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Diversity of *Varthemia candicans* phytochemicals in response to growth habitat

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Abstract

Aerial parts of *Varthemia candicans* were collected seasonally for one year (winter, spring, summer and autumn) from Wadi Habbes (rocky habitat) and Sand Dunes habitat, West Marsa Matrouh, Egypt. The results of the plant photochemical analysis cleared out that the amount of soluble carbohydrates in the study habitats during the wet seasons (winter and autumn) was higher than that of the dry season, however the content of insoluble carbohydrates recorded a reverse trend. HPLC analysis of free sugars detected the occurrence of glucoronic acid, raffinose, glucose, galactose, fructose and fucose. The most abundant free sugar in WH was glucoronic acid but in SD was raffinose. Also, HPLC analysis of combined sugars detected the presence of glucose, mannose, fructose and maltose with the commonness of maltose in the two study habitats. The amount of soluble amino acids and soluble proteins were greater in SD habitat than WH habitat during all seasons, except autumn season. Free amino acids in WH habitat revealed the richness of the plant with asparagines, but in SD habitat with proline, but in case of protein amino acids proline was common in WH and aspartic acid was common in SD habitat. The total lipids content was greater in SD habitat than in WH. GC-MS analysis of fatty acids revealed that Hexadecanoic acid methyl ester was common in the plant aerial parts in WH habitat, however the fatty acid 6-Acetyl-8methoxy-2,2-dimethyl-2H-chromen-5-ol was common in the plant aerial parts in SD habitat.

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Introduction

The Western Mediterranean Coastal land of Egypt is relatively rich and diverse and it is one of the richest phyto-geographical regions in Egypt because of its relatively high rainfall, it contains about 50% of the total flora of Egypt (Abbas *et al.*, 2008). Sand dunes along the Western Mediterranean coast of Egypt are formed of loose oval pseudo-oolitic grains of calcium carbonate. These dunes are close to the sea and as a result they are humid, exposed to northerly winds and affected by sea spray. Plants growing in sand dunes are highly adapted and have the ability to grow vertically and tolerate the exposure of their underground organs to sever water stress of calcareous sandy soil. The most important land-use in this area is the grazing (Abbas *et al.*, 2008). The plant species in the Western Mediterranean desert area exhibit different growth and productivity characteristics (Elhaak, 1986).

The genus *Jasonia* (=*Varthemia*) is a member of Asteraceae, is a small genus with about five species mainly distributed in the Mediterranean region (Bremer *et al.*, 1994). In Egypt, the genus *Varthemia* comprises three species (Täckholm, 1974) namely *V. candicans, V. montana* and *V. iphionoides*. El-Kady (1993) reported that *V. candicans* is an aromatic perennial plant and recorded it in rocky places, semi-dry land and sand dunes in Egypt. Ali (2012) reported that *V. candicans* is an unpalatable sub-shrub with a defense strategy depending on the odour generated by the essential oils of the plant. In Egypt, *V. candicans* is mainly distributed in Mareotic Sector, Isthmic Desert, Galala Desert, Libyan Desert, Nubian Desert and Gebel Oweinat (Hepper and Friis, 1994 and Boulos, 2009).

The plant is used in the earlier times in the folk medicine to treat some diseases. Earlier work on the chemistry of *V. candicans* revealed the presence of several sesquiterpenes and sesquiterpene-lactone derivatives (De Pascuai *et al.*, 1980) eudesmanoic acids and eudesmanolides (Ahmed *et al.*, 1994) guaianolides and pseudoguaianolides (Ahmed *et al.*, 1993) together with several polymethoxylated flavonoids and some coumarins (Ahmed *et al.*, 1994). Plant sesquiterpenes are known to show diverse biological and pharmacological actions, including anti-inflammatory activity (Recio *et al.*, 2000). Recently, Ahmed *et al.* (2013) reported that the high content of terpenes, sesquiterpenes and flavonoids in the ethanolic extract of *V. candicans* could be responsible for the anti-cholinesterase activity, anti-inflammatory action, antioxidant capacity and neurotrophic effect as well as anti-amyloidogenic potential of these extracts. This suggests that *V. candicans* ethanolic extract may effectively ameliorate the inflammation and neurodegeneration characterizing Alzheimer's disease in the male rats (Ahmed *et al.*, 2013).

The aim of this study is to investigate the effect of two different habitats of the plant on the chemical composition giving the plant its medicinal importance represented in soluble and insoluble carbohydrates, free and combined sugars, soluble amino acids and proteins, type of amino and fatty acids, lipid profile, phenolics and alkakoids.

Materials and methods

Collection of Plant Material

The fresh aerial parts (shoot system) of *Varthemia candicans* were collected at winter, spring, summer and autumn seasons (2011-2012 season) from the first habitat, Wadi Habbes (master up stream rocky portion of Wadi Habbes habitats) situated about 18 km West of Marsa Matrouh. The second habitat was the Consolidated Oolitic Sand Dunes, situated about 180 km West of Marsa Matrouh (the coastal of Abo Zereba or west of Barany). The plant samples were washed, weighted as fresh, then dried in an oven at 60°C to constant weight, then finally ground to fine powder and stored in paper bags for further analysis.

Total soluble and insoluble carbohydrates content

The total carbohydrates in aerial parts of *Varthemia candicans* were extracted and hydrolyzed by 2M HCl for 2-5 hrs at 100°C. After neutralization, extracted

sugars were estimated using the general phenol-sulfuric acid assay (DuBois *et al*., 1956) and expressed as mg/g d.wt of the plant.

Determination of Free Sugars

Free sugars were extracted with 80% ethyl alcohol from 10g of the plant dried material and filtered. The alcoholic extract was clarified on amberlite IR 120 resin column and then evaporated. The residue was redissolved in 3ml of 10% aqueous isopropanol for chromatographic investigation according to Chaplin and Kennedy (1994).

Determination of Combined Sugars

Combined sugars were extracted by refluxing of the plant dry powder for 3 hours with 6N HCl. The acid was evaporated under vacuum at 45°C and the residue was dissolved in 10% isopropanol for chromatographic investigation as previously mentioned.

HPLC analysis of sugars

Samples (free and combined sugars) were filtered through a 0.45 µm membrane. Analysis of the carbohydrate in the filtrate was performed using HPLC, Shimadzu Class-VPV 5.03 (Kyoto, Japan) equipped with refractive index RID-10A Shimadzu detector, LC-16ADVP binary pump, DCou-14 A degasser and Shodex PL Hi-Plex Pb column (Sc 1011 No. H706081), Guard column Sc-Lc Shodex, and heater set at 80°C. Separation and quantitation were carried out on an amino-bonded column with a mobile phase of CH_3CN and H_2O (80:20 v/v).

Determination of total soluble proteins and free amino acids

The total soluble proteins content of the plant dried samples were estimated quantitatively using the method described by Bradford (1976) and the protein content was calculated as mg/g d.wt. Total free amino acids were assayed by the method described by Lee and Takahashi (1966) and amino acids content was calculated as mg/g d.wt.

Free amino acids and protein amino acids

The investigation of free amino acids and protein-amino acids after the hydrolysis of *V. candicans* were accomplished according to Pellet and Young (1980) by using Amino Acid Analyzer technique (Sykam System 7130 Amino Acid Reagent organizer).

Total lipids content and Saponifiable matter

Total lipids of the plant aerial parts were extracted with petroleum ether (40-60): ether (1:1) using Soxhlet apparatus. The lipids were quantified according to Christie (1982). After the removal of the unsaponifiable fraction with ether, the soapy solution was converted into the corresponding free fatty acids by means of 2.5% sulphuric acid. When the fatty acids were completely liberated they were removed by extracting with ether. Then ether extract was dried over anhydrous Na_2SO_4. The extracted fatty acids were converted to the corresponding methyl esters using ethereal solution of diazomethane (Farag *et al*., 1986). The methyl esters of the fatty acids were analyzed with gas chromatographic apparatus. The fractionation of fatty acid methyl esters was conducted using GC-MS analyzer HP 6890 Series Gas Chromatograph System with an HP 5973 Mass Selective Detector. The column is TR-FAME (Thermo 260 M142 P) (30 m, 0.25 mm ID, 0.25um Film) (70% Cyanopropyl –Polysilphphenylene siloxane) capillary column with injector Temperature 200°C and temperature transfer line 250°C. The carrier gas was He (1.5 ml/min) and the ionization energy was 70eV. The sample inject was 1µl (5 µl/1 ml solvent).

Identification of the different constituents was performed by comparison of the relative retention times and mass spectra with those of authentic reference compound and probability merge search libraries software of NIST11.L, wiley7n.l and Pest l (Adams, 2004).

Statistical Analysis

Statistical analysis of the obtained results was carried out according to the technique adopted by Norusis (2006 and 2007) using SPSS statistical package.

Results and discussion

Total soluble and insoluble carbohydrates

The variation in carbohydrates content in the aerial parts of *V. candicans* in WH and SD habitats during the dry and wet seasons indicated changes in soluble and insoluble carbohydrates with high significant value (P<0.001) with the changes in the study habitats, seasons and the interaction between the habitats and seasons (Table 1). The highest amount of total soluble carbohydrates in WH (215 mg/g d.wt) was in winter and the lowest content (38 mg/g d.wt) was in spring. Total insoluble carbohydrates in this habitat recorded the highest content (352 mg/g d.wt) in summer and the lowest one (130 mg/g d.wt) was in winter. So, in this habitat, the amount of soluble carbohydrates in the wet seasons (winter and autumn) was higher than that of the dry season, however the content of the insoluble carbohydrates recorded a reverse trend.

Table 1. The content of total soluble and insoluble carbohydrates in *V. candicans* aerial parts in the study habitats during the period of investigation.

Season		Habitat					
		Wadi Habbes			Sand Dunes		
		Soluble	Insoluble	Ratio	Soluble	Insoluble	Ratio
Wet seasons	Winter	215 ±13	130±15	1.65	121 ±7	271 ±64	0.44
	Spring	38±6	268±70	0.14	13±4	418±66	0.03
Dry seasons	Summer	89±9	352±78	0.25	139±26	108±45	1.28
	Autumn	173±16	311±33	0.55	152±6	449±17	0.34
	Mean	128.75	265.25	0.65	106.25	311.50	0.52

The highest amount of total soluble carbohydrates in SD (152 mg/g d.wt) was in autumn and the lowest content (13 mg/g d.wt) was in spring. Total insoluble carbohydrates in this habitat recorded the highest content (449 mg/g d.wt) in autumn and the minimum one (108 mg/g d.wt) was in summer. The trend of soluble and insoluble carbohydrates in this habitat is like that of the former habitats. So our results support the hypothesis that the change in growth habitat and growth season has a major effect on the metabolic pathways of the plant tissues.

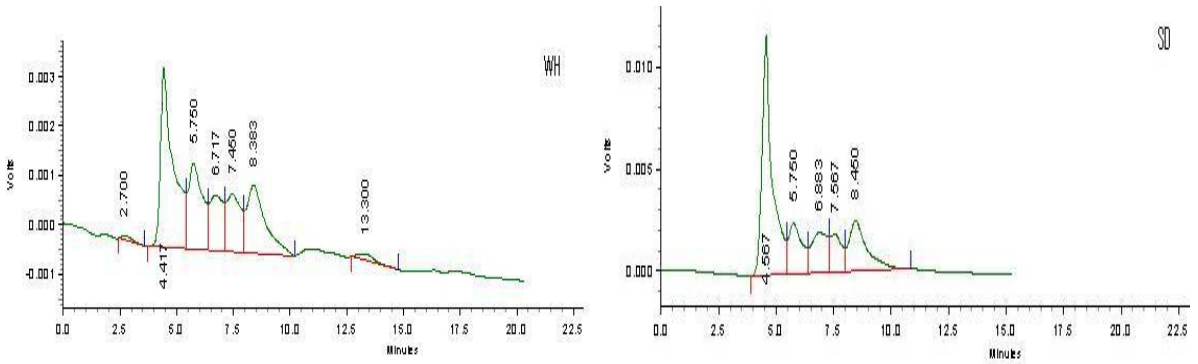
Prado *et al.* (2000) and Gill *et al.* (2001) reported that the accumulation of sugars in plants is enhanced in response to a variety of environmental stresses. Siddique *et al.* (2000) found that the adaptation of plants to water stress has been attributed to the stress-induced increase in carbohydrate levels. Hanson and Hitz (1982) concluded that plant cells and tissues show several metabolic responses to water stress, some of which may have adaptive implication. These responses are usually described at one or another of three levels: (a) perturbation of whole metabolic pathways leading to accumulation or depletion of metabolites, (b) alterations in activities of conveniently assayable enzymes, and (c) changes in the pattern of synthesis of proteins of unknown function. These changes include the accumulation of carbohydrates, organic and amino acids, quaternary ammonium compounds and ABA. The accumulation of osmolytes may ensure the maintenance of the structural integrity of membranes (Conroy *et al.*, 1988). There are some evidences that plants are more tolerant to water shortage when water is withheld under conditions that favour osmotic adjustment (Moinuddin and Chopra, 2004).

Free sugars

Data represented in Table (2) and high performance liquid chromatography **(**HPLC**)** photogram (1) show the difference in free and combined sugars composition in the aerial parts of *V. candicans* growing in WH and SD habitats. The detected free sugars in the two habitats were glucoronic, raffinose, glucose, galactose, fructose and fucose.

Table 2. Free and combined sugars (%) of the aerial parts of *V. candicans* in the study habitats using HPLC technique.

No	R.T	Sugar	Habitat			
			Wadi Habbes		Sand Dunes	
			Free	Combined	Free	Combined
1	2.700	Unknown	0.969	-	-	-
2	4.417	Glucornic	39.227	-	-	-
3	4.567	Raffinose	-	-	57.241	-
4	5.750	Glucose	18.768	2.415	12.153	14.160
5	6.717	Galactose	12.124	-	9.479	-
6	7.133	Mannose	-	0.354	-	-
7	7.450	Fructose	12.727	0.281	9.037	-
8	8.383	Fucose	14.763	-	12.090	-
9	9.717	Unknown	-	0.405	-	-
10	11.650	Maltose	-	96.545	-	85.840
11	13.300	Unknown	1.421	-	-	-



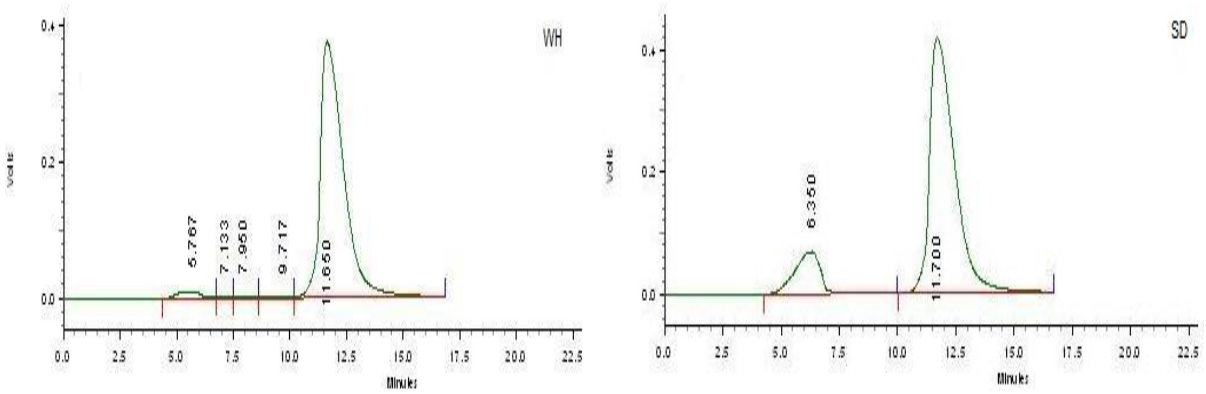
Photogram 1. Free sugars (%) of the aerial parts of *V. candicans* in the study habitats using HPLC technique.

The plant growing in WH habitat was characterized by the presence of five free monosaccharides; glucoronic acid, glucose, galactose, fructose and fucose. The most abundant free sugar was glucornic acid (39.3%), while the most scant free sugar was galactose (12.1%). Also, the plant growing in SD habitat was characterized by the presence of five sugars, four free monosaccharides; glucose, galactose, fructose and fucose and one trisaccharide; raffinose. The most abundant free sugar was the trisaccharide raffinose (57.2%) and the most scant one was the monosaccharide fructose (9.0%). The obtained results showed that the plant aerial parts in the two habitats have four common monosaccharides. In WH habitat the free monosaccharide glucoronic acid is found and replaced in SD habitat by the trisaccharide raffinose. Also, in this habitat, two unknown sugars were detected but with small percentages.

The accumulation of free soluble sugars seem to play an important role in osmotic regulation of cells (Bolarin *et al.*, 1995) and regulate the expression of some genes (Yu *et al.*, 1996). Also, these sugars appear to be central to the development of desiccation tolerance (Hoekstra *et al.*, 2001). On the other hand, Herbinger *et al.* (2002) concluded that the decrease in soil moisture content resulted in the decrease of photosynthesis, which was associated with an increase in respiration rate and led to the reduction in carbohydrates concentration in plant and hence the breakdown of starch into free soluble sugars needed for osmoregulation (Elhaak and El Sayed, 1990).

Combined sugars

The combined sugars (glycosides) of the aerial parts of *V. candicans* were determined after hydrolysis using HPLC (Table 2 and Photogram 2). The detected sugars were the monosaccharides glucose, mannose and fructose and the disaccharide maltose.



Photogram 2. Combined sugars (%) of the aerial parts of *V. candicans* in the study habitats using HPLC technique.

The plant growing in WH habitat was characterized by the presence of three combined monosugars glucose and the disaccharide maltose in the plant in the two habitats, in addition to the two monosaccharides mannose and fructose in the plant of WH habitat. The most abundant combined sugar in the plant aerial parts in this habitat was the disaccharide maltose (96.5%), while the most inadequate sugar was the monosaccharide fructose (0.3%). However, the plant growing in SD habitat was characterized by lower number of combined sugars, one combined monosaccharide; glucose (14.2%) and one disaccharide, maltose (85.8%). The data revealed that the two monosaccharides mannose and fructose have been vanished in SD habitat, so the plant compensated the deficiency of these two sugars by the abundance of the monosaccharide glucose.

Li *et al.* (2013) found a slight increase in soluble sugar concentration in *Eremosparton songoricum* with the increase in the osmotic potential. They reported that one of the osmotic stress defense mechanisms is the accumulation of organic osmolytes (such as proline, soluble sugars, glycine betaine, and organic acids) in the cytoplasm to maintain the plant water potential during drought stress. The severity of drought is unpredictable as it depends on many factors such as occurrence and distribution of rainfall, evaporative demands and moisture storing capacity of soils (Wery *et al.*, 1994). One of the most common stress tolerance strategies in plants is the overproduction of different types of compatible organic solutes (Serraj and Sinclair, 2002). Singh (2004) proved that a greater accumulation of sugar lowers the osmotic potential of cells and reduces loss of turgidity in plants. The other possible role of sugar may be as a readily available energy source.

Soluble amino acids and soluble protein contents

Data in Table (3) represent the variation in soluble amino acids and soluble protein contents in *V. candicans* aerial parts during the study seasons in the studied habitats (WH and SD). The variation in the contents of soluble amino acids and soluble proteins varied statistically with highly significant value ($P<0.001$) by each habitats, seasons and the interaction between them. The content of soluble amino acids of WH plant varied slightly between a maximum value (6.4 mg/g d.wt) in autumn and a minimum value (6.2 mg/g d.wt) in winter. On the opposite, the soluble amino acids content in SD habitat plants recorded the highest content (6.7 mg/g d.wt) in spring and the lowest one (6.2 mg/g d.wt) in autumn. However, the content of soluble protein in the WH habitat recorded the maximum content (32.8 mg/g d.wt) in summer and the minimum one (9.5 mg/g d.wt) in winter through the period of investigation. Also, the amount of soluble proteins in SD reached to its highest content (48.9 mg/g d.wt) in spring and the lowest one (12.2 mg/g d.wt) in autumn

throughout the period of investigation. In general, both soluble amino acids and proteins were greater in SD habitat than in the WH habitat during all seasons, except autumn.

Table 3. Free amino acids and free protein contents (mg/g d.wt) in the aerial parts of *V. candicans* in the study habitats during the period of investigation.

Season		Habitat					
		Wadi Habbes			Sand Dunes		
		Amino acids	Protein	Ratio	Amino acids	Protein	Ratio
Wet seasons	Winter	6.2 ±0.02	9.5 ±0.96	0.65	6.5 ±0.00	17.8 ±0.76	0.37
	Spring	6.3 ±0.08	25.9 ±2.21	0.25	6.7 ±0.07	48.9 ±3.09	0.14
Dry seasons	Summer	6.2 ±0.04	32.8 ±2.18	0.19	6.3 ±0.05	35.7 ±4.63	0.18
	Autumn	6.4 ±0.03	17.3 ±4.54	0.37	6.2 ±0.02	12.2 ±2.31	0.51
	Mean	6.30	21.38	0.36	6.43	28.65	0.30

Vyas *et al.* (1996) observed that water stress causes both reductions in the rate of protein synthesis as well as changes in the type of proteins produced. He also reported that increasing water stress progressively decreased soluble protein content in *Vigna aconitifolia* leaves. Garg *et al.* (2001) also found that increasing water stress progressively decreased plant water potential, leaf area, net photosynthetic rate, starch and soluble protein contents and nitrate reductase activity in *Vigna aconitifolia*. Al-Jebory (2012) reported that the content of protein in *Pisum sativum* decreased with increasing of drought stress, whereas proline content increased with increasing of drought stress. The alternation in protein synthesis or degradation is one of the fundamental metabolic processes that may influence water stress tolerance (Jiang and Huang, 2002). Both quantitative and qualitative changes of proteins have been detected during drought stress (Ahire *et al.*, 2005 and Kottapalli *et al.*, 2009).

Free and protein amino acids

The separation of free amino acids and protein amino acids of the aerial parts of *Varthemia candicans* were achieved using amino acid analyzer and each component was detected quantitatively (Table 4). The data illustrated that the aerial parts of the plant in the WH habitat exhibited twenty seven free amino acids and twenty five in SD habitat. The most abundant free amino acid in WH habitat was asparagine (63.576 µg/g d.wt), however the most abundant one in SD habitat was proline (136.649 µg/g d.wt). The lowest concentrations of free amino acids (0.065 and 0.064 µg/g d.wt) given by hydroxy-proline in WH and SD habitat, respectively.

Table 4. Free and protein amino acids content of *V. candicans* aerial parts in the study habitats (WH and SD) using amino acid analyzer.

No	Amino acid	Free amino acids (µg)			Protein amino acids (mg)		
		RT	Habitat		RT	Habitat	
			WH	SD		WH	SD
1	Phospho-serine	4.075	0.605	0.491	-	-	-
2	Aspartic acid	17.563	2.012	7.102	9.053	54.015	108.577
3	Hydroxy-Proline	23.275	0.065	0. 064	-	-	-
4	Threonine	25.317	1.591	3.520	10.347	5.615	12.225
5	Serine	27.752	4.880	3.944	11.192	17.335	32.401
6	Asparagine	32.683	63.576	95.287	-	-	-
7	Glutamine	36.704	0.183	1.533	-	-	-
8	α-Aminoadepic acid	41.579	0.723	1.121	-	-	-
9	Proline	46.048	24.913	136.649	15.048	102.189	42.238
10	Glycine	49.011	2.703	0.795	19.576	24.853	51.471
11	Alanine	51.259	10.255	18.170	21.016	22.819	38.665
12	Citrulline	55.501	-	0.163	-	-	-
13	α-Aminobutyric acid	55.736	-	0.144	-	-	-

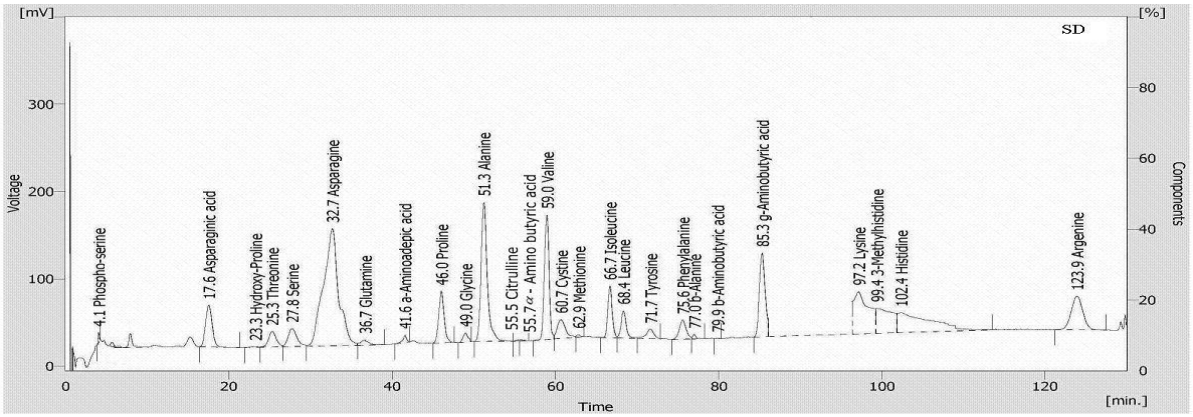
No	Amino acid	Free amino acids (µg)			Protein amino acids (mg)		
		RT	Habitat		RT	Habitat	
			WH	SD		WH	SD
14	Valine	58.987	4.573	16.391	23.611	8.446	13.929
15	Cystine	60.704	9.500	8.189	22.613	2.250	-
16	Methionine	62.888	0.291	0.378	25.683	5.509	9.982
17	Isoleucine	66.704	1.811	7.022	27.717	5.186	9.990
18	Leucine	68.352	1.087	4.305	28.667	17.013	32.267
19	Tyrosine	71.675	0.921	3.393	31.016	6.960	14.038
20	Phenylalanine	75.616	0.159	5.681	32.227	7.007	14.906
21	β-Alanine	77.019	2.166	1.377	-	-	-
22	β-Aminobutyric acid	79.853	1.729	0.129	-	-	-
23	γ-Aminobutyric acid	85.333	0.181	14.362	-	-	-
24	Lysine	97.165	63.037	21.580	38.549	12.437	19.301
25	3-Methylhistidine	99.389	6.499	19.693	-	-	-
26	Histidine	102.365	53.555	24.187	35.203	37.810	55.706
27	Argenine	123.928	9.524	18.070	42.749	16.417	23.491
28	Glutamic acid	-	-	-	12.816	39.481	61.783
Total number of amino acids		-	**25**	**27**	**-**	**17**	**16**

Also, all of the detected amino acids were found in the aerial parts of the plant growing in SD habitat, but the two amino acids citrulline and α-aminobutyric acid were vanished in WH habitat. It is also important to note that the content of most detected amino acids in SD habitat was greater than the total content of free amino acids in WH habitat.

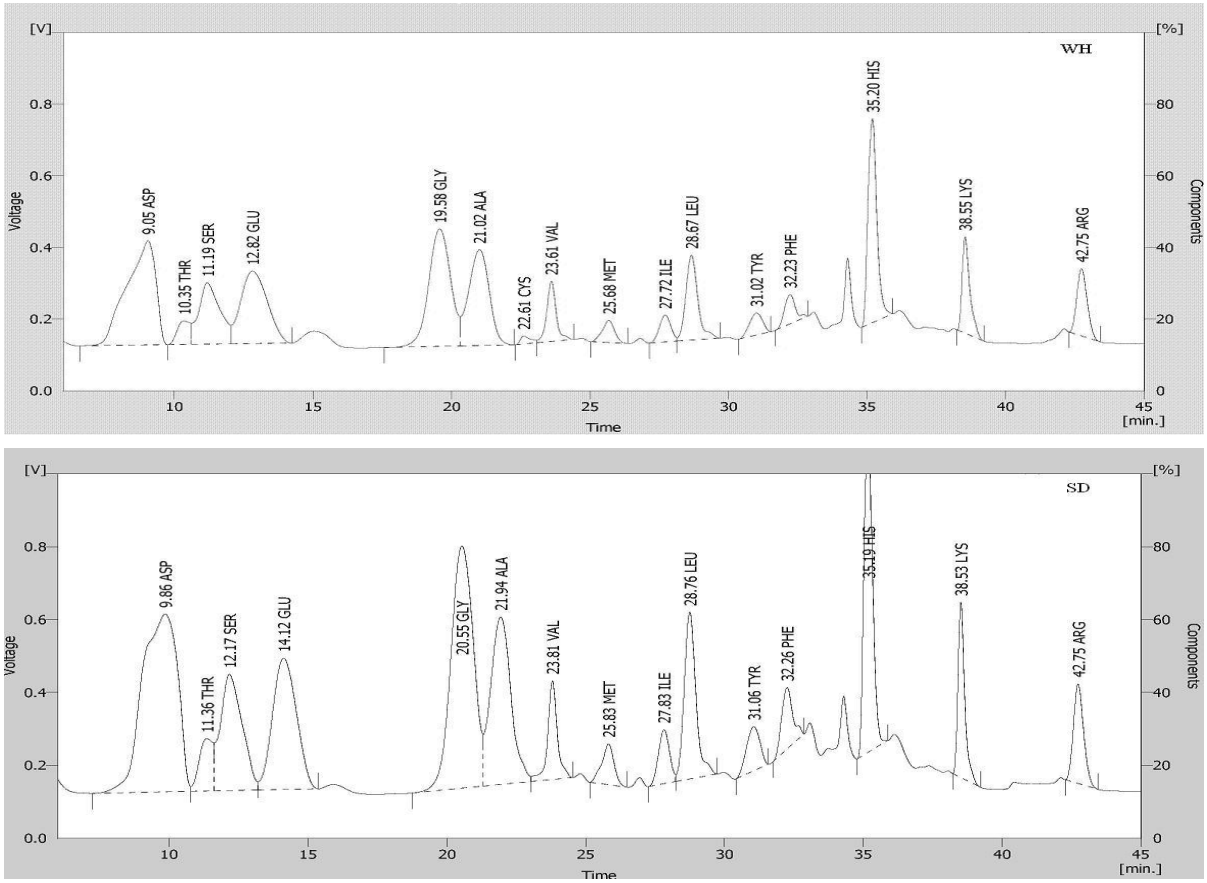
The investigation of hydrolyzed protein-amino acids in the aerial parts of *V. candicans* growing in the two study habitats (WH and SD) was achieved (Table 5 and Photogram 3a and b). The data reflected that there were seventeen amino acids with different concentrations in the two habitats. The most abundant protein-amino acid in WH habitat was proline (102.189 mg) and the scarcer one was cystine (2.250 mg). However, the most abundant protein-amino acid in SD habitat was aspartic acid (108.577 mg) and the less abundant protein-amino acids were methionine and isoleucine (9.982 and 9.990 mg). The data also revealed that the amino acid cystine was unique and characteristic to the plant growing in WH habitat.

Table 5. Total lipids content of the aerial parts of *Varthemia candicans* in two different habitats (WH and SD) during the period of investigation (2011-2012).

Season	Total lipids (mg/g d.wt)	
	Wadi Habbes	Sand Dunes
Winter	10.870 ±0.91	21.110 ±0.69
Spring	52.855 ±0.78	87.330 ±1.00
Summer	71.945 ±0.84	82.135 ±0.85
Autumn	31.555 ±1.00	52.420 ±0.76
Mean	41.806±26.41	60.749±30.57



Photogram 3a. Free amino acids of *V. candicans* aerial parts in the study habitats (WH and SD) using amino acid analyzer.



Photogram 3b. Protein amino acids of *V. candicans* aerial parts in the study habitats (WH and SD) using amino acid analyzer.

Proline is one of the osmolytes, which increase faster than other amino acids in plants under water deficit stress and help the plants to maintain cell turgor pressure (Valentovic *et al.*, 2006). Thus, proline accumulation can be used as a criterion for drought resistance assessment (Gunes *et al.*, 2008). Najaphy *et al.* (2010) explained that the increased proline content in chickpea leaves exposed to water deficit was due to protein breakdown. Increasing proline content of leaves with decreasing available water means that an efficient mechanism for osmotic regulation, stabilizing sub-cellular structures and cellular adaptation to water stress was provided (Valentovic *et al.*, 2006 and Gunes *et al.*, 2008).

Asparagine and glutamine connect the two important metabolic cycles of the plant, the carbon and nitrogen cycles, and they have an influence both on sugars and proteins. In plants, aspartate is the precursor to several amino acids, including methionine, threonine and isoleucine (Eid *et al.*, 2011). do Amarante *et al.* (2006) proved that legumes use asparagine rather than ureides as nitrogen transport compounds in the xylem. Asparagine also accumulates under conditions of stress as proline in many plant species as soybean (King and Purcell, 2005), alfalfa (Fougere *et al.*, 1991), pearl millet (Kusaka *et al.*, 2005) and wheat (Carillo *et al.*, 2005). This may be a direct biological response to the stress conditions, for example, by contributing to the maintenance of osmotic pressure or indirect result of the restriction of protein synthesis under stress conditions (Lea and Miflin, 2003).

Total lipids content

The total lipids content of *V. candicans* aerial parts in the study habitats (WH and SD) was recorded in Table (5). The recorded data were statistically highly significant ($P<0.001$) and revealed that the total lipids content was greater in SD habitat than in WH

one during all seasons. The highest lipid content in SD habitat (87.330 mg/g d.wt) and in WH habitat (71.945 mg/g d.wt) was recorded during spring and summer, respectively. However, the lowest lipid content in the two habitats (10.870 and 21.110 mg/g d.wt, respectively) was recorded during winter season.

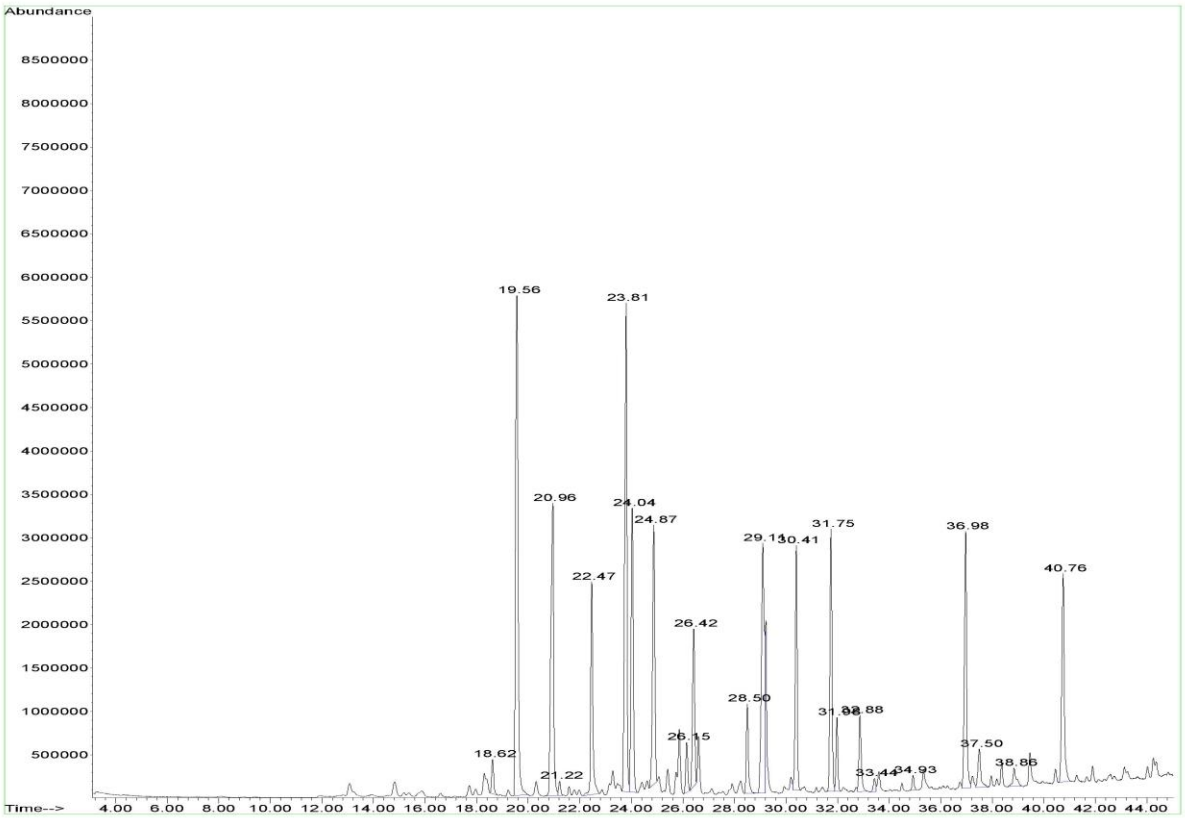
Membranes are main targets of degradative processes induced by drought and it has been shown that, under water stress, a decrease in membrane lipid content is correlated to an inhibition of lipid biosynthesis (Monteiro *et al.*, 1990) and a stimulation of lipolytic and peroxidative activities (Matos *et al.*, 2001).

Fatty acids constituents

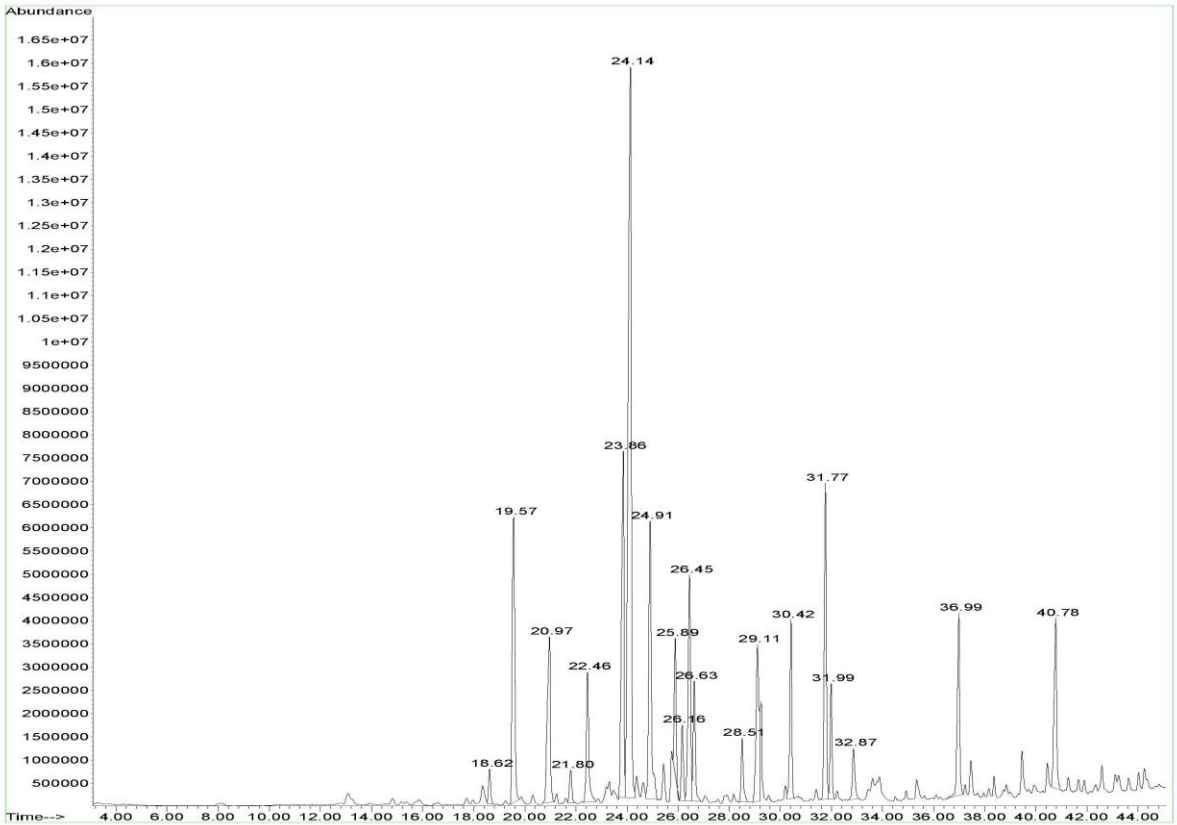
The fatty acids content of the aerial parts of *V. candicans* in the study habitats, WH and SD, estimated using GC-MS technique was calculated and tabulated (Table 6 and Photogram 4a and b). The obtained results revealed that *V. candicans* growing in the two habitats contained twenty eight fatty acids; twenty four fatty acids were identified and four fatty acids remained nameless.

Table 6. GC-MS analysis of fatty acids composition of the aerial parts of *V. candicans* in the study habitats.

No	RT (min)	IUPAC Name	Habitat	
			WH (%)	SD (%)
1	18.62	Methyl tetradecanoate	0.77	0.77
2	19.57	Unknown	14.14	7.71
3	20.96	Trimethylurea	9.08	5.23
4	21.22	Pentadecanoic acid, methyl ester	0.33	-
5	21.80	Unknown	-	0.82
6	22.46	Unknown	-	3.32
7	22.81	Unknown	12.01	-
8	23.81	Hexadecanoic acid, methyl ester	12.01	8.62
9	24.04	2,5,10-Trimethyl-6,7,8,9-tetradehydrocyclopentadecenone	7.17	-
10	24.14	6-Acetyl-8methoxy-2,2-dimethyl-2H-chromen-5-ol	-	23.92
11	24.88	3-Oxo-isocostic acid	6.69	7.61
12	25.89	8,9-Dihydrocyclohepta[a]phenalene-6 (10H) - one	-	2.53
13	26.15	Hexadecanoic acid, 15-methyl-, methyl ester	1.11	1.67
14	26.42	Ethanone	3.77	-
15	26.45	2,2,4-Trimethyl-4,5-dihydro-1,3,8H-azulene-6,7-dicarboxylic anhydride	-	5.77
16	26.63	Methyl ester of Encecalol	-	2.71
17	28.50	Stearic acid, methyl ester	2.05	1.43
18	29.11	6-Octadecadienoic acid, methyl ester (Z)- (CAS)	8.33	4.76
19	30.41	9,12-Octadecadienoic acid, methyl ester	5.28	3.71
20	31.75	Benzene, hexaethyl-	6.00	7.02
21	31.98	9,12,15-Octadecatrienoic acid, methyl ester	1.48	2.20
22	32.88	Eicosanoic acid, methyl ester	1.94	1.35
23	33.44	Cis-11-Eicosenoic acid, methyl ester	0.33	-
24	34.94	Heneicosanoic acid, methyl ester	0.32	-
25	36.98	Docosenoic acid, methyl ester	6.51	4.37
26	37.50	13-Docosenoic acid, methyl ester, (Z)-	1.20	-
27	38.86	Tricosanoic acid, methyl ester	0.68	-
28	40.76	Tetracosanoic acid, methyl ester	5.74	4.51



Photogram 4a. GC-MS analysis of fatty acids composition of the aerial parts of *V. candicans* in WH habitat.



Photogram 4b. GC-MS analysis of fatty acids composition of the aerial parts of *V. candicans* in SD habitat.

The fatty acid Hexadecanoic acid methyl ester presented the higher percentage (12.01%) in the aerial parts of the plant growing in WH habitat, however the fatty acid 6-Acetyl-8methoxy-2,2-dimethyl-2H-chromen-5-ol presented the highest percentage (23.92%) in the aerial parts of the plant growing in SD habitat. On the other hand, the fatty acid Heneicosanoic acid methyl ester represented the lowest percentage (0.32%) in the aerial parts in WH habitat; however the fatty acid Methyl tetradecanoate represented the lowest percentage (0.77%) in SD habitat.

The results cleared out that the aerial parts of the plant in WH habitat exhibited unique characteristic fatty acids like Pentadecanoic acid methyl ester, 2,5,10-Trimethyl-6,7,8,9-tetradehydrocyclopentadecenone, Cis-11-Eicosenoic acid methyl ester, Heneicosanoic acid methyl ester, 13-Docosenoic acid, methyl ester, (Z) and Tricosanoic acid methyl ester. Also, the plant growing in SD habitat exhibited some unique fatty acids like 6-Acetyl-8methoxy-2,2-dimethyl-2H-chromen-5-ol, 8,9-Dihydrocyclohepta[a]phenalene-6 (10H) – one, Methyl ester of Encecalol and 2,2,4-Trimethyl-4,5-dihydro-1,3,8H-azulene-6,7-dicarboxylic anhydride. The two habitats mirrored distinction in number and type of fatty acids composition in the aerial parts of the plant. The plant growing in WH habitat contained 22 fatty acids, while in SD habitat it contained 20 fatty acid.

Lipids contain a wide range of molecules with many important functions, which include fatty acids that play multiple roles in cells. Polyunsaturated fatty acids are an important source of energy for ATP synthesis, are essential cellular membrane components, and are also precursors of important molecules that have specific and central regulatory roles in living organisms (Das, 2006).

Hexadecanoic acid (Palmitic acid) is the most common saturated fatty acid in animals, plants and microorganisms (Gunstone *et al*., 2007). Excess carbohydrates in the body are converted to palmitic acid and it is a major body component of animals, it comprise 21–30% of human depot fat (Kingsbury *et al*., 1961) and it is a major, but highly variable, lipid component of human breast milk (Jensen *et al*., 1978). Palmitate negatively feeds back on acetyl-CoA carboxylase (ACC), which is responsible for converting acetyl-CoA to malonyl-CoA, which in turn is used to add to the growing acyl chain, thus preventing further palmitate generation. In biology, some proteins are modified by the addition of a palmitoyl group in a process known as palmitoylation which is important for membrane localization of many proteins. Wei *et al*. (2011) reported that Octadecatrienoic acid, Pentadecanoic acid, Heneicosanoic acid, Oleic acid, β-sitosterol and Phytol may be responsible for the antioxidant activity of *Adrographis paiculata* leaf extract.

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