.36 ± 0.62 a
	In vitro	Control	141.43 ± 0.47 d	54.36 ± 0.51 a
		50 NaCl	196.20 ± 0.87 c	39.01 ± 0.30 c
		100 NaCl	142.83 ± 1.87 d	42.24 ± 0.29 b
		200 NaCl	86.65 ± 1.04 e	35.78 ± 0.64 d

Each value is the means ± SE of 3 replicates measurement.

Means followed by different lower-case letters in the columns showed significant differences, by the Tukey's test, at $p \leq 0.05$

a: GAs, gallic acid equivalents (y=0.0356x-0.0047 R2=0.997)

b: QEs, quercetin equivalents (y=0.0619x-0.0043 R2=0.9991)

Table 5. shows the antimicrobial activities of the *in vivo* and *in vitro* plant extracts. Inactive samples are not included in the table. The *in vivo* plant extracts found to be active in terms of antimicrobial effect, especially, female parts are more active than male parts. The inhibition zone diameters of *in vivo* samples range from 8 mm to 16mm, and the MIC values from 100 μg/ml to 800 μg/ml. The best activity was 16 mm inhibition zone diameter and 100 μg/ml MIC value recorded by female leaf extract against *C. albicans* and by male root extract against *P. aeruginosa*.

We could not see the same activity in *in vitro* samples. Most of the *in vitro* samples did not exhibit any activity. However, 100 mM NaCl leaf extract exhibited moderate antimicrobial activity (inhibition zone diameter between 12-20 mm) against *S. aureus* (17 ± 0.6 zone diameter, 50 ± 4.0 MIC value) and *C. albicans* (15 ± 0.0 zone diameter, 100 ± 5.0 MIC value). While antimicrobial activity was not observed in root control, 100 and 200 mM NaCl applications induced the activity against *E. coli* and *P. aeruginosa*. Similarly, we see that 50, 100 and 200 mM NaCl applications on the leaf increase the antimicrobial activity compared to control.

Table 5. Inhibition zone diameters and MIC values of *in vivo* and *in vitro* samples

Explant type				*E. coli*	*P. aeruginosa*	*S. aureus*	*S. pyogenes*	*C. albicans*
ROOT	*In vivo*	Male	DD	9 ± 0.3 c	16 ± 3.0 a	9 ± 0.6 c	10 ± 0.0 b	13 ± 0.3 c
			MIC	500 ± 6.0 B	100 ± 0.11 C	500 ± 17.0 A	500 ± 12 B	200 ± 5 A
		Female	DD	15 ± 0.9 b	13 ± 0.60 b	12 ± 0.6 b	10 ± 0.1 b	14 ± 0.2 b
			MIC	100 ± 3.0 C	200 ± 2.0 B	400 ± 17.0 B	500 ± 0.14 B	200 ± 1 A
	In vitro	100 NaCl	DD	7.66 ± 0.3 c	7 ± 0.00 c	NZ	7 ± 0 c	NZ
			MIC	>1000 A	>1000 A	-	>1000 A	-
		200 NaCl	DD	7 ± 0 c	NZ	NZ	NZ	NZ
			MIC	>1000 A	-	-	-	-
PC			DD	20 ± 0.6 a	NZ	35 ± 0.0 a	19 ± 0.1 a	30 ± 0.0 a
			MIC	7.815 ± 0.1 D	-	1.95 ± 0.3 C	7.815 ± 0.2 C	3.125 ± 0.1 B
STEM	*In vitro*	Male	DD	10 ± 0.3 b	9 ± 0.6 b	10 ± 0.0 b	9 ± 0.2 b	7 ± 0.0 c
			MIC	500 ± 10 A	500 ± 16 A	500 ± 6 A	500 ± 0.0 B	>1000 A
		Female	DD	10 ± 0.5 b	15.03 ± 0.4 a	9 ± 0.2 c	7.83 ± 0.2 c	13 ± 0.6 b
			MIC	500 ± 29 A	100 ± 3 B	500 ± 10 A	800 ± 6 A	200 ± 1.0 B
PC			DD	20 ± 0.6 a	NZ	35 ± 0.0 a	19 ± 0.6 a	30 ± 0.0 a
			MIC	7.815 ± 0.1 B	-	1.95 ± 0.3 B	7.815 ± 0.2 C	3.125 ± 0.1 C
LEAF	*In vivo*	Male	DD	9 ± 0.00 c	13.03 ± 0.4 a	12 ± 0.6 c	11 ± 0.3 c	9 ± 0.0 e
			MIC	500 ± 20.0 B	200 ± 8.0 D	400 ± 7.0 A	400 ± 2.0 B	500 ± 3.0 A
		Female	DD	13 ± 0.6 b	10 ± 0.3 c	13 ± 0.6 c	15.03 ± 0.4 b	16 ± 0.0 b
			MIC	200 ± 8.0 C	500 ± 0.0 B	200 ± 9.0 B	100 ± 6.0 C	100 ± 5.0 B
	In vitro	Control	DD	NZ	7 ± 0.00 d	NZ	NZ	NZ
			MIC	-	>1000 A	-	-	-
		50 NaCl	DD	9 ± 0.2 c	10 ± 0.1 c	12 ± 0.3 c	9 ± 0.0 d	10 ± 0.4 d
			MIC	500 ± 18.0 B	500 ± 2.0 B	400 ± 6.0 A	500 ± 12.0 A	500 ± 0.19 A
		100 NaCl	DD	10 ± 0.2 c	11 ± 0.57 b	17 ± 0.6 b	12 ± 0.6 c	15 ± 0.0 c
			MIC	500 ± 10.0 B	400 ± 5.2 C	50 ± 4.0 C	400 ± 5.0 B	100 ± 2.0 B
		200 NaCl	DD	7 ± 0.00 d	NZ	NZ	NZ	NZ
			MIC	>1000 A	-	-	-	-
PC			DD	20 ± 0.6 a	NZ	35 ± 0.0 a	19 ± 0.6 a	30 ± 0.0 a
			MIC	7.815 ± 0.12 D	-	1.95 ± 0.3 D	7.815 ± 0.2 D	3.125 ± 0.07 C

Each value is the means ± SE of 3 replicates measurement. Means followed by different lower-case letters in the columns for DD and upper-case letters in the columns for MIC showed significant differences, by the Tukey's test, at $p \leq 0.05$

Each disc contains 1.5 mg extract. DD: disc diameter in mm. MIC: minimum inhibitory concentration in µg/ml. NZ: No zone C: control of *in vitro* leaf. PC: positive control (ampicillin and nystatin).

Discussion

Under stress conditions, including salinity, the speed of reactive oxygen species (ROS) will increase, and the resulting stress can cause harmful oxidation of various plant components. To prevent oxidation, ROS concentration in the plant cells is kept in check by several scavenging antioxidant compounds (Apel and Hirt, 2004).

Various adaptation tolls have been developed by higher plants for extreme conditions through cascade biosynthesis for both enzymatic and non-enzymatic antioxidants to protect themselves against ROS production by detoxification systems under salinity stress (Arzani and Ashraf, 2016; Golkar and Taghizadeh, 2018). Phenolic compounds are among such antioxidants, and several studies have reported changes in their concentrations in plants upon salinity stress (Yuan *et al.*, 2010; Falleh *et al.*, 2012; Golkar and Taghizadeh,

2018; Golkar *et al.*, 2019). This change was observed as both increasing (Ksouri *et al.*, 2007) and decreasing depending on the stress levels (Navarro *et al.*, 2006). Besides, it has been reported that these phenolic components are in different concentrations in different parts of the same plant, with their increases positively correlated with increases in antioxidant activity (Hatamnia *et al.*, 2016; Golkar and Taghizadeh, 2018; Golkar *et al.*, 2019). The results of these previous studies are comparable to those obtained in our current study. Previous research has indicated that plants react to salinity by increasing their antioxidant activity potentials (Hernandez and Almansa, 2002; Falleh *et al.*, 2012; Javed and Gürel, 2019) through the metabolites commonly found in them that demonstrate antioxidant activity and are effective defence mechanisms against oxidative stress from free radicals (Sacchetti *et al.*, 2005). Moreover, the increases in these metabolites also enhance tolerance levels in plants against oxidative stress coming from increases in salinity (Ksouri *et al.*, 2007).

Several different methods were developed to measure the antioxidant capacity, which has a different substrate, function, polarity, source, and mechanism. Besides, the extraction method, parts of the plant, age of the plant, and the plant's environmental conditions are important factors for measuring antioxidant activity (Brewer, 2011; Esmaeili *et al.*, 2015). In this study, three methods by which to measure antioxidant activity in plants 2,2-azino-bis (ethylbenzene-thiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and cupric ion reducing antioxidant capacity (CUPRAC) were used, the best results of which were obtained from the ABTS measurements.

Pistacia species contains several different bioactive compounds, such as triterpenes, essential oils, and phenolic compounds, and different ratios of these compounds are found in different parts of the plant, such as its resin, stem, leaves, and fruit (Kaliora *et al.*, 2004; Assimopoulou, 2005; Tilkat *et al.*, 2018); consequently, we attributed the differences in antioxidant activities determined from our results to these concentration differences. The overall evaluation of our results indicates that plant samples *in vivo* demonstrated higher antioxidant activity and phenolic ingredients than their counterparts *in vitro*. Esmaeili *et al.* (2015) have examined the total phenolic content (TPC), flavonoid content (TFC), and antioxidant activity *in vitro*, *in vivo*, and callus tissue from *Trifolium pratense*. All TPC, TFC, and antioxidant activities were in the order of *in vitro* > callus > *in vivo*. Parsaeimehr *et al.* (2010) have demonstrated results comparable to those in our *in vitro* and *in vivo* studies. Similarly, we have deduced that *in vivo* samples have higher potential than *in vitro* samples regarding the total phenolic and flavonoid content, antioxidant and antimicrobial activity.

In the literature, the studies comparing mostly between wild plants and plants grown in an *in vitro* culture medium are encountered. However, there is no study comparing antioxidant and antimicrobial activity between wild plants and plants treated with salt *in vitro* up to now. So, this is the first study to be performed in this sense.

Variation in the amount and diversity of secondary plant metabolites under abiotic stress has been reported by several studies (Navarro *et al.*, 2006; Valifard *et al.*, 2014). The antimicrobial activity most probably affiliated to the chemical structure as well as the amount of the most abundant compounds and interaction of major and minor active compounds (Dorman and Deans, 2000; Delaquis *et al.*, 2002; Mazari *et al.*, 2010). Salinity could affect the antimicrobial activity as it has significant effects on the quality and quantity of the different composition of secondary metabolites. According to our results, the antimicrobial activity exhibited diversity with organs type, and salt stress level and these results may be explained by the possible variations of the chemical structure of *P. khinjuk* plant grown under NaCl stress.

In many studies comparing antimicrobial activities of *in vitro* and *in vivo* samples, different results have been obtained indicating that activity increases, decreases or remains constant (Taran *et al.*, 2010; Cuce *et al.*, 2017; Ahmed *et al.*, 2018; Taghizadeh *et al.*, 2018). Contrary to our results, Salem *et al.* (2014) reported that salinity reduces antimicrobial activity in Carthamus tinctorius plant. When we compare *in vivo* and *in vitro* control samples; it is clear that the *in vivo* samples are more active, while the activity appears to be increased up to 100 mM NaCl when comparing salt-eluted samples with *in vitro* control samples. We consider that the activity has decreased as a result of damage in the physiological mechanism by the *in vitro* elicitation. Briefly, in terms of antimicrobial activity, female plant extracts are more active than all other tested extracts.

As a result, although increased salinity values significantly reduced antimicrobial activity, it is determined that 100 mM NaCl applications to *in vitro* leaf extracts exhibited moderate antimicrobial activity against *S. aureus* and *C. albicans*.

Conclusions

Our study aimed to reveal the antioxidant and antimicrobial activities of the different plant parts (root, stem and leaf) of the *Pistacia khinjuk* Stocks plant both *in vitro* and *in vivo*, and to determine whether these activities increase or not, especially with salt elicitations.

As a result of our study, it was determined that the antioxidant and antimicrobial activities of all *in vivo* samples had close or higher activity than the standard BHT, BHA, AA, and *in vivo* samples had higher activity in terms of both parameters compared to *in vitro* samples. Changes in activities were found in positive correlation with the increase or decrease in the total amount of phenolic and flavonoid substances. It is clear that salinity can change the metabolic profile of plants, and this metabolic profile change causes them to differ in their antimicrobial and antioxidant activities.

Authors' Contributions

EAT: *In vitro* propagation of the plant, NaCl elicitation to in vitro plants, experimental design and preparation of the manuscript. NH: Antimicrobial studies as well as preparation of the manuscript. İSK: Conducted antioxidant studies and preparation of the manuscript. VS: Conducted the statistical analysis and preparation of manuscript according to journal rules. All authors read and approved the final manuscript.

Acknowledgements

The study was funded by a grant from BTUBAP-Batman University Research Project Council BTÜBAP-2018-FED-1. We also thank Israt Jahan for linguistically reviewing the manuscript.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Ahmed ABA, Magesh J, Idris SN, Mubarak EN, Taha RM (2018). Chemical constituents and antimicrobial activity of essential oils from micropropagation and field grown plants of *Wedelia biflora* L. Journal of Essential Oil-Bearing Plants 21(6):1712-1724. *https://doi.org/10.1080/0972060X.2018.1538819*

Ahmed S, Saeed-Ul-Hassan S, Islam M, Qureshi F, Waheed I, Munawar I (2017). Anti-oxidant activity of *Pistachia khinjuk* supported by phytochemical investigation. Acta Poloniae Pharmaceutica 74:173-78.

Apak R, Güclü K, Özyürek M, Karademir SE (2004). A novel total antioxidant capacity index for dietary polyphenols, vitamin C and E, using their cupric ion reducing capability in the presence of neocuproine: The CUPRAC method. Journal of Agriculture Food Chemistry 52:7970-7981. *https://doi.org/10.1021/jf048741x*

Apel K, Hirt H (2004). Reactive oxygen species: Metabolism, oxidative stress and signal transduction. Annual Review of Plant Biology 55:373-399. *https://doi.org/10.1146/annurev.arplant.55.031903.141701*

Arzani A, Ashraf, M (2016). Smart engineering of genetic resources for enhanced salinity tolerance in crop plants. Critical Review Plant Science 35:146-189. *https://doi.org/10.1080/07352689.2016.1245056*

Assimopoulou AN, Papageorgiou VP (2005). GC-MS analysis of penta- and tetra-cyclic triterpenes from resins of *Pistacia* species. Part II. *Pistacia terebinthus* var. c*hia*. Biomedical Chromatography 19:586-605. *https://doi.org.10.1002/bmc.484*

Ayaz Tilkat E, Kaplan A, Hoşer A, Tilkat E (2017). *In vitro* şartlarda yetiştirilen Buttum (*Pistacia khinjuk* Stocks)'da çözünür karbonhidrat değerleri ile antioksidan peroksidaz aktivitesi üzerine tuz stresinin etkileri. Batman Üniversitesi Yaşam Bilimleri Dergisi 7(2):90-97. *https://hdl.handle.net/20.500.12402/1769*

Ayaz Tilkat E, Kaplan A, Tilkat E, Bağlamış G, Onay A (2019). Effects of salt stress on morpho-physiological and biochemical characters of Lentisk (*P. lentiscus* L.). International Journal of Nature and Life Sciences 3(1):20-31. *https://dergipark.org.tr/tr/pub/ijnls/issue/43966/533316*

Benmahioul B, Daguin F, Kaid-Harche M (2009) Effects of salt stress on germination and *in vitro* growth of pistachio (*Pistacia vera* L.). Comptes Rendus Biologies 332(8):752-8. *https://doi.org/10.1016/j.crvi.2009.03.008*

Blois MS (1958). Antioxidant determinations by the use of a stable free radical. Nature 181:1199-1200. *https://doi.org/10.1038/1811199a0*

Brewer MS (2011). Natural antioxidants: Sources, compounds, mechanisms of action, and potential applications. Comprehensive Reviews in Food Science and Food Safety 10:221-247. *https://doi.org/10.1111/j.1541-4337.2011.00156.x*

Chelli-Chaabouni A, Mosbah AB, Maalej M, Gargouri K, Gargouri-Bouzid R, Drira N (2010). *In vitro* salinity tolerance of two pistachio rootstocks: *Pistacia vera* L. and *P. atlantica* Desf. Environmental and Experimental Botany 69(3):302-312. *https://doi.org/10.1016/j.envexpbot.2010.05.010*

Cuce M, Bekircan T, Laghari AH, Sökmen M, Sökmen A, Önay Uçar E, Kılıç AO (2017). Antioxidant phenolic constituents, antimicrobial and cytotoxic properties of *Stachys annua* L. from both natural resources and micropropagated plantlets. Indian Journal of Traditional Knowledge 16(3):407-416.

Delaquis PJ, Stanich K, Girard B, Mazza G (2002). Antimicrobial activity of individual and mixed fractions of dill, citaro, coriander and eucalyptus essential oils. International Journal of Food Microbiology 74:101-109. *https://doi.org/10.1016/s0168-1605(01)00734-6*

Dorman HJD, Deans SG (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology 88:308-316. *https://doi.org/10.1046/j.1365-2672.2000. 00969.x*

Esmaeili AK, Taha RM, Mohajer S, Banisalam B (2015). Antioxidant activity and total phenolic and flavonoid content of various solvent extracts from *in vivo* and *in vitro* grown *Trifolium pratense* L. (Red Clover). BioMed Research International 643285:11. *https://doi.org/10.1155/2015/643285*

Esmat A, Al-Abbasi FA, Algandaby MM, Moussa AY, Labib RM, Ayoub NA, Abdel-Naim AB (2012). Anti-inflammatory activity of *Pistacia khinjuk* in different experimental models: Isolation and characterization of its flavonoids and galloylated sugars. Journal of Medicinal Food 15(3):278-287. *https://doi.org/10.1089/jmf.2011.0099*

Falleh H, Jalleli I, Ksouri R, Boulaaba M, Guyot S, Magné C, Abdelly C (2012). Effect of salt treatment on phenolic compounds and antioxidant activity of two *Mesembryanthemum edule* provenances. Plant Physiology and Biochemistry 52:1-8. *https://doi.org/10.1016/j.plaphy.2011.11.001*

Giaginis C, Theocharis S (2011). Current evidence on the anticancer potential of Chios mastic gum. Nutrition Cancer 63(8):1174-84. *https://doi.org/10.1080/01635581.2011.607546*

Golkar P, Taghizadeh M (2018). *In vitro* evaluation of phenolic and osmolyte compounds, ionic content, and antioxidant activity in safflower (*Carthamus tinctorius* L.) under salinity stress. Plant Cell Tissue Organ Culture 134:357-368. *https://doi.org/10.1007/s11240-018-1427-4*

Golkar P, Taghizadeh M, Yousefian Z (2019) The effects of chitosan and salicylic acid on elicitation of secondary metabolites and antioxidant activity of safflower under *in vitro* salinity stress. Plant Cell, Tissue and Organ Culture 137:575-585. *https://doi.org/10.1007/s11240-019-01592-9*

Hacibekiroglu I, Yılmaz-Köseoglu P, Nesrin N, Ersin K, Veysel T, Ufuk K (2015). *In vitro* biological activities and fatty acid profiles of *Pistacia terebinthus* fruits and *Pistacia khinjuk* seeds. Natural Product Research 29(5):444-446. *https://doi.org/10.1080/14786419.2014.947492*

Hatamnia AA, Rostamzad A, Malekzadeh P, Darvishzadeh R, Abbaspour N, Hosseini M, Nourollahi K, Mehr RS. 2016). Antioxidant activity of different parts of *Pistacia khinjuk* Stocks fruit and its correlation to phenolic composition. Natural Product Research 30(12):1445-1450. *https://doi.org/10.1080/14786419.2015.1060593*

Hazrati S, Govahi M, Ebadi MT, Habibzadeh F (2020). Comparison and evaluation of oil content, composition and antioxidant properties of *Pistacia atlantica* and *Pistacia khinjuk* grown as wild. International Journal of Horticultural Science and Technology 7(2):165-174. *https://doi.org/10.22059/ijhst.2020.287550.316*

Hernandez JA, Almansa MS (2002). Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. Physiologia Plantarum 115(2):251-257. *https://doi.org/10.1034/j.1399-3054.2002.1150211.x*

Holley RA, Patel D (2005). Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. Food Microbiology 22:273-292. *Https://doi.org/10.1016/j.fm.2004.08.006*

Hosseinzadeh H, Sajadi Tabassi SA, Milani Moghadam N, Rashedinia M, Mehri S (2012). Antioxidant activity of *Pistacia vera* fruits, leaves and gum extracts. Iranian Journal of Pharmaceutical Research 11(3):879-887.

Javed R, Gurel E (2019). Salt stress by NaCl alters the physiology and biochemistry of tissue culture-grown *Stevia rebaudiana* Bertoni, Turkish Journal of Agriculture Forestry 43:11-20. *https://doi.org/10.3906/tar-1711-71*

Kaliora AC, Mylona A, Chiou A, Petsios DG, Andrikopoulos NK (2004). Detection and identification of simple phenolics in *Pistacia lentiscus* resin. Journal of Liquid Chromatography Relation Technology 27:289-300. *https://doi.org/10.1081/JLC-120027100*

Ksouri R, Megdiche W, Debez A, Falleh M, Grignon C, Abdelly C (2007). Salinity effect on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*. Plant Physiology and Biochemistry 45:244-248. *https://doi.org/10.1016/j.plaphy.2007.02.001*

Mazari K, Bendimerad N, Bekhechi C, Fernandez X (2010). Chemical composition and antimicrobial activity of essential oils isolated from Algerian *Juniperus phoenicea* L. and *Cupressus sempervirens* L. Journal of Medicinal Plant Research 4:959-964. *http://www.academicjournals.org/JMPR*

Mirian M, Behrooeian M, Gahanadian M, Dana N, Sadeghi-Aliabadi H (2014). Cytotoxity and antiangiogenic effects of *Rhus coriaria*, *Pistacia vera* and *Pistacia khinjuk* oleoresin methanol extracts. Research in Pharmaceutical Sciences 10(3):233-240.

Mohagheghzadeh A, Faridi P, Ghasemi Y (2010). Analysis of mount atlas mastic smoke: A potential food preservative. Fitoterapia 81:577-580. *https://doi.org/10.1016/j.fitote.2010.01.022*

Moreno MIN, Isla MI, Sampietro AR, Vattuone MA (2000). Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. Journal of Ethnopharmacology 71(1-2):109-114. *https://doi.org/10.1016/s0378-8741(99)00189-0*

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiology 15:473-497. *https://doi.org.10.1111/j.1399-3054.1962.tb08052.x*

National Committee for Clinical and Laboratory Standard (2009). Performance Standards for Antimicrobial Susceptibility Testing; 20th Informational Supplement. CLSI document M100-S19. CLSI, Wayne, PA: NCCSL.

National Committee for Clinical Laboratory Standards (1997). Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A6. Wayne, PA: NCCLS.

Navarro JM, Flores P, Garrido C, Martinez V (2006). Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. Food Chemistry 96:66-73. *https://doi.org/10.1016/j.foodchem.2005.01.057*

Ongphimai N (2013). Phenolic acids content and antioxidant capacity of fruit extracts from Thailand. Chiang Mai Journal of Science 40(4):636-642.

Parsaeimehr A, Sargsyan E, Javidnia K (2010). A comparative study of the antibacterial, antifungal and antioxidant activity and total content of phenolic compounds of cell cultures and wild plants of three endemic species of Ephedra. Molecules 15(3):1668-1678. *https://doi.org/10.3390/molecules15031668*

Rahneshan Z, Nasibi F, Moghadam AA (2018). Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (*Pistacia vera* L.) rootstocks, Journal of Plant Interactions 13(1)7:73-82. *https://doi.org/10.1080/17429145.2018.1424355*

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice EC (1999). Antioxidant activity applying an improved ABTS radical cation decolourization assay. Free Radical Biology and Medicine 26:1231-1237. *https://doi.org/10.1016/s0891-5849(98)00315-3*

Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, Bruni R (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chemistry 91:621-632. *https://doi.org/10.1016/j.foodchem.2004.06.031*

Salem MZM, Salem AZM, Camacha LM Ali HM (2013). Antimicrobial activities and phytochemical composition of extracts of Ficus species: An over view. African Journal of Microbiology Research 7(33):4207-4219. *https://doi.org/10.5897/AJMR2013.5570*

Salem N, Msaada K, Dhifi W, Limam F, Marzouk B (2014). Effect of salinity on plant growth and biological activities of *Carthamus tinctorius* L. extracts at two flowering stages. Acta Physiologiae Plantarum 36(2):433-445. *https://doi.org/10.1007/s11738-013-1424-5*

Slinkard K, Singleton VL (1977). Total phenol analyses: Automation and comparison with manual methods. American Journal of Enology and Viticulture 28:49-55.

Taghizadeh SF, Davarynejad G, Asili J, Riahi-Zanjani B, Nemati SH, Karimi G (2018). Chemical composition, antibacterial, antioxidant and cytotoxic evaluation of the essential oil from pistachio (*Pistacia khinjuk*) hull. Microbial Pathogenesis 124:76-81. *https://doi.org/10.1016/j.micpath.2018.08.039*

Tahvilian R, Moradi R, Zhale H, Zangeneh MM, Zangeneh A, Yazdani H, Hajialiani M (2016). Ethnomedicinal plants: Study on antifungal activity of essential oil of *Pistacia khinjuk* (combined with the dominance γ-Terpinene) against *Candida albicans*. International Journal of Pharmaceutical and Clinical Research 8(10):1369-1373. *www.ijpcr.com*

Taran M, Sharifi M, Azizi E, Khanahmadi M (2010). Antimicrobial activity of the leaves of *Pistacia khinjuk*. Journal of Medicinal Plants 9(6):81-85.

Tilkat E, Ertaş A, Surmuş-Aşan H, Yılmaz MA, Süzerer V (2018). TÜBİTAK KBAG 114Z842 *Pistacia lentiscus* L.'un ı*n vitro* sürgün, kallus ve hücre süspansiyon kültürlerinde antikanser aktivite gösteren kimyasal bileşenlerin üretilmesi. [The production of chemical components perfoming anti-anticancer activity in *in vitro* culture of shoots, callus and suspension of *Pistacia lentiscus* L.] TÜBİTAK Final Report pp 241 (In Turkish) *https://app.trdizin.gov.tr/publication/project/detail/TVRjMk5UTXo=*

Tilkat E, Işıkalan Ç, Onay A (2005). *In vitro* propagation of khinjuk pistachio (*Pistacia khinjuk* Stocks) through seedling apical shoot tip culture. Propagation of Ornamental Plants 5:124-128. *http://www.journal-pop.org/2005_5_3_124-128.html*

Topçu G, Ay M, Bilici A, Sarıkürkcü C, Öztürk M, Ulubelen A (2007). A new flavone from antioxidant extracts of *Pistacia terebinthus*. Food Chemistry 103(3):816-822. *https://doi.org/10.1016/j.foodchem.2006.09.028*

Valifard M, Mohsenzadeh S, Kholdebarina B, Rowshanb V (2014). Effects of salt stress on volatile compounds, total phenolic content and antioxidant activities of *Salvia mirzayanii*. South African Journal of Botany 93:92-97. *https://doi.org/10.1016/j.sajb.2014.04.002*

Wei T, Sun H, Zhao X, Hou J, Hou A, Zhao Q (2002). Scavenging of reactive oxygen species and prevention of oxidative neuronal cell damage by a novel gallotannin, pistafolia A. Life Sciences 70:1889-1899. *https://doi.org/10.1016/S0024-3205(02)01494-7*

Yuan G, Wang X, Guo R, Wang Q (2010). Effect of salt stress on phenolic compounds, glucosinolates, myrosinase and antioxidant activity in radish sprouts. Food Chemistry 121:1014-1019. *https://doi.org/10.1016/j.foodchem.2010.01.040*