International Journal of Environment **Volume : 09 | Issue : 02| April- June | 2020**
EISSN: 2706-7939 ISSN: 2077-4508 **Pages: 104-116**
DOI: 10.36632/ije/2020.9.2.7

Seasonal impact on photosynthetic pigments, antioxidant activity and total phenolic content in *Ajuga iva* (L.) Schreb. grown in Sidi Barrani Desert, Egypt

Taghried M. El-Lamey

Department of Plant Ecology and Ranges, Desert Research Center, Egypt

Received: 30 Mar. 2020 / Accepted 10 May 2020 / Publication date: 15 May 2020

ABSTRACT

Seasonal variations have a significant effect on plant metabolites. The aim of this work was to study the effect of seasonal variations on the yield of antioxidant compounds and the activity of some antioxidant enzymes in *Ajuga iva* plants collected from Sidi Barrani Desert, Egypt. The aqueous extract of leaves and flowers samples were analyzed for total phenolic and flavonids content and antioxidant activity (radical scavenging activity (DPPH), ferric-reducing antioxidant (FRAP) and Trolox equivalent antioxidant capacity (TEAC). The extracts of summer samples displayed the highest contents of total phenol (82.61±0.81 mg GAE/g FW) and total flavonoids (16.3±0.39 mg QE)/g FW), and the greatest DPPH radical scavenging potency with an IC_{50} value of (2.47±0.04 mg/ml). There was a high significant correlation between antioxidant capacity and total phenols (DPPH, r=0.931; FRAP, r=0.958) and total flavonoids (DPPH, r=0.933; FRAP, r=0.956) were observed. The activities of polyphenoloxidase (PPO) and catalase were significantly affected by stress conditions and increased to 5.19±0.15 (µmol catechol min^{-1} g FW^{-1}) and 166±3.09 (µmol H_2O_2 decomposed min^{-1} g^{-1} FW) in summer, respectively. The production of these compounds can be correlated to the changes in the rate of rainfall, climatic temperature and the duration of sunlight .Thus, seasonal variations in climatic factors should be taken into consideration when harvesting the plant to isolate antioxidant constituents or to use the crude extracts. As it is possible, based on the results, to predict and hypothesize the chemical features of *Ajuga* in rationale with changes in climatic factors.

***Keywords*:** Ajuga iva, Antioxidants, Enzymes, Chlorophyll, Flavonoids; Phenolics, Seasonal variations

Introduction

Drought stress is considered as one of the environmental stresses that plants suffer from. The combined effect of heat, drought stresses and elevated level of CO_2 causes the initiation of reactive oxygen species (ROS) which trigger multiple stresses in crops. The production of ROS promotes the lipid peroxidation and impairs several cell building materials like carbohydrates, lipids, proteins, and nucleic acid, which lead to the disturbance of plant development, photosynthesis and respiration processes and consequently causes the cell death (Ahmad *et al.,* 2018).

Many studies revealed that there is a significant correlation between the high responses of the plant to antioxidative defences to stress and the increase in its ability to tolerance to environmental stresses. As tolerant species tend to maintain lower concentrations of O_2^- than susceptible species, thereby decreasing the risk of oxidative injury (Quartacci *et al*., 1994). To prevent damage to cellular components by ROS, plants have developed the scavenging mechanism of ROS categorized as enzymatic and non-enzymatic antioxidants (Blokhina *et al*., 2003; Reddy *et al*., 2004; Demiral and Turkan, 2005). The primary components of this antioxidant include carotenoids, glutathione, ascorbate ,tocopherols and a number of secondary products such as phenolics, flavonoids, terpenoides, anthocyanins, etc. which help them to maintain their physiology well. In addition to enzymes such as catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD) and peroxidases (Foyer and Halliwell, 1976). Many components of this antioxidant defense system can be found in various subcellular compartments (Hernandez *et al*., 2000). For instance, phenolic compounds can be accumulate in the plant cells during stress conditions as a protective substance from the negative effects of stress. They can scavenge reactive oxygen species (ROS) and free radicals (FR) (Amarowicz *et al*., 2004; Caillet *et al*., 2006; Amarowicz and Weidner 2009) and form complexes with the metals which catalyze oxygenation reaction and inhibit activity of oxidizing enzymes (Sokół-Łętowska 1997).

Corresponding Author: Taghried Mohammed El-Lamey, Department of Plant Ecology and Ranges, Desert Research Center, Egypt**.** E-mail: lameytaghried_drc@yahoo.com

The genus of *Ajuga* belongs to family Lamiaceae, that composed of 301 species of flowering plants. *Ajuga* is herbaceous plant native to Europe, Asia, Northern Africa, and distributed widely across much of the temperate world (Yalcin and Kaya, 2006; Israili and Lyoussi, 2009).

Many phytochemical studies have been reported the presence of several pharmaceutical compounds in *Ajuga* species as essential oils, diterpenoids, triterpenes, neo-clerodane-diterpenes, sterols, iridoid glycosides, flavonoids. Therefore, *Ajuga* species are widely used for the treatment of hyperglycemia, hypertension, analgesia, fever (Pal and Pawar, 2011), pneumonia, acute and chronic pharyngitis (Kirtikar and Basu,1962; Agrawal and Tamrakar,2005).

Several climate factors such as water availability, temperature, duration and intensity of sunlight, have a significant effect on the nature and quantity of metabolites (Ramakrishna and Ravishankar, 2011; Arbona *et al*., 2013; Shulaev *et al*., 2008; Jakobsen and Olsen, 1994).Thus, plants may show changes in the production of different metabolite classes under conditions of stress induced by climate factors (i.e. drought, high temperatures, freezing, high intensity of solar radiation).

Therefore, the aim of this study was to investigate the effect of seasonal variation on the yield of antioxidant compounds and the activity of some antioxidant enzymes in *Ajuga iva* plants, and to study the relationships between seasonal changes in temperature, duration in sunlight and the rate of rainfall and plant metabolism.

Materials and Methods

1. Plant description

Ajuga iva (L.) Scherb.: Woolly perennials flowers in dense leafy spikes. Corolla purple or yellow with minute reduced upper limb and an elongated lower limb consisting of 2 small lateral lobes and long central bifid lobe. Stem 15-20 cm. high, densely hairy or fleecy with crowded linear revolute-margined leaves. (Täckholm, 1974).

2. Plant material

The *Ajuga iva* plant was collected in Winter season (February) and in Summer season (July) from Sidi Barani desert , Marsa Matrouh governorate. The samples of *Ajuga* plant were identified by experts of Egyptian flora in Desert Research Center.

3. Chemicals and reagents

Folin–Ciocalteu's phenol reagent was purchased from Sigma–Aldrich, sodium acetate, aluminum chloride, ferrous sulfate, ferric chloride, sodium carbonate, sodium hydroxide, sodium nitrate and acetone were purchased from ADWIC, Egypt. 2,4,6-Tris(1-pyridyl)-5-triazine (TPTZ), and 2,2-diphenyl-1-pycridazil (DPPH. 90%) were purchased from the Fluka company (Switzerland). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) which is a hydrophilic analogue of vitamin E, ABTS (2,2-azino-bis3 ethylbenzothiazoline-6-sulfonic acid) , quercetin , gallic acid and all Buffer solutions were supplied by Sigma–Aldrich.

4. Ecological studies

4.1. The study area

The *Ajuga* plant was collected from Sidi Barrani Desert, Marsa Matruh governorate. The Study area is lied between Latitudes 31°14'57.2"N and Longitudes 25°40'19.6"E at the distance about 70Km apart from the center of Sidi Barrani City.

4.2. Soil physical and chemical analysis

Soil samples were collected from the soil supporting the investigated plant at 3 random points at 0-30 cm depth. Soil physical properties and soil texture (Granulometric analysis) were determined through mechanical analysis by the sieve method (Jackson, 1967). For soil chemical analysis, the electrical conductivity (EC) and pH for each sample were determined as a 1:2.5 dilution in deionized water according to Page (1987). The content of chloride (Cl) was determined by titrating the soil solution against silver nitrate (0.5N) and using 1% potassium chromate as an indicator (Jackson, 1967). The contents of sodium and potassium in the soil solution were determined by using flame photometer (Jenway, PFP-7 and the concentrations of magnesium (Mg) and calcium (Ca), were determined by

titration with ethylene diamine tetra-acetic acid (EDTA) according to the method of Rowell (1994). However, the content of carbonate (CO_3) and bicarbonate ions (HCO_3) was determined by titration, using 0.1N HCl and methyl orange as an indicator (Rowell, 1994).

5. Physiological studies

5.1. Determination of photosynthetic pigments

The contents of chlorophyll-a (Chl a), chlorophyll-b (Chl b) and carotenoids were determined by spectrophotometric method (Sumanta *et al*., 2014). Accurately weighted 0.5g of fresh plant leaf sample was taken, and homogenized in tissue homogenizer with 10 ml of 80% acetone. The homogenate was centrifuged at 10,000 rpm for 15 min at 4^0C. The supernatant was separated and filtrated, and then 0.5ml of it was mixed with 4.5ml of solvent. The solution mixture was analyzed for chlorophyll a, chlorophyll b and carotenoids content .The contents of chlorophyll-a, chlorophyll-b and carotenoids were calculated according to the following equations:

Chl a=12.25A663.2 – 279A646.8

Chl b=21.5A646.8 – 5.1A663.2

C x+c=(1000A470 – 1.82Ca –85.02Cb)/198

Where: A = Absorbance, Chl a = chlorophyll a, Chl b = chlorophyll b, C x+c = carotenoids and the results were expressed as (µg/ml).

5.2. Phytochemical and antioxidant assays

5.2.1. Extraction procedure

The leaves and flowers were cut in small pieces. Then, one gm of each sample in three replicates was soaked in 20 mL of hot distilled water to prepare the aqueous extract. The extracts were allowed to stand for 24 hrs before being filtered with Whatman No. 1 filter paper. The extract was then stored at 4 ^{0}C till analysis (Ijeh *et al*., 2005).

5.2.2. Determination of total phenol content (TPC)

Total phenol content (TPC) of samples was determined according to spectrophotometric procedure (Singleton 1998). Briefly, 100 µL of plant extract was mixed with 1.0 mL of distilled water and 0.5 mL Folin–Ciocalteu's reagent(1:10 v/v). After mixing, 1.5 mL of 2% aqueous sodium bicarbonate was added and the mixture was allowed to stand for 30 min with vigorously in a darkroom, absorbance was read at 765 nm against a blank in a spectrophotometer. TPC was calculated by a calibration curve of gallic acid in methanol (8-80 mg/L) and the results were expressed as milligrams gallic acid equivalent per gram fresh weight used in extraction (mg GAE/g FW).

5.2.3. Determination of total flavonoids content (TFC)

Total flavonoids content (TFC) was determined using the aluminium chloride ($AlCl_3$) method using quercetin as the standard (Ordonez *et al*., 2006). In this regard, the plant extract (100 µL) was added to 0.3 mL of distilled water followed by addition of 0.03 mL of $NaNO_3$ (5% w/v). After 5 min. at 25 ^{0}C, $AlCl_3$ (0.03 mL, 10%) was added. After further 5 min., the reaction mixture was treated with 0.2 mL of NaOH (1 mM). Finally, the reaction mixture was diluted to 1 mL with water and the absorbance was measured at 510 nm using UV-Vis Shimadzu (UV-1601, PC) spectrophotometer. The results were expressed as mg quercetin (mgQE)/g FW.

5.2.4. DPPH radical scavenging capacity.

The antioxidant activity of the extract was measured as described by Gebhard (2001) using the 1, 1'–diphenyl– 2–picrylhydrazyl ($DPPH^0$) free radical scavenging capacity. Briefly, 3 ml of methanol solution of 0.004 % DPPH was added to the test tube containing 1.0 mL of properly diluted plant extract in different concentrations. Mixture was then vortexed for 1 min at 800 rpm and kept in the dark for 30 min at room temperature. Absorbance of samples was measured at 517 nm.

The radical scavenging activity of the samples (antioxidant activity) was expressed as percent inhibition of DPPH0 radical as following:

$$\% \text{ Inhibition} = [(A \text{ control} - A \text{ sample})/A \text{ control})] \times 100$$

Where: A control: is the absorbance of the control at 30 min; A sample is the absorbance of the samples in the presence of the extract at 30 min. Results were expressed as mg sample/mL in the form of IC_{50}, determined by linear regression of IC and extract concentration at 50% inhibition.

5.2.5. Ferric-reducing antioxidant power

Ferric-reducing antioxidant power (FRAP) was determined by the spectrophotometric method previously described by Benzie and Strain (1996). Three stock solutions were prepared: a 300 mM acetate buffer (3.1 g sodium acetate and 16 mL glacial acetic acid), pH 3.6, 10 mM TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine) solution in 40 mM HCl, and 20 mM ferric chloride solution Working solution was prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution and 2.5 mL of $FeCl_3 \bullet 6H_2O$solution. This working solution was then warmed to 37˚C. A 75 mL aliquot of properly diluted plant extract in different concentrations was mixed with 1.425 mL of working solution. After mixing in the dark condition for 30 min., the absorbance of the colored product [ferrous tripyridyltriazine complex] were then taken at 593 nm.

An antioxidant capable of donating a single electron to the ferric-TPTZ (Fe (III)-TPTZ) complex would cause the reduction of this complex into the blue ferrous-TPTZ (Fe (II)-TPTZ) complex which absorbs strongly at 593 nm. The higher the FRP value, the greater was the reducing power, thus the greater the antioxidant activity.

% reducing power = [(A sample - A control)/A control] X 100, A sample is the absorbance of the sample in the presence of the extract and A control is the absorbance of the control. The result was expressed as mg sample/mL in the form of IC_{50}, determined by linear regression of IC and extract concentration at 50% reduction. The result was expressed as IC_{50} which corresponds to the concentration of the extract necessary to reduce 50% of ferric ferrous complex.

5.2.6. Trolox equivalent antioxidant capacity assay

The ABTS free radical-scavenging activity of plant extracts was determined according to the methods described by Arnao *et al*., (2001). The radical cation $ABTS^{\bullet+}$ was produced by reacting equal quantities of a 7 mM aqueous solution of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) with 2.45 mM potassium persulfate for 16 h at room temperature (25°C) in the dark. The working solution was then prepared by diluting 1 mL ABTS solution with 60 mL of ethanol: water (50:50, v/v) to obtain an absorbance of 1.0± 0.02 units at 734 nm using the spectrophotometer. Different concentrations of plant extract were allowed to react with 4.95 mL of the radical cation $ABTS^{\bullet+}$ solution for 1 h in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer after 10 min. The standard curve was prepared using Trolox. Results were expressed as mM Trolox equivalents per gram of fresh samples (mM TE/g FW). Additional dilution was needed if the ABTS value measured was over the linear range of the standard.IC_{50} values calculated denote the concentration of the sample required to decrease the absorbance at 734 nm by 50%.

5.2.7. Determination of polyphenoloxidase (PPO) activity

The plant tissue (about 1.0 g) was homogenized with 20 mM Tris–HCl buffer, pH 7.2 using a homogenizer. The homogenate was centrifuged at 21000 xg for 10 min at 4 ^{0}C. The supernatant was designated as crude extract and stored at −20 ^{0}C for further analysis. The activity PPO was determined by the method of (Liu *et al*., 2005). The standard reaction mixture contained 1.5 ml of 40 mmol/l catechol and 2.3 ml of 0.1 mol/l phosphate buffer (pH 6.5) in a 10 ml test tube, and was placed in a 25 °C water bath for 5 minutes. Then, 0.2 ml of crude enzyme was added to the test tube and mixed thoroughly. Immediately, the increase in absorbance was measured at 420 nm with a UV-Vis Shimadzu (UV-1601, PC) spectrophotometer. The reaction time for PPO was 3 min. The activity of PPO was calculated based on the molar extinction coefficient of 3400 mM–1 cm–1 for catechol and expressed in μmol min^{-1} g FW^{-1}.

5.2.8. Determination of peroxidase (POD) activity

About (0.3g) of plant samples were mixed with disodium phosphate buffer (pH-7). Each homogenate was transferred to centrifuge tubes and was centrifuged at 4 ^{0}C for 20 min. at 3000 xg. Peroxidase was determined using the method of Srinivas *et al*. (1999) by following the formation of tetraguaiacol by measuring the absorbance at 470 nm, and using an extinction coefficient of 26.6 mM^{-}

^{1} cm^{-1} to calculate the amount of tetraguaiacol. The 1 ml reaction mixture contained 20 mM phosphate buffer, pH 6.0, 5 mM 2-methoxy phenol (guaiacol), 1 mM hydrogen peroxide with an appropriate aliquot of enzyme extract. The reaction was carried out for 3 min. One unit of peroxidase activity represents the amount of enzyme catalysing the oxidation of 1 µmol of guaiacol in 1 min.

5.2.9. Determination of catalase) (CAT) activity

The activity of catalase was determined by the method of Korolyuk *et al*. (1988). leaves or flowers (100 mg) were extracted by homogenization in 400 µl of 50 mM Potassium dihydrogen phosphate buffer (pH 7.0) containing 0.1% (v/v) Triton X-100 and 1% (w/v) PVP-40 .The homogenate was centrifuged at 13 000 g for 15 min. The assay consisted of 100 µl of extract, 890 µl 50 mM KH_2PO_4, and 10µl of 10mM H_2O_2, and the decomposition of H_2O_2 was followed at 240nm ($\varepsilon = 0.04$ mM^{-1} cm^{-1}).

Statistical analysis

All experimental measurements were carried out in triplicate and are expressed as average of three measurements ± standard deviation. Pearson's correlation coefficients were calculated among phenolics, flavonoids and antioxidant activities, and interpreted according to Taylor (1990).The application of PLSR Partial Least Squares Regression using XLSTAT 2020.3.1.27, allowed to identify the compounds whose concentrations in plant samples were changing more with climatic temperature, rainfall rate or duration of sunlight, and used to investigate the correlations between some climatic factors and the production of photosynthetic pigments, phenolic and flavonoids and the antioxidant enzymes. Also, to identify the more influent variables contributing more to the prediction of the production responses by using variable importance in prediction (VIP) method.

Results and Discussion

1. Climatic conditions

As Shown in Fig. 1, the average high temperatures during the period of study in 2019 were 19.6, 17.9 and 18.1°C in December, January and February, respectively. Whereas, the recorded average high temperatures during spring in April, May and June were 22.2, 24.1 and 27°C, respectively. The warmest month (with the highest average high temperature) was August (29°C). The month with the lowest average high temperature was January (17.9°C). The average rainfall in winter months; December, January and February were 33, 39 and 17mm, while in spring months, its values were 5, 3 and 0 mm in April, Mayand June, respectively. The month with the highest rainfall was January (39mm).The dry period extended from June to August. Meanwhile, the average duration of sunlight in December, January and February were 10.1, 10.4 and 11.1h, respectively. The month with the longest days was June (average sunlight: 14.2h), while the month with the shortest days was December (average sunlight: 10.1h).

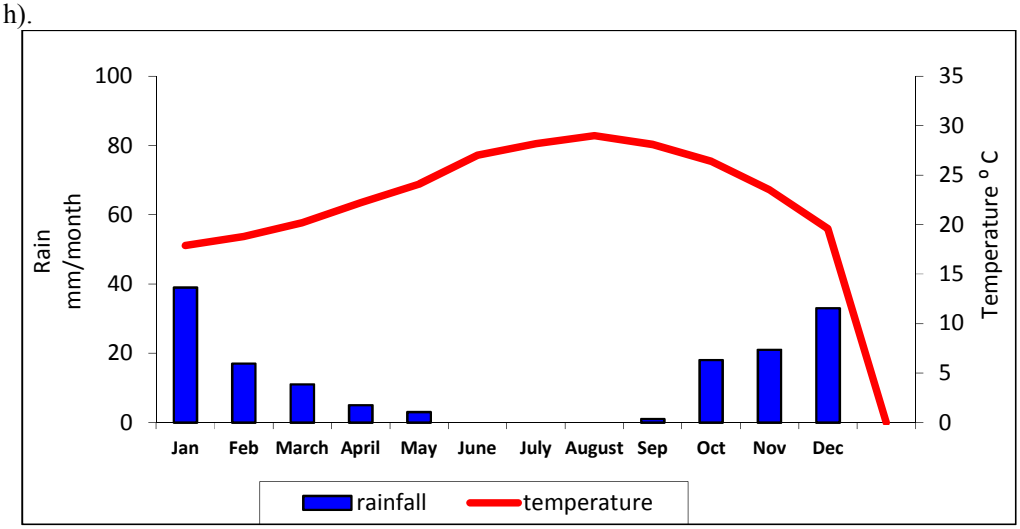

Fig.1: Climatic data of Sidi Barrani Desert

2. The soil physical and chemicals properties

As shown in Table 1, the soil of Sidi Barrani Desert is Loamy Sand in nature, as the percentage of sand in soil sample taking at depth (0-30) was 62.41%. The soil was rich with silt (32.07 %) and contained a low percentage from clay (5.32%).

Table 1: Soil physical properties

Soil Particles Distribution			Soil Texture Class
Sand%	**Silt%**	**Clay%**	
62.41	32.07	5.32	Loamy Sand

The result of the chemical analysis of soil solution revealed that the soil PH was slightly alkaline (8.80). Its soluble salts content was 0.45 dS/m and the percentage of total calcium carbonate was 29.28%. According to Moore *et al*., (1990), the presence of silt and clay particles make the carbonates very active, which may cause a reduction in the availability of phosphorous, zinc, manganese and copper.

Table 2: Soil chemical properties

Soil Depth (cm)	pH 1:2.5	EC dS/m	Cation (milliequivalent/Liter)				Anion(milliequivalent/Liter)			% $CaCO_3$
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Cl^{-}	CO_3^{-}	HCO_3^{-}	
0 – 30	8.80	0.45	2.50	1.15	0.61	0.40	1.30	-	3.40	29.28

3. Effect of season on the photosynthetic pigments in *Ajuga* leaves

Chlorophyll a and chlorophyll b are essential pigments of the plant photosystems (Richardson *et al*., 2002), as they are the main source of energy and their production is mainly depended on penetration of sun light (Srichaikul *et al*., 2011). Thus, their concentrations indicate to the content of chloroplast, performance of photosynthesis and energy utilization rate. Moreover, chlorophyll has antioxidant capacity and stored in chloroplast of green leaf, stems, flowers and roots (Srichaikul *et al*., 2011; Hasanuzzaman *et al*., 2013)

As shown in Table 3 and Fig.2, the content of chlorophyll b (Chl b) was significantly affected by seasons, as its value increased from (0.036±0.003 µg/ml) in winter to (0.790±0.003 µg/ml) in summer. Whereas the concentration of primary photosynthetic pigment Chl a in summer sample was 2 times lower than that in winter. Considerable reduction in Chl content due to water deficit was related to plant drought tolerance and previously reported for some crop species and tolerant genotypes (Sairam *et al*., 1997)

Meanwhile, Demmig-Adams and Adams (1996) reported that during excess excitation of chlorophyll, carotenoids are generated and involved in quenching of $^1O_2$ and peroxyl radicals. Another study performed by Knox and Dodge (1985) revealed that the inhibition of cartotenoid biosynthesis by using norflurazon, increased the production of $^1O_2$ and caused photo-oxidative damage. Thus, maintaining a high carotenoid concentration may protect plants from $^1O_2$ damage during environmental stresses.

The obtained data also indicated that there was a significant reduction in the ratio of Chl a to Chl b from 2.977±0.435 in winter to 0.070±0.008 in summer. Similarly, Ghorbanli *et al*. (2013) reported that the ratio of chlorophyll a to b reduced in resistant species of tomato under water deficit condition and this indicated that photosystem II may protect the plant against heat and water stresses.

Table 3: Effect of season on the photosynthetic pigments in *Ajuga* leaves

Seasons	Chl a (µg/ml)	Chl b (µg/ml)	Carotenoids (µg/ml)	Chl a/ Chl b
Winter	0.108±0.008^a	0.036±0.003^b	0.072±0.004^a	2.977±0.435^a
Summer	0.055±0.006^b	0.790±0.003^a	0.080±0.003^a	0.070±0.008^b

Values are expressed as mean ± SD (n = 3). In each column values followed by different letters are significantly different at $p < 0.05$

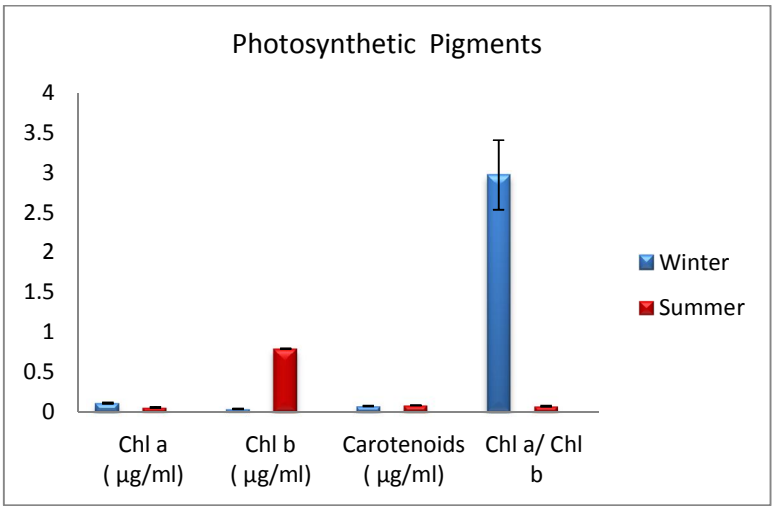

Fig. 2: Seasonal variations in the content of photosynthetic pigments in *Ajuga* leaves

The relationships between the seasonality of certain climatic factors (temperature, rainfall rate and the period of sunlight) and the content of photosynthetic pigments based on the partial least squares regression analysis (PLSR)showed that there was an inverse correlation between the climatic variations in temperature and Chl a (Y = - 0.003X +0.195, r = - 0.951), as well as the duration of sunlight (Y = - 0.006X+0.195, r = - 0.886), and a direct correlation between rainfall and Chl a (Y = 0.001X+0.195, r = 0.832). On contrary, the correlation between the seasonal variations in temperature and the content of Chl b (Y = 0.037X -1.196,r = 0.960) and carotenoids (r = 0.687) was direct while the correlation between rainfall rate and the content of Chl b (r = - 0.8940) and carotenoids (Y = 0.0003X+0.061,r = - 0.800) was inverse. Whereas the correlation between the duration of sunlight and carotenoids (Y = 0.001X+ 0.061, r = 0.579) was direct and moderate. According to the value of variable importance in prediction (VIP) (Fig.3), the duration of sunlight was more influent variables contributing more to the prediction and hypothesis of the chemical features of *Ajuga* (1.047), followed by the rainfall rate (1.004) and climatic temperature (0.946).

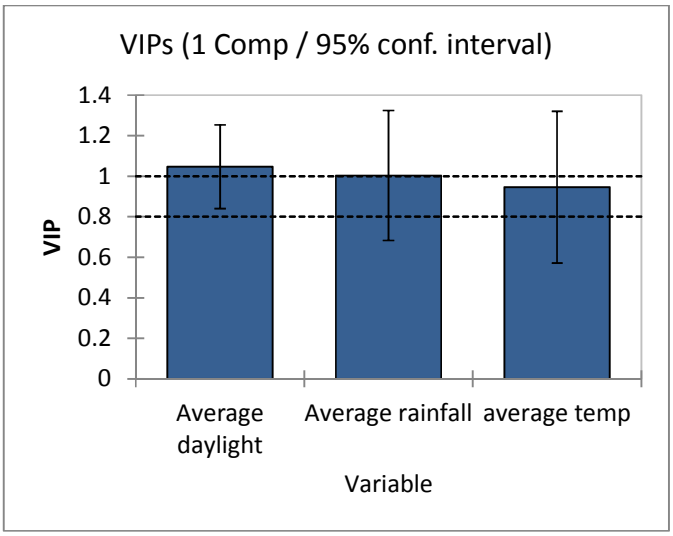

Fig. 3: Value of Variable Importance in Projection (VIP)

4. Effect of season on the antioxidant compounds and antioxidant capacity

4.1. Phenolic and flavonoids compounds in *Ajuga* leaves

As shown in Table4, there was a highly significant correlation (r=0.931) and linear positive relationships (R^2=0.867) between total phenolic content and radical scavenging activity (DPPH), as well

as between total phenolic content and ferric reducing antioxidant power (FRAP), as r=0.958 and R^2=0.918. Similarly, a highly significant correlation (r=0.933) and linear positive relationships (R^2=0.871) were recorded between total flavonoids content and (DPPH), as well as between total flavonoids content and (FRAP) (r=0.956) (R^2=0.915). The obtained results also revealed that there was a highly significant correlation (r=0.995) and linear positive relationships (R^2=0.991) between total phenolic content and Trolox equivalent antioxidant Capacity (TEAC), as well as between flavonoids and TEAC, as r=0.981 and R2=0.963. Similarly, de Oliveira *et al*. (2012) demonstrated a strong correlation between total polyphenol content and the antioxidant activity of *Sidastrum micranthum* (R^2=0.929), as well as of *Wissadula Periplocifolia* (R^2=0.814) samples, while Liu *et al*.(2009) demonstrated the same correlation of *Ilex kudingChl a* extract (r >0.812).

Table 4: Pearson's correlation coefficients (r) and regression coefficients among antioxidant compounds (total phenolic and total flavonoids) and antioxidant activity (DPPH, FRAP and TEAC assays)

Antioxidant compounds	DPPH% (mg/ml)	FRAP% (mg/ml)	TEAC (mM TE/g FW)
Total phenols (mg GAE/g FW)	y = 100.908x + 29.337 R^2 = 0.867 r=0.931**	y = 102.00x + 21.324 R^2 = 0.918 r=0.958**	y = - 0.023x + 2.163 R^2 = 0.991 r= 0.995 **
Total flavonoids (mgQE)/g FW	y = 509.847x + 28.728 R^2 = 0.871 r=0.933**	y = 513.337x + 20.855 R^2 = 0.915 r=0.956**	y = - 0.129x + 2.383 R^2 = 0.963 r= - 0.981 **

**Correlation is highly significant at the 0.01 level (2-tailed)

As shown in Table 5 and Fig.4, the contents of total phenolic and flavonoids were significantly affected by season, as their values increased from (62.4±0.45mg GAE/g FW)) and (12.8±0.09 mg QE)/g FW) in winter to (82.6±0.81 mg GAE/g FW) and (16.3±0.39 mg QE)/g FW) in summer ,respectively. These results are in agreement with the previous study of Ahmed *et al*., (2012) on *Melilotus indicus*, which indicated the significant effect of seasonal variation on the biosynthesis of antioxidant free radical scavenging compounds.

The potential of antioxidant capacity of *Ajuga* extracts were determined based on free radical scavenging activity (DPPH), ferric-reducing antioxidant Power (FRAP) and Trolox equivalent antioxidant capacity assays (TEAC). DPPH assay is based on the scavenging of $DPPH^{\bullet}$ radical through the addition of a radical species or an antioxidant that decolourizes the DPPH solution (Krishnaiah *et al*., 2011), while In FRAP assay, the antioxidant capacity is measured on the basis of the ability to reduce ferric (III) ions to ferrous ions (II) and the results were expressed as IC_{50}, which corresponds the concentration required to reduce 50% of ferric tripyridyltriazine complex.

The obtained results showed that the lower values of IC_{50} of DPPH (2.47±0.04 mg/ml), FRAP (3.40 ±0.03 mg/ml) and TEAC (0.26±0.03 mM TE/g FW) of summer extract, corresponds to a higher antioxidant capacity and a higher $ABTS^{\bullet+}$radical scavenging activities. Which implies that the antioxidant compounds in *Ajuga* were able to scavenge free radical and reduce oxidant (ferric ions). Several studies (Amarowicz *et al*., 2000, 2004; Negro *et al*. 2003; Caillet *et al*., 2006; Amarowicz and Weidner 2009), demonstrated that phenolic compounds can scavenge free radicals and reactive oxygen species (ROS), as well as they have metal chelating properties to form complexes with the metals which catalyze oxygenation reaction and inhibit activity of oxidizing enzymes (Sokół-Łętowska 1997). Similarly, other (Tabart *et al*., 2006;Tabart *et al*.,2007; Maizura *et al*.,2011) demonstrated a highly positive correlation between total phenolic and antioxidant activity based on the DPPH, TEAC and FRAP assays. Therefore, in this study, highly significant correlations and linear positive relationships among the contents of total phenolic and flavonoids and antioxidant activity based on the DPPH, TEAC and FRAP assays, indicate that these compounds were the major contributors to the antioxidant activity of *Ajuga* extracts and the potential of antioxidant capacity was strongly correlated with their concentrations.

The results of partial least squares regression analysis (PLSR) revealed that there were direct strong correlations among the seasonal variations in temperature as well as the duration of sunlight and the yield of phenolic (Y = 0.973X + 29.703, r = 0.873; Y = 2.087X + 29.703, r = 0.953, respectively) and flavonoids compounds (Y = 0.166 X + 7.270, r = 0.845; Y = 0.357X + 7.270, r = 0.928, respectively

). Whereas the correlation between the rainfall rate and yield of total phenolic (Y = - 0.217X +29.703, r = - 0.884) and total flavonoids (y = - 0.037X + 7.270, r = - 0.870) was inverse. It can be observed that samples collected during the dry period in summer season were rich in polyphenolic compounds (phenol, flavonoids). Others (Harbowy and Balentine ,1997; Sezai *et al*.,2008) reported that the variations of climatic and ecological conditions, such as the duration and intensity of sunlight, have significant effect on the nature and quantity of natural products produced in the plant body. This can be attributed to the well known tendency of these compounds to reduce free radical levels, to inhibit the production of reactive oxygen species and to protect the plant tissues from their damaging effects. Moreover, several flavonoids are induced by abiotic stress and act as potential inhibitors to lipid peroxidation (Potapovich and Kostyuk, 2003) and lipoxygenase enzyme, which converts polyunsaturated fatty acids to oxygen-containing derivatives (Nijveldt *et al*., 2001).

Table 5: Effect of season on the content of total phenolic, total flavonoids and antioxidant activity (DPPH, FRAP and TEAC assays)

Seasons	Total Phenolic (mg GAE/g FW)	Total Flavonoids (mg QE)/g FW	DPPH (IC_{50}=mg/ml)	FRAP (IC_{50}=mg/ml)	TEAC (mM TE/g FW)
Winter	62.4±0.45^{b}	12.8±0.09^{b}	3.28±0.03^{a}	4.50±0.03^{a}	0.72±0.01^{a}
Summer	82.6±0.81^{a}	16.3±0.39^{a}	2.47±0.04^{b}	3.40±0.03^{b}	0.26±0.03^{b}

Values are expressed as mean ± SD (n = 3). In each column values followed by different letters are significantly different at $p < 0.05$

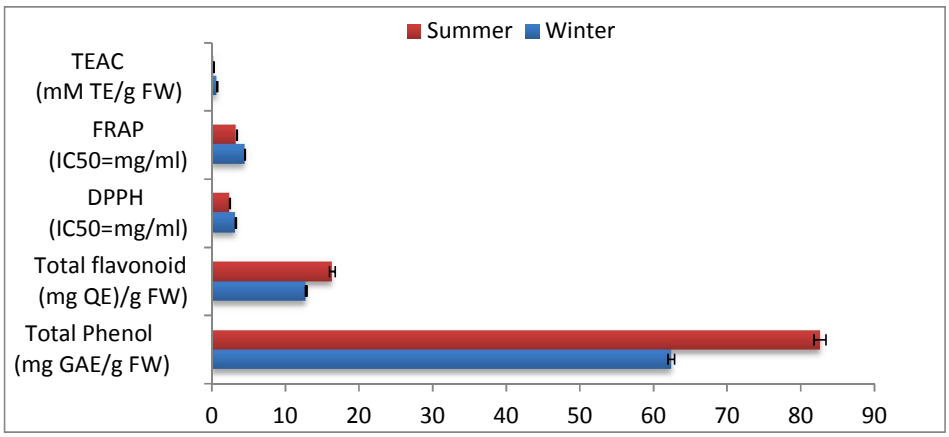

Fig. 4: Seasonal variations in the content of total phenolic, total flavonoids and antioxidant activity

4.2. Antioxidant enzymes

The obtained results revealed that there was a significant effect of seasonal variation on antioxidant enzymes such as polyphenoloxidase (PPO) and catalase (CAT) (Table 6, Fig.5).The activity of PPO affected by stress condition in summer and increased from 3.92±0.22 to 5.19±0.15 (µmol catechol min^{-1} g FW^{-1}). Meanwhile, the activity of catalase was significantly increased from 147±2.68 to 166.2±3.09 (µmol H_2O_2 decomposed min^{-1} g^{-1} FW) in summer. Meloni *et al*. (2003) reported that antioxidant enzymes are the most important components in the scavenging system of ROS, as catalase, and a variety of general peroxidases (POD) (Chang *et al*., 1984) catalyze the breakdown of H_2O_2. Therefore, these enzymes may help in eliminating the damaging effects of toxic oxygen species.

Table 6: Effect of season on the activity of antioxidant enzymes in *Ajuga* leaves

Seasons	Polyphenoloxidase (PPO) (µmol catechol min^{-1} g FW^{-1})	Peroxidase (POD) (µmol of guaiacol min^{-1} g FW^{-1})	Catalase (CAT) µmol H2O2 decomposed min^{-1} g^{-1} FW
Winter	3.92±0.22^{b}	30.7±1.18^{a}	147±2.68^{b}
Summer	5.19±0.15^{a}	31.7±0.79^{a}	166±3.09^{a}

Values are expressed as mean ± SD (n = 3). In each column values followed by different letters are significantly different at $p < 0.05$

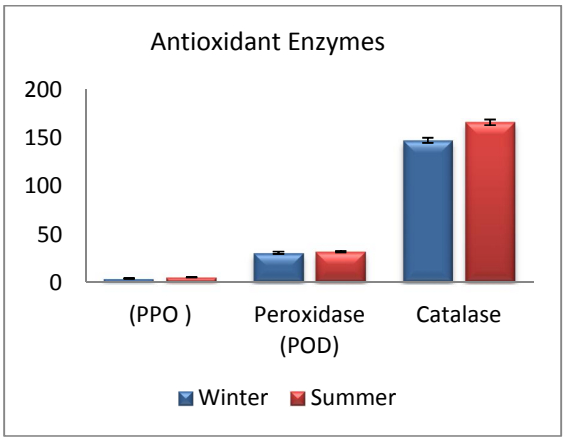

Fig. 5: Seasonal variations in the activity of antioxidant enzymes

The results of partial least squares regression analysis (PLSR), indicated that there was a strong direct correlation coefficient between climatic temperature and the activity of PPO (Y = 0.064X + 1.748, r = 0.857), as well as the activity of catalase (Y = 0.929X + 115.940, r = 0.873). Similarly, the correlation between the period of sunlight and the activity of the same enzymes was highly significant and linear positive relationships (Y = 0.137X+ 1.748, r = 0.960; Y = 1.993X + 115.940, r = 0.918, respectively). Meanwhile, the correlations between POD and climatic temperature, (Y = 0.066 X + 28.289, r = 0.539) as well as the duration of sunlight (Y = 0.143X + 28.289, r = 0.673) were moderate. Kusyuran *et al*. (2016) reported that antioxidant enzyme are correlated with higher stress tolerance and can be use to identifying the tolerant and sensitive plant genotypes, as the high level of antioxidative enzyme activities were detected in the tolerant plant genotype.

5. The antioxidant compounds and antioxidant capacity of *Ajuga* flowers

As shown in Table 7, the content of total phenolic and total flavonoids in *Ajuga* flowers extract were 67.3±0.32 (mg GAE/g FW) and 14.2±0.61 (mg QE)/g FW, respectively. The obtained results indicated that flowers extract had a significant antioxidant activity, as DPPH with an IC_{50} at (3.04±0.01 mg/ml) and FRAP with an IC_{50} at (4.18±0.02mg/ml), while TEAC with an IC_{50} at (0.65±0.04 mg/ml). The high content of flowers extract from antioxidant compounds, may help to improve the antioxidant capacity of plant and assist in reducing free radical and in protecting the plant tissues from damaging effects of reactive oxygen species, which induced by abiotic stress in summer season.

Table 7: The content of total phenolic, total flavonoids and antioxidant activity (DPPH, FRAP, and TEAC assays) in *Ajuga* flowers

Total Phenolic (mg GAE/g FW)	Total Flavonoids (mg QE)/g FW	DPPH (IC_{50}=mg/ml)	FRAP (IC_{50}=mg/ml)	TEAC (mM TE/g FW)
67.3±0.32	14.2±0.61	3.04±0.01	4.18±0.02	0.65±0.04

Values are expressed as mean ± SD (n = 3)

As shown in Table 8, the activity of PPO, POD and Catalase enzymes was 1.67±0.09 (µmol catechol min^{-1} g FW^{-1}), 19.6±0.60 (µmol of guaiacol min^{-1} g FW^{-1}) and 125.5±1.04 (µmol H_2O_2 decomposed min^{-1} g^{-1} FW).

Table 8: the activity of antioxidant enzymes in *Ajuga* flowers

Polyphenoloxidase (PPO) (µmol catechol min^{-1} g FW^{-1})	Peroxidase (POD) (µmol of guaiacol min^{-1} g FW^{-1})	Catalase (CAT) (µmol H2O2 decomposed min^{-1} g^{-1} FW)
1.67±0.09	19.6±0.60	125.5±1.04

Values are expressed as mean ± SD (n = 3)

Conclusion

The results indicated that the production of some secondary metabolites in *Ajuga iva* was significantly influenced by seasonal variations in temperature, the rate of rainfall and the duration of sunlight. As they had negative effects on photosynthetic pigments and caused significant reduction in Chl a and the ratio of Chl a to Chl b in summer season. However, the biosynthesis of phenolic and flavonoids was significantly affected by season and significantly increased under stress conditions in summer season. Which may help in improving tolerance of *Ajuga* to oxidative stress and protecting the plant tissues from the damaging effects of free radicals.

It was observed that antioxidant activities such as radical scavenging activity (DPPH) and ferric reducing antioxidant power (FRAP) of *Ajuga* extracts also showed variations with the seasonal change and had strong direct correlations with the yield of total phenolic and flavonoids..

In conclusion, the results of partial least squares regression analysis (PLSR) showed that the contents of photosynthetic pigments, total phenolic and flavonoids, as well as, the activity of catalase and PPO enzymes were significantly correlated with climatic factors, such as the duration of sunlight, rainfall and temperature. Whereas, the duration of sunlight was more influent variables contributing more to the prediction and hypothesis of the chemical features of *Ajuga iva*, followed by the rainfall rate and climatic temperature. Thus, seasonal variations in climatic factors should be taken into consideration when harvesting the plant to isolate antioxidant constituents or to use the crude extracts.

The great antioxidant activity of extracts of *Ajuga iva* leaves and flowers indicates the value of plant as a natural source of antioxidants that could be used in the treatment of diseases with free radical origin.

References

Agrawal, S.S. and B.P. Tamrakar, 2005. Clinically useful herbal drugs, Edn 1, Ahuja publishing house, 233-235.

Ahmed, D., H. Baig and S. Zara, 2012. Seasonal variation of phenolics, flavonoids, antioxidant and lipid peroxidation inhibitory activity of methanolic extract of *Melilotus indicus* and its sub-fractions in different solvents. International Journal of Phytomedicine, 4, 326-332.

Ahmad Z., S. Anjum , E.A. Waraich, M.A. Ayub, T. Ahmad, R.M.S. Tariq, R. Ahmad, and M.A. Iqbal, 2018. Growth, physiology, and biochemical activities of plant responses with foliar potassium application under drought stress- a review, Journal of Plant Nutrition, 41(13): 1734-1743. DOI:10.1080/01904167.2018.1459688

Amarowicz, R., R.B. Pegg, P. Rahimi-Moghaddam, B. Barl, and J.A. Weil, 2004. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chem., 84(4): 551–562. DOI: 10.1016/S0308-8146(03)00278-4

Amarowicz, R. and S. Weidner, 2001. Content of phenolic acids in rye caryopses determined using DAD-HPLC method. Czech. J. Food Sci., 19: 201-205.

Arbona, V., M. Manzi, C. de Ollas and A. Gómez-Cadenas, 2013. Metabolomics as a tool to investigate abiotic stress tolerance in plants. Int. J. Mol. Sci., 14: 4885–4911. DOI: 10.3390/ijms14034885.

Arnao, M.B., A. Cano and M. Acosta, 2001.The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem., 73: 239–244. DOI: 10.1016/S0308-8146(00)00324-1

Benzie, I.F.F. and J.J. Strain, 1996. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. Analytical Biochemistry, 239: 70-76. DOI: 10.1006/abio.1996.0292.

Blokhina, O., E. Virolainen-Arne and K.V. Fagerstedt, 2003. Antioxidants, oxidative damage and oxygen deprivation stress: A review. Ann. Bot., 91:179-194. DOI: 10.1093/aob/mcf118

Caillet, S., S. Salmieri and M. Lacroix, 2006. Evaluation of free radical-scavenging properties of commercial grape phenol extracts by a fast colorimetric method. Food Chem., 95: 1-8.

Chang, H., B.Z. Sigel and S.M. Sigel, 1984. Salinity induced changes in isoperoxidase in taro, *Colocasia Esculenta.* Phytochem, 23: 233-235.

de Oliveira A.M.F., L.S. Pinheiro, C.K.S. Pereira, W.N. Matias, R.A. Gomes, O.S. Chaves, M.F.V. de Souza, R.N. de Almeida and T.S. de Assis, 2012. Total phenolic content and antioxidant activity of some Malvaceae family species. Antioxidants, 1:33–43. DOI: 10.3390/antiox1010033

Demirel, T. and I. Turkan, 2005. Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. Environmental and Experimental Botany, 53: 247–257.

Demmig-Adams, B. and W.W.I.I. Adams, 1996. The role of xanthophylls cycle carotenoids in the protection of photosynthesis. Trends in Plant Science, 1: 21–26. DOI: 10.1016/S1360-1385 (96)80019-7

Foyer, C.H. and B. Halliwell, 1976. The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta, 133 : 21 –25

Gebhard, R., 2001.Anticholestatic activity of flavonoids from artichoke (*Cynara scolymus* L.) and their metabolites. Med Sci Mon., 7: 316-320. PMID: 12211745

Ghorbanli, M., M. Gafarabad, T.A.N.N.A.Z. Amirkian, and B.A. Mamaghani, 2013. Investigation of proline, total protein, chlorophyll, ascorbate and dehydroascorbate changes under drought stress in Akria and Mobil tomato cultivars. Iran J. Plant Physiol., 3(2): 651–658.

Harbowy, M.E. and D.A. Balentine, 1997. Tea Chemistry, Crit. Rev. Plant Sci., 16: 415-480.

Hernandez, J.A., P. Mullineaux, and F. Sevilla, 2000. Tolerance of pea (*Pisum sativum* L.) to long term stress is associated with induction of antioxidant defences. Plant, Cell and Environment, 23: 853–862.

Hasanuzzaman, M., K. Nahar, M.M. Alam, R. Roychowdhury and M. Fujita, 2013. Physiological, Biochemical, and Molecular Mechanisms of Heat Stress Tolerance in Plants. Int. J. Mol. Sci., 14: 9643-9684. DOI: 10.3390/ijms14059643.

Ijeh, I.I., O.D. Omodamiro and I.J. Nwanna, 2005. antimicrobial effects of aqueous and ethanolic fraction of two spices, *Ocimum gratissimum* and *Xylopia aethiopica*. Afr. J. Biotechnol., 4: 953-456.

Israili, Z.H. and B.L. Youssi, 2009. Ethnopharmacology of the Plants of Genus *Ajuga*, Pak. J Pharm. Sci., 22(4): 425-462.

Jackson, M. L., 1967. Soil Chemical Analysis. Hall of India Private, New Delhi, India. Printice. Hall Inc., N. J., 248 pp.

Jakobsen, H. and C. Olsen, 1994. Influence of climatic factors on emission of flower volatiles in situ. Planta, 192: 365–371.

Kirtikar, K.R. and B.D. Basu, 1963. Indian Medicinal Plants, Edn 2, Allahabad, India: Lalit Mohan Basu., 1961-1962.

Knox, J.P., and A.D. Dodge, 1985. Singlet oxygen and plants. Phytochemistry, 24: 889–896.

Korolyuk, M.A., L.I. Ivanova and I.T. Majorova, 1988. Methods of definition of catalase activity. Lab Manuals, 1: 16-19.

Krishnaiah, D., R. Sarbatly and R. Nithyanandam, 2011. A review of the antioxidant potential of medicinalplant species. Food Bioprod. Process, 89: 217–233.

Kusvuran, S., S. Kıran and Ş.Ş. Ellialtıoğlu, 2016. Antioxidant enzyme activities and abiotic stress tolerance relationship in vegeTable crops. In: Abiotic and biotic stress in plants – recent advances and future perspectives, Shanker, A.K., Shanker, Ch. (eds). InTech. pp. 481–506.

Liu, H., W. Jiang, Y. Bi and Y. Luo, 2005. Postharvest BTH treatment induces resistance of peach (*Prunus persica* L. cv. Jiubao) fruit to infection by *Penicillium expansum* and enhances activity of fruit defense mechanisms. Postharvest Biology and Technology, 35: 263–269.

Maizura, M., A. Aminah and W.M. Aida, 2011.Total phenolic content and antioxidant activity of kesum (*Polygonum minus*), ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) extract. International Food Research Journal, 18: 526-531.

Meloni, D.A., M.A. Oliva, C.A. Martinez, and J. Cambraia, 2003. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ. Exp. Bot., 49: 69-76. DOI: 10.1016/S0098-8472(02)00058-8

Moore, T. J., R. C. Hartwig and R. H. Loeppert, 1990. Steady-state procedure for determining the effective particle-size distribution of soil carbonates. Soil Sci. Soc. Am. J., 54: 55–59.

Najjaa, H., A. Boubakri, A.B. and Arfa, *et al*., 2018. Salinity and drought stresses improve antioxidant potential of *Allium roseum* L., an edible medicinal plant. Acta Physiologiae Plantarum, 40 (11): 195.

Negro, C., L. Tommasi and A. Miceli, 2003. Phenolic compounds and antioxidant activity from red grape marc extracts. Bioresour. Technol., 87: 41-44. DOI: 10.1016/s0960-8524(02)00202-x

Nijveldt, R.J., E.van Nood, D.E.C. van Hoorn, P.G. Boelens, K. van Norren and P.A.M. van Leeuwen, 2001.Flavonoids: a review of probable mechanisms of action and potential applications. American Society for Nutrition, 74: 418–425.

Ordonez, A., J. Gomez and M. Vattuone, 2006. Antioxidant activities of Sechiumedule (Jacq) Swart extracts. Food Chem., 97: 452-458. DOI: 10.1016/j.foodchem.2005.05.024

Page, A.L., 1987. Methods of soil analysis, part2.nchemical and microbiological properties agronomy monograph. No. 9. American Society of Agronomy Inc., Madison., 167-179.

Pal, A. and R.S. Pawar, 2011. A study on *Ajuga bracteosa* wall ex. Benth for analgesic activity. Int. J. Cur. Bio. Med. Sci., 12–14.

Potapovich, A.I. and V.A. Kostyuk, 2003.Comparative study of antioxidant properties and cytoprotective activity of flavonoids. Biochemistry, 68: 514–519.

Quartacci, M.F., C.L.M. Sgherri, C. Pinzino and F. Navari-Izzo, 1994. Superoxide radical production.n in wheat plants differently sensitive to drought. Proc. Rog. Soc. (Edinbrugh), 102: 287- 290. DOI: 10.1017/S0269727000014251

Ramakrishna, A. and G.A. Ravishankar, 2011. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal. Behav., 6: 1720–1731. DOI: 10.4161/psb.6.11.17613

Reddy, A.R., Chaitanya, K.V., Vivekanandan, M., 2004. Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. J Plant Physiol., 161: 1189 –1202. DOI**:** 10.12691/wjar-4-5-5

Richardson, A.D., S. P. Duigan and G.P. Berlyn, 2002. An evaluation of noninvasive methods to estimate foliar chlorophyll content. New Phytologist., 153: 185-194.

Rowell, D.L., 1994. Soil science: methods and application. Longman Publishers, Singapore, 350.

Sairam, R.K., P.S. Deshmukh, D.S. Shukla, 1997. Tolerance of Drought and Temperature Stress in Relation to Increased Antioxidant Enzyme Activity in Wheat, J. Agron. Crop Sci. 178: 171–178.

Sezai, E., O. Emine, O. Ozlem, S. Memnune and N. Gungor, 2008. Seasonal variation of total phenolic, antioxidant activity, plant nutritional elements, and fatty acids in tea leaves (*Camellia sinensis* var. *sinensis* clone derepazari 7) grown in Turkey, Pharmaceutical Biology, 46 (10-11): 683-687. DOI:10.1080/13880200802215818

Shulaev, V., D. Cortes, G. Miller and R. Mittler, 2008. Metabolomics for plant stress response. Physiol. Plant. 132, 199–208. DOI:10.1111/j.1399-3054.2007.01025.x

Singleton, V.L., 1998.Wine polyphenols. In: Linskens, H.F., Jackson, J.F. (Eds.), Modern Methods of Plant Analysis. New Series, vol. 6. Springer-Verlag, Berlin, 173–219.

Sokół-Łętowska A., 1997. Próby opracowania i zastosowania preparatów związków fenolowych wybranych surowców roślinnych jako przeciwutleniaczy. Zeszyty Naukowe Akademii Rolniczej we Wrocławiu. Technologia Żywności XI, 319: 99-115.

Srichaikul, B., R. Bunsang, S. Samappito, S. Butkhup and G. Bakker, 2011. Comparative study of chlorophyll content in leaves of Thai Morus alba Linn. Species. Plant Science Research, 3: 17-20.

Srinivas, N.D., K.R. Rashmi and K.S. Raghavarao, 1999. Extraction and purification of a plant peroxidase by aqueous two-phase extraction coupled with gel filtration. Process Biochem, 43-8.

Sumanta, N., C.I. Haque , J. Nishika and R. Suprakash, 2014. Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents. Research Journal of Chemical Sciences, 4(9): 63–69.

Tabart, J., C. Kevers , J. Pincemail, J.O. Defraigne and J. Dommes, 2006. Antioxidant capacity of black currant varies with organ, season, and cultivar. J. Agr. Food Chem., 54:6271–6276. DOI: 10.1021/jf061112y

Tabart, J., C. Kevers, A. Sipel, J. Pincemail, J.O. Defraigne, and J. Dommes, 2007. Optimisation of extraction of phenolics and antioxidants from black currant leaves and buds and of stability during storage. Food Chem., 105: 1268–1275.

Tackholm, V., 1974. Student Flora of Egypt. 2nd Ed. Cairo University Cooperative printing Company, Beirut.

Taylor, R., 1990. Interpretation of the correlation coefficient: a basic review. The Journal of Defense Modeling and Simulation, 1: 35–39.

Yalcin, F.N. and D. Kaya, 2006. Ethnobotany, Pharmacology and Phytochemistry of the Genus *Lamium* (Lamiaceae), FABAD J Pharm. Sci., 31: 43-52.