

## The Environmental Fluctuations of some Bioactive Nutraceutical Compounds in *Zilla spinosa* Inhabiting Arid Habitats

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### Abstract

*Zilla spinosa* is one of the dominated woody perennial shrubs widespread in the Egyptian Red sea coastal desert, belonging to family Brassicaceae. *Z. spinosa* is used as a folk medicine and for heating by local people. *Z. spinosa* inhabits arid habitats exposed to adverse climatic changes which influence the production of the bioactive natural products. The natural secondary products have significant importance for plant acclimatization to the arid habitats beside their significant practical application in medicinal, nutritive and industrial purposes. The accumulation levels of some natural products including phenols, tannins, glucosinolates, flavonoids, saponins, proanthocyanidins and cardiac glycosides were measured in *Z. spinosa* inhabiting different locations of Wadi Hagul during spring and summer seasons. The results of the current study showed that *Z. spinosa* grown in the adverse environment has adapted to cope with extreme temperature, water deficit and geoclimate changes especially in summer, by enhancing the accumulation of some antioxidant compounds including phenols, tannins, glucosinolates, flavonoids, saponins, cardiac glycosides, concomitant with increments in the total antioxidant capacity and PAL activity. Consequently, *Z. spinosa* shrubs inhabiting the arid environment is a promising new source of saponins, glucosinolates, cardiac glycosides, phenols and flavonoids which could participate in drug development and exploration of alternative strategies to increase productivity of wild plants.

**Keywords:** arid habitats, natural products, seasonal climatic changes, *Zilla spinosa*

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### Introduction

Wadis are the most prevalent ecosystems in the world mountainous deserts (Fossati *et al.*, 1999). The wadi system such as wadi Hagul comprises various habitats (Abdel Rahman and Batanouny, 1965). Vegetation in the wadi is frequently subjected to adverse seasonal climatic changes that negatively affected their setting up, growth and development, as well as canopy level. Water supply is the most prominent factor that can influence plant metabolism and anabolism (Akinci, 1997). Generally, water deficit stimulates the production of reactive oxygen species (ROS) in plants, which causes damage in membrane lipids, nucleic acids, proteins, pigments (Noctor *et al.*, 2014), and consequently disturb the normal metabolic activities of plants.

Plants cope with the environmental adverse effects by modulating the production and accumulation of a number

of secondary metabolites. Environmental stressors increased secondary metabolites production and accumulation on the expense of primary metabolites and growth. Therefore, alterations in the local geoclimate and seasonal changes including light, temperature and humidity affect the composition of secondary metabolites (Ramakrishna and Ravishankar, 2011). Secondary metabolites including phenolic compounds, saponins, alkaloids and other nitrogenous compounds are sometimes participating in plant defense strategies against various stresses (Ramakrishna and Ravishankar, 2011). Wahid and Close (2007) reported that phenolics significantly contributed in abiotic stress tolerance as they serve as antioxidants (Balasundram *et al.*, 2006). The exposure to either cold or heat stresses promote overproduction of phenolic compounds in wheat plants (Khattab *et al.*, 2012). Similarly, drought stress stimulated the production and accumulation of total phenols and flavonoids in different plant species (Emam *et al.* 2014; Al Hassan *et al.*, 2015). The quantitative and qualitative variations in flavonoid content of

*Flavoparmelia caperata* (L.) Hale and *Physcia dubia* (Hoffm.) Lettau plants were also recognized depending on growth stage, degree of senescence, season and geographical location (Chaves *et al.*, 2003).

In addition, the production and accumulation of saponins in plants is affected by the environmental conditions including light, temperature, humidity, soil fertility, local geoclimate and seasonal changes, as well as cultivation techniques. A positive correlation between saponins concentrations and abiotic stresses was observed (De Costa *et al.*, 2013). It was indicated that the increment in the synthesis of saponins in response to stresses, might be an indicator for their involvement in plant tolerance under sever stressful conditions (Lin *et al.*, 2009; Szakiel *et al.*, 2011; Mahmood *et al.*, 2014).

Furthermore, glucosinolates are secondary metabolites which occurred in family Brassicaceae (Fahey *et al.*, 2001; Wittstock and Halkier, 2002). The accumulation of glucosinolates is regulated by plant development and tissue type (Reichert *et al.*, 2002; Brown *et al.*, 2003) as environmental stresses (Bidart-Bouzat *et al.*, 2005; Kim and Jander, 2007; Wentzell and Kliebenstein, 2008). The induction of glucosinolates accumulation by drought conditions has been reported by Schreiner *et al.* (2009). The glucosinolate content of different plant species generally increased under drought and temperature stress conditions (Radovich *et al.*, 2005; Schreiner *et al.* 2009; Justen and Fritz, 2013; Charron *et al.*, 2005).

The synthesis and accumulation of secondary metabolites in plants is influenced by environmental conditions, such as light intensity, soil minerals, drought, salinity and seasonality (Waterman and Mole, 1989). Hydric stress usually induced the accumulation of various secondary metabolites including glycosides (Gouvea *et al.*, 2012). Moreover, Sahin *et al.* (2013) reported that cardenolide production significantly increased in *Digitalis* plants exposed to some nutrient deficiency.

The seasonal fluctuations in the natural secondary metabolites in *Zilla* plants inhabiting the arid environment are not clear. Thus, the present study aimed to elucidate the suitable environment for greater natural products accumulation. Simultaneously, it was of interest to interpret the ecophysiological roles of natural secondary metabolites in this distinct desert plant adaptive strategy. Moreover, the natural variation in the levels of secondary metabolites may explore the ecological-plant interactions with their environment and may help in providing alternative strategies to increase productivity of wild plants utilized in drug and food industries.

## Materials and Methods

### Study area

Wadi Hagul is an extensive wadi occupying the valley depression between Gebel Ataqa to the North and Gebel Kahaliya to the South, Egypt. Its main channel extends for about 35 Km and collects drainage water on both sides. With reference to the vegetation and geological features of wadi Hagul, three main sectors may be distinguished, upstream, midstream and downstream (Fig. 1). Moreover,

the climate of the wadi area has been described as arid to extremely arid (Zahran, 2010).

### Climate of the study area

Climatic means of Suez metrological station were studied; the nearest station to wadi Hagul had been measured during the period between 2010-2014 to throw light on the climatic conditions prevailing in the surveyed wadi (Table 1). The climatic condition of the study areas was of the arid type, with mild winter temperatures. January was the coldest month, while July and August were the hottest ones. The relatively highest amount of annual rainfall was recorded during April, whereas the highest relative humidity value was recorded during December. Moreover, the maximum wind velocity was measured during October.

### Plant material

*Z. spinosa* shrubs with similar age and size were collected from three main sectors (upstream, midstream and downstream) of wadi Hagul from spring (April) til summer (July) seasons, depending on the vegetative organ under study.

### Extraction and determination of secondary metabolites

#### Extraction

Aerial parts and the root system of *Z. spinosa* were collected from wadi Hagul, in April (the flowering stage) and June (fruiting stage), after drying under shade for 15 days, were ground to a powder. The dried powder (100 g) was extracted with distilled water or ethyl alcohol, methyl alcohol, petroleum ether, ethyl acetate at 4 °C. After 72 hr, the extracts were filtered and the filtrates were concentrated on rotary evaporator under reduced pressure at 30 °C. Then, the crude concentrated extracts were completed to final volume with water (30 ml) and then subjected to phytochemical analysis (Harborne, 1973). The qualitative phytochemical analysis was carried out to determine the suitable solvent for the maximum quantitative estimation of secondary metabolites.

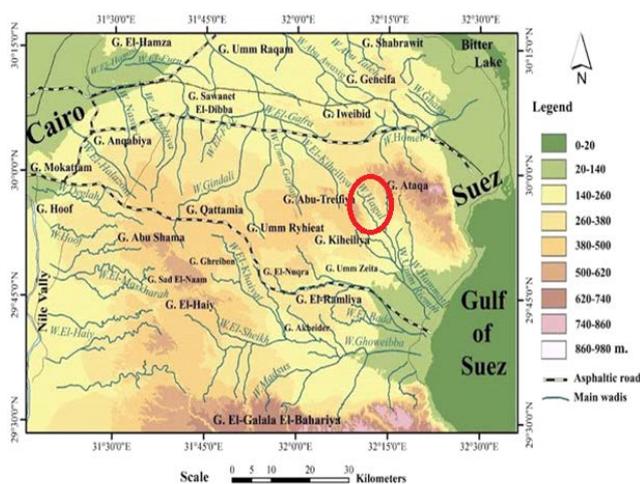


Fig. 1. Map of Cairo-Suez road showing the locations of the studied area of wadi Hagul

Table 1. The meteorological data (climatic condition) of wadi Hagul during 2010-2014

Wind velocity Km/h	Months	Relative humidity%	Rain fall mm/month	Mean minimum temp (°C)	Mean maximum temp (°C)
7.02	January	59	3.4	10	20
7.65	February	58	3.7	11	21
7.46	March	54	3.2	13	25
8.36	April	47	17.7	16	28
7.46	May	45	0	19	32
8.81	June	48	0	22	36
7.89	July	52	0.5	24	38
7.78	August	54	0	24	38
8.3	September	56	0	22	34
8.56	October	58	0.2	19	30
6.8	November	60	1.7	15	26
6.21	December	62	3.9	11	22

### Phytochemical screening

Phytochemical screening was carried out to test the presence of tannins, flavonoids, terpenoids, saponins, quinones and anthroquinones in all plant extracts following standard protocol of Makkar (1993).

### Quantitative estimation of secondary metabolites

#### Measurement of total phenolics and tannins

The total phenolics and tannins were measured by using Folin-Ciocalteu method, based on the method described by Makkar *et al.* (1993).

#### Measurement of condensed tannins (Proanthocyanidins)

The total proanthocyanidins were determined by using vanillin reagent as described by Price *et al.* (1978). The absorbance was measured at 500 nm by a Shimadzu UV-265 spectrophotometer (Japan).

#### Extraction and estimation of total flavonoids content

The aluminium chloride colorimetric method described by Harborne (1998) was used for determination of total flavonoids in the extracts. The total flavonoid contents were calculated as  $\mu\text{g/g}$  fresh weight from standard curve of quercetin.

#### Determination of saponins

The total saponins was calculated from the diosgenin standard solution by using vanillin reagent according to the method of Francis *et al.* (2002).

#### Determination of glucosinolate

Glucosinolate was determined by the spectrophotometric method described by Makkar *et al.* (1993).

#### Determination of cardiac glycosides

Cardiac glycoside content in the sample was evaluated using Buljet's reagent as described by El-Olemy (1994).

#### Assay of phenylalanine ammonia-lyase activity (PAL) EC 4.3.1.5

The activity of PAL was assayed as described by He *et al.* (2001). The reaction mixture consisted of 100  $\mu\text{L}$  crude enzyme extract and 900  $\mu\text{L}$  of 6  $\mu\text{mol}$  phenylalanine in 500

mM TrisHCl buffer (pH 8.5). The mixture was incubated at 37 °C for 1 hr and measured spectrophotometrically at 290 nm using UV-spectrophotometer (Spectronic 601, Milton Roy Company). PAL activity was expressed as mM of trans-cinnamic acid/g fresh weight/min.

#### Total antioxidant capacity

The total antioxidant capacity of the extracts was evaluated by the phosphomolybdenum method according to the procedure described by Prieto *et al.* (1999).

#### Statistical analysis

Statistical analysis was done by determining the variance and the means separated by Duncans multiple range test ( $p > 0.05$ ) as described by Snedecor and Cochran (1980). The software SAS (SAS Institute, Cary, N.C.) were used for analysis.

## Results

### Characteristics of wadi Hagul habitats

The restricted amounts of precipitation, associated with extremely high summer temperatures and high evaporation rates, create a harsh environment for vegetation in arid area, particularly at the Wadi's edges within the study area. The high percentage of soil moisture content was detected during the wet period of April, which was related to the fall of rain season; however, it was significantly reduced in the dry season (August). The intensification of soil water deficit in wadi Hagul during summer, particularly at the area edges (Khattab *et al.*, 2014), was concomitant with low precipitation and high temperature (Table 2).

### Seasonal qualitative and quantitative analysis of phytochemicals of *Zilla spinosa* parts inhabiting wadi Hagoul

Seasonal variation pronounced in different qualitative phytochemical screening of crude aqueous extracts of *Z. spinosa* parts was done to detect the presence of secondary metabolites. The extracts of *Zilla* aerial portions and roots revealed the presence of phenols, flavonoids, triterpenoids, steroids, alkaloids, saponins, coumarins, glucosinolate, proanthocyanidines and cardiac glycosides. The best and more relevant observed results are assayed in the aqueous extracts, particularly during the dry season, which were

Table 2. Soil reaction, electrical conductivity and chemical analysis of soil samples from wadi Hagol, during summer (sum) and spring (spr)

Location	% of soil water content		Soluble cations meq								CaCO <sub>3</sub> %	
	Spr	Sum	Mg <sup>2+</sup>		Na <sup>+</sup>		Ca <sup>2+</sup>		K <sup>+</sup>		Spr	Sum
			Spr	Sum	Spr	Sum	Spr	Sum	Spr	Sum		
Upstream	1.45	0.75	0.86	2.45	2.80	8.10	2.06	2.94	0.214	0.226	7.9	15.5
			0.53	1.56	1.10	6.33	2.42	3.12	0.100	0.133	5.7	8.10
Midstream	2.90	2.75	1.98	4.16	5.43	12.86	5.40	6.24	0.214	0.346	17.3	19.3
			0.12	0.56	0.37	0.95	0.296	0.777	0.100	0.171	13.1	14.9
Down stream	0.90	0.36	0.26	0.60	0.37	0.96	0.41	0.81	0.143	0.154	40.4	41.7
			0.73	0.21	0.10	0.68	0.15	0.295	0.100	0.105	48.7	51.2

Table 3. Preliminary phytochemical screening of *Zilla spinosa* plants grown in different habitats of wadi Hagoul during spring and summer season

Solvent	Compound	Spring		Summer	
		Root	Shoot	Root	Shoot
Water	Phenolic	++ ve	++ ve	++ ve	+++ ve
	Flavonoids	+ ve	++ ve	+ ve	++ ve
	Coumarins	+ ve	+ ve	+ ve	++ ve
	Triterpenoid	+ ve	++ ve	+ ve	++ ve
	Alkaloids	+ ve	++ ve	+ ve	++ ve
	Steroids	+ ve	++ ve	+ ve	++ ve
	Tannins	+ ve	+ ve	+ ve	+ ve
	Saponins	++ ve	++ ve	+++ ve	++ ve
	Cardiac glycosides	++ ve	+++ ve	++ ve	+++ ve
	Glucosinolate	++ ve	++ ve	++ ve	+++ ve
Acid	+ve	+ ve	+ ve	+ ve	
Ethyl alcohol	Phenolic	+ ve	++ ve	+ ve	++ ve
	Flavonoids	+ ve	++ ve	+ ve	++ ve
	Coumarins	+ ve	+ ve	+ ve	++ ve
	Triterpenoid	+ ve	+ ve	+ ve	+ ve
	Alkaloids	- ve	- ve	- ve	- ve
	Steroids	+ ve	+ ve	+ ve	+ ve
	Tannins	- ve	- ve	- ve	- ve
	Saponins	+ ve	+ ve	+ ve	+ ve
	Cardiac glycosides	+ ve	++ ve	+ ve	++ ve
	Glucosinolate	+ ve	++ ve	+ ve	++ ve
Acid	+ ve	+ ve	+ ve	+ ve	
Methyl alcohol	Phenolic	++ ve	++ ve	++ ve	+++ ve
	Flavonoids	+ ve	++ ve	+ ve	++ ve
	Coumarins	+ ve	+ ve	+ ve	+++ ve
	Triterpenoid	+ ve	+ ve	+ ve	+ ve
	Alkaloids	- ve	- ve	- ve	- ve
	Steroids	+ ve	+ ve	+ ve	+ ve
	Tannins	- ve	- ve	- ve	- ve
	Saponins	+ ve	+ ve	+ ve	+ ve
	Cardiac glycosides	+ ve	+ ve	+ ve	+ ve
	Glucosinolate	++ ve	++ ve	++ ve	++ ve
Acid	+ve	+ ve	+ ve	+ ve	
Petroleum ether	Phenolic	+ ve	++ ve	+ ve	+ ve
	Flavonoids	+ ve	++ ve	+ ve	+ ve
	Coumarins	- ve	- ve	- ve	- ve
	Triterpenoid	+ ve	++ ve	+ ve	++ ve
	Alkaloids	- ve	- ve	- ve	- ve
	Steroids	+ ve	e	+ ve	+ ve
	Tannins	- ve	- ve	- ve	- ve
	Saponins	+ ve	+ ve	+ ve	+ ve
	Cardiac glycosides	+ ve	+ ve	+ ve	+ ve
	Glucosinolate	+ ve	+ ve	+ ve	+ ve
Acid	+ ve	+ ve	+ ve	+ ve	

-ve = Negative results; +ve = Positive results; ++ve = Moderately positive results; +++ve = strongly positive results

thereafter used in the quantification of the secondary metabolites (Table 3). The greater qualitative secondary metabolites contents were detected in *Z. spinosa* aerial portions as compared with their roots. Therefore, the secondary metabolites levels were measured in the aerial portions along wadi Hagul habitats.

*Quantitative analysis of secondary metabolites*

The important secondary metabolites such as phenolics, flavonoids, saponins, tannins, proanthocyanidines and glucosinolates were quantified by using spectrophotometric assay.

*Seasonal and spatial variation in total phenols*

*Z. spinosa* aerial portions accumulated higher amount of total phenols in dry summer season compared to those harvested in spring (Table 4). The greatest levels of phenols accumulation were observed in *Zilla* aerial portions inhabiting the edges of the wadi, particularly at the downstream locations, during the dry season.

*Seasonal and spatial variation in flavonoids*

The quantitative estimation of the seasonal and spatial changes in the total flavonoids illustrated that *Zilla* aerial portions exhibited greater levels of flavonoids during summer seasons (Table 4). The maximum levels of flavonoids were measured in *Zilla* aerial portions inhabiting upstream and downstream wadi Hagul during summer season.

*Seasonal and spatial variation in tannins*

*Z. spinosa* aerial portions attained higher tannins levels in the dry season, particularly at the edges of the wadi (Table 4). The aerial portions of *Zilla* plants exhibited greater amounts of tannins during summer seasons. In addition, the maximum value of tannins level was attained in

downstream inhabiting *Zilla* aerial portions during the summer season.

*Seasonal and spatial variation in proanthocyanidines (condensed tannins)*

The amount of pro-anthocyanidines was significantly increased in *Zilla* aerial portions harvested in the dry season compared to those of spring. The maximum increase in the accumulation of pro-anthocyanidines was measured in *Zilla* aerial portions inhabiting the edges of wadi Hagul during dry periods (Table 4). However, the increment in the total pro-anthocyanidines was more pronounced in *Zilla* inhabiting the downstream during summer season.

*Seasonal and spatial changes in saponins*

Saponins are the most common secondary metabolites accumulated in *Zilla* aerial portions (Table 5). It was noticed that the highest amount of saponins was observed in *Zilla* aerial portions inhabiting the downstream location followed by the first location during the dry season compared with this of the second stand.

*Seasonal and spatial changes of glucosinolates*

The total glucosinolates content increased significantly in summer harvested *Zilla* aerial portions particularly at the edges of wadi Hagul (Table 5). The maximum amount of glucosinolates was achieved in *Zilla* aerial portions located downstream the wadi (6318.42 µg/g dw).

*Seasonal and spatial variation in cardiac glycosides*

Data presented in Table 5 clearly indicated that the accumulation of total cardiac glycosides was greater in *Zilla* aerial portions during summer season. The maximum increase in cardiac glycosides was attained in *Zilla* aerial portions grown at the edges of wadi Hagul specially at the third location, in summer.

Table 4. Seasonal changes in total phenols, flavonoids, total tannins and proanthocyanidines (µg/g dw) contents of *Zilla spinosa* aerial portions grown in different habitats of wadi Hagul

Season	Location	Parameter (µg g <sup>-1</sup> )			
		Total phenols	Flavonoids	Tannins	Pro-anthocyanidines
Spring	Upstream	3623.74±75.63 <sup>c</sup>	3386.10±174.62 <sup>c</sup>	211.77±5.55 <sup>d</sup>	242.65±1.63 <sup>bc</sup>
	Midstream	2147.47±67.76 <sup>d</sup>	876.67±133.67 <sup>c</sup>	176.51±14.22 <sup>c</sup>	558.82±1.63 <sup>a</sup>
	Downstream	2723.23±36.5 <sup>cd</sup>	2634.17±178.47 <sup>d</sup>	224.02±4.66 <sup>d</sup>	248.37±1.25 <sup>bc</sup>
Summer	Upstream	5622.90±11.80 <sup>b</sup>	5874.98±38.8 <sup>b</sup>	295.20±4.115 <sup>b</sup>	214.22±4.71 <sup>c</sup>
	Midstream	4292.93±77.2 <sup>c</sup>	1754.12±32.1 <sup>de</sup>	248.00±12.31 <sup>c</sup>	356.21±2.87 <sup>b</sup>
	Downstream	83 <sup>8</sup> 7.21±89.2 <sup>a</sup>	6318.42±46.2 <sup>a</sup>	520.95±6.44 <sup>a</sup>	177.94±1.47 <sup>c</sup>

Each value is a mean of three replicates ± SD

Table 5. Seasonal changes in glucosinalates, cardiac glycosides, saponins , the total antioxidant capacity and PAL activity of *Zilla spinosa* aerial portions grown in different habitats of wadi Hagul

Season	Location	Parameter				
		Saponins (µg g <sup>-1</sup> )	Glucosinolates (µgg <sup>-1</sup> )	% of cardiac glycosides	Total antioxidant capacity (µg g <sup>-1</sup> )	PAL (mM of trans-cinnamic acid/g FW/min)
Spring	Upstream	14762.00±48.01 <sup>a</sup>	5276.87±38.8 <sup>c</sup>	1.180 ±0.022 <sup>a</sup>	0.020±0.0008 <sup>c</sup>	0.70±0.067 <sup>b</sup>
	Midstream	7084.00±29.14 <sup>c</sup>	4819.24±38.7 <sup>cd</sup>	0.38± 0.023 <sup>bc</sup>	0.004±0.0004 <sup>e</sup>	0.41±0.007 <sup>c</sup>
	Downstream	9775.00±38.15 <sup>b</sup>	5877.79±38.8 <sup>b</sup>	0.54±0. 015 <sup>b</sup>	0.010±0.0022 <sup>d</sup>	0.66±0.01 <sup>b</sup>
Summer	Upstream	5016.00±38.15 <sup>c</sup>	5874.99±32.1 <sup>b</sup>	0.58± 0.011 <sup>b</sup>	0.043±0.0008 <sup>a</sup>	0.81±0.007 <sup>b</sup>
	Midstream	3696.00±38.14 <sup>f</sup>	5754.12±38.8 <sup>b</sup>	0.41±0.009 <sup>bc</sup>	0.0274±0.0004 <sup>b</sup>	0.51±0.008 <sup>c</sup>
	Downstream	8844.00±38.10 <sup>b</sup>	6318.42±23.5 <sup>a</sup>	1.26± 0.004 <sup>a</sup>	0.045±0.0.0004 <sup>a</sup>	1.1±0.01 <sup>a</sup>

Each value is a mean of three replicates ± SD

*Seasonal and spatial variation in PAL activity*

In addition, the increments in PAL activity was more pronounced in aerial portions inhabiting the edges of wadi Hagul particularly at the third downstream stand during summer followed by the first location during spring (Table 5).

*Seasonal and spatial variation in the total antioxidant capacity*

The total antioxidant values of aqueous extracts of *Zilla* aerial portions were greater in the three investigated sites during summer season. It was obvious from the obtained data that the greatest magnitude of the total antioxidant value was measured in *Zilla* aerial portions inhabiting the third stand particularly during the dry period (Table 5).

**Discussion**

The mountainous desert is characterized by aggressive climatological condition. The restricted amounts of freshwater, in combination with extremely high summer temperatures and high evaporation rates, creates a harsh habitat for vegetation. Topography affects soil type and thus habitat and weather pattern including fluctuation in seasonal temperature and precipitation (Ruggiero *et al.*, 1994), as well as soil moisture level across arid environment (Walker, 2000). In addition, the spatial pattern plays a central role in plant community dynamics including succession, adaptation, maintenance of species density and competition, as well as biomass (Sofy and Mohamed, 2012). Woody plants exhibit marked seasonal metabolic fluctuations triggered by day length, light intensity, temperature extremes and thereby reduction in soil moisture content and plant water status particularly during the dry season (Larcher, 2003; Ramakrishna and Ravishankar, 2011). The synthesis and accumulation of secondary metabolites including phenols, flavonoids, saponins, tannins, glucosinolate and cardiac glycosids fluctuated seasonally in the arid environment inhabiting plants.

The preliminary qualitative secondary metabolites screening of the crude extracts of *Zilla* was done to assess the presence of bioactive components. The stem and root extracts of *Z. spinosa* have revealed the presence of phenols, flavonoids, saponins, steroids, glucosinolate, cardiac glycosides, tannins and alkaloids, detected generally, in all used solvents. However, in the present investigation all the screened secondary metabolites were found to be better extracted by water. Seasonal variation was pronounced in different secondary metabolites content of *Zilla* inhabiting wadi Hagul. The environmental stressors pushed all metabolic processes towards the synthesis of secondary metabolites as isoprenoids, phenols, flavonoids or alkaloids due to the closure of stomata and consequently reduction in the endogenous CO<sub>2</sub> level and the consumption of reduction equivalents (NADPH<sup>+</sup>H<sup>+</sup>) utilized in the CO<sub>2</sub>-fixation via Calvin cycle (Ramakrishna and Ravishankar, 2011; Al-Gabbiesh *et al.*, 2015).

In addition, the quantitative analysis indicated that phenols accumulated in relatively large amounts in *Zilla spinosa* aerial portions inhabiting the edges of wadi Hagul particularly during the dry season. This can be possibly be

related to the depletion of soil moisture content at the edges of wadi Hagul and the unfavorable environmental conditions (high temperature and strong light radiation). The accumulation of phenolics in stressed plants may be due to the activation of their biosynthesis and/or inhibition of their oxidation, which could be an acclimation mechanism of the plant against stress (Kefeli *et al.*, 2003). Likewise, water stress can stimulate the accumulation of phenolic compounds in desert plants in summer season by hydrolyzing the glycosides (Gehlot *et al.*, 2011). Moreover, the spatial variations in phenols accumulation were parallel to the changes in PAL activity in *Zilla* aerial portions inhabiting different locations along wadi Hagul during spring and summer seasons. Arbona *et al.* (2013) reported that heat induced PAL activity and the production of phenolic and at the same time reduced their oxidation, contributing to heat stress acclimation. Polyphenol synthesis and accumulation is generally stimulated in response to biotic or abiotic stresses (Muthukumarasamy *et al.*, 2000; Rajabbeigi *et al.*, 2013; Patel and Patel, 2014). Phenolic compounds confer ROS scavenging ability (Pokorny *et al.*, 2001; Saeed *et al.*, 2012) and enhanced plant tolerance under harsh environmental conditions. Likewise, the longer exposure to stronger sunlight stimulated phenols biosynthesis (Sezai *et al.*, 2008). Furthermore, deficiencies in nutrients in wadi Hagul habitats, particularly during summer, led to the accumulation of phenol precursor, phenyl propanoids and lignification (Dixon and Paiva, 1995) and thus induced plant acclimation against stress (Arbona *et al.*, 2013).

In addition, flavonoids are one of the main phenolic compounds of plants which have potent antioxidant activities (Bravo, 199; Nunes *et al.*, 2012) and thus, they are involved in the protection of plants against stresses (Winkel-Shirley, 2001). Summer harvested *Z. spinosa* aerial portions attained greater amount of flavonoids particularly at the third location of Wadi Hagul (downstream). The increments in the total flavonoids during the dry hot season particularly at third site may be associated to the extent of environmental stressful conditions imposed by high temperature, low soil humidity and strong solar radiation. The climatic and ecological variations, such as the duration and intensity of sunlight, have significant effect on the nature and quality of secondary plant metabolites (Sezai *et al.*, 2008). The total flavonoids and phenolic contents increased in *Melilotus indicus* and *Lactuca sativa* L. exposed to abiotic stresses (Ahmed *et al.*, 2012; Rajabbeigi, *et al.*, 2013). Flavonoids are efficient antioxidant (Tahara, 2007) and able to neutralize the reactive oxygen species under stress conditions (Gould, 2004), so nullify the stress injuries of *Z. spinosa* subjected to temporal and spatial variations. *Tecomella undulate* accumulated greater amount of flavonoids during summer season (Patel and Patel, 2014). Flavonoids are involved in the resistance of plant and also provide a number of functions in the ecology of plants.

Saponins, like flavonoids, tannins and terpenes, are defensive secondary metabolites that allow plants to cope with the environmental conditions (storing and conserving water, resisting predators and surviving severe weather conditions (Harlev *et al.*, 2012). Saponin is one of the most common compounds measured in *Z. spinosa* plants. The

greatest saponins level was measured in *Zilla* aerial portions collected during the dry period especially at the third location. The seasonal and spatial variations in saponins in *Zilla* may be due to the differential synthesis and/or accumulation of saponins and their aglycones, depending upon species or genotype, age and environmental conditions (Inderjit and Foy, 1999). The greatest accumulation of saponins in *Zilla* parts particularly at the third location was positively related to the depletion in soil moisture content (Khattab et al., 2014). This observation agreed with the results reported previously, that drought and salinity stimulated the production of total saponins in several plant species (De Costa et al., 2013). The increase in saponins levels could be related to its protective role against oxidative stress (Lin et al., 2009). Many desert plants contain substantial amounts of steroids and triterpene saponins which make them survive severe weather conditions (Ramakrishna and Ravishanker, 2011).

Glucosinolates are nitrogen and sulphur containing compounds derived from amino acids. The quantitative distribution of glucosinolates in the aerial portions was greater during the hot dry summer. *Z. spinosa* inhabiting the third location exhibited the maximum levels of glucosinolates during the dry period. The increments in glucosinolates were concomitant with the reduction in amino acids content (precursors of glucosinolates, unpublished results). Glucosinolates significantly increased in drought stressed *Brassica oleracea* and *Brassica carinata* (Radovich et al., 2005; Schreiner et al., 2009). The actual function of the accumulation of glucosinolates on a biotic stress tolerance is not known yet (Arbona et al., 2013).

Cardiac glycoside is one of the secondary metabolites which can play a role in the adaptation of plants to their environment (Sahin et al., 2013). The highest production of cardiac glycosides was recorded in *Zilla* aerial portions inhabiting wadi Hagul edges. The levels of cardiac glycosides accumulation were negatively related to the total soluble sugars levels in *Zilla* grown at the three locations along wadi Hagul (unpublished results). The increments in the cardiac glycosides might be related to the reduction in the rate of plant tissues growth which concomitant with the depletion in nutrient and soil moisture content. The depletion in cardiac glycosides in *Zilla* grown midstream might be attributed to the increase in soil moisture and nutrients, which participate in fast growth rate. Such results are similar to those reported by Sahin et al. (2013) who noted that deficiencies in Ca and Mg results in the greater accumulation of cardenolides in *Digitalis* species. The synthesis and accumulation of secondary metabolites such as cardiac glycosides is affected by abiotic environmental factors such as light intensity, soil minerals, osmotic stresses (drought and salinity) and seasonality (Waterman and Mole, 1989).

The activation of PAL enzyme is a common response of plant cells to biotic and abiotic stresses and may also function as antioxidants and play a major role in secondary metabolites biosynthesis (Haslam, 1998). PAL activity was markedly increased in summer harvested *Zilla* aerial portions particularly at downstream. The increments in PAL activity were concomitant with increments in total phenols and flavonoids. Similar results have been reached by Sung et al. (2005), Ashraf (2011) and Rajabbeigi et al. (2013). PAL plays a key role in linking primary metabolism

to secondary metabolism, particularly of phenylpropanoid metabolism (Zon et al., 2002).

The seasonal and spatial changes in the activity and total levels of antioxidants in *Zilla* aerial portions can be estimated. The greatest reducing antioxidant power measured in the third location harvested *Zilla* aerial portions during the dry period was concomitant with the increments in the magnitudes of the antioxidants such as phenols, flavonoids and tannins which may suggest the involvement of antioxidants in the main strategy of adaptation of *Zilla* to arid environment. Similar results have been reached by Ahmed et al. (2012) who observed increments in the total antioxidant capacity of *Melilotus indicus* extract harvested during the hot dry season, which might be due to the greater accumulation of antioxidants including phenols and flavonoids. Similarly, phenolic acids and flavonoids were the major contributors for the increments in the total antioxidant activity in *Torilis leptophylla* (Saeed et al., 2012). Phenols and flavonoids have been shown to be highly effective scavengers of most oxidizing molecules (Nunes et al., 2012).

## Conclusions

The greatest accumulation in natural bioactive secondary metabolites which serve as antioxidants was attained in *Z. spinosa* experienced dry hot summer environment. Consequently, *Z. spinosa* is a promising new source of nutraceutical molecules which can be utilized in food and drug industries.

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