ASSESMENT OF PRIMARY METABOLITES OF *HELICHRYSUM CONGLOBATUM* (VIV.) STEUD. DURING DIFFERENT GROWTH SEASONS

ABD EL-MOATY, H. I.[1,2*] – SOROUR, W. A.[3,4] – ABU-KHUDIR, R.[5,6] – HAMAD, R. S.[1,7] – ALTHUMAIRY, D.[1] – HADID, M. L.[8] – MOHAMED, A. A. A.[4]

[1]*Biological Sciences Department, College of Science, King Faisal University, P.O. Box: 380 Al-Ahsa 31982, Saudi Arabia*

[2]*Medicinal and Aromatic Plants Department, Desert Research Center El-Mataria, Cairo 11753, Egypt*

[3]*Department of Basic Sciences, Preparatory Year Deanship, King Faisal University, Al-Ahsa 31982, Saudi Arabia*

[4]*Department of Botany, Faculty of Science, Aswan University, Aswan 81528, Egypt*

[5]*Chemistry Department, College of Science, King Faisal University, Al-Hofuf, Al-Ahsa 31982, Saudi Arabia*

[6]*Chemistry Department, Biochemistry Branch, Faculty of Science, Tanta University, Tanta 31527, Egypt*

[7]*Central Laboratory, Theodor Bilharz Research Institute, Giza, Egypt*

[8]*Department of Agribusiness and Consumer Science, College of Agricultural and Food Science, King Faisal University, Al-Hofuf, Al-Ahsa, Saudi Arabia*

**Corresponding author*
e-mail: hitorkey@kfu.edu.sa; phone: +966-54-504-1183

(Received 24th Feb 2023; accepted 27th Apr 2023)

Abstract. *Helichrysum conglobatum* (Asteraceae) is an important economic and medicinal plant. The proximate composition of different plant parts was determined. Sugars, amino acids, and lipids have been analyzed using HPLC, Amino Acid Analyzer and GLC respectively. Seasonal changes in plant moisture content, ash, organic matter, crude fiber, carbohydrate, nitrogen, protein and lipid were monitored. Ash content, (acid soluble, acid insoluble, water-soluble, water-insoluble) and crude fiber contents gradually increased from winter to summer. Water content, organic matter, total carbohydrates, soluble and insoluble carbs, total nitrogen, total protein and total lipids decreased gradually from winter to summer. Drought decreased photosynthesis consequently reduced the total carbohydrates. The decrease in total nitrogen and total protein is possibly linked to the buildup of some amino acids (like proline), that regulates cell osmoregulation. High content of lipids in winter can probably be accredited to the augmented metabolic rate, which elevated carbohydrate content, which converted to lipids by oxidation reaction. Glucose was the highest free sugar detected while ribose and rhamnose were the highest combined sugars. Serine was the major free amino acid. Phenylalanine, aspartic and arginine were the major protein amino acids. The physical and chemical properties of lipids, saponifiable, unsaponifiable matter fractions, saturated and unsaturated fatty acids were investigated.

Keywords: *Asteraceae, carbohydrates, proteins, lipids, nutritional composition*

Introduction

The western Mediterranean desert of Egypt is characterized by the high diversity of many wild plants. Fluctuations in climatic and edaphic conditions lead to the buildup of high concentrations of beneficial plant metabolites (Hendawy, et al., 2019; Abdel-Rahman and Migahid, 2019), that thrive in less adverse conditions (Ebifa-Othieno, et al., 2020). Many studies have discussed the effects of climate and other environmental factors on plant nutrients and plant growth. The protein and other metabolite content of plants are affected by climate change. Plants that experience different types of stress build up more nutrients that are beneficial to humans than plants that grow under less stressful conditions (Abd El-Moaty, et al., 2022; Ebifa-Othieno, et al., 2020). Although there is a sporadic short rain shower in winter and autumn, the shoreline basin of the Mediterranean in Egypt experiences a warm coastal desert climate (Meigs, 1973). The warmest summer month has an average temperature of less than 30 °C and the coldest month of winter has an average temperature above 10 °C (Galal et al., 2019; UNESCO, 1977). More than 500 species of *Helichrysum* can be found in Africa, Asia, Australia, and Europe (Galbany-Casals et al., 2006). Three species of the Genus *Helichrysum's* wild species are recorded in Egypt. *Helichrysum conglobatum* (Viv.) Steud. (Syns. *Gnaphalium conglobatum* Viv., *Helichrysum siculum* Boiss.), *Helichrysum orientale* (L.) (Syn. *Gnaphalium orientale* L.) and *Helichrysum glumaceum* DC., (Syn. *Achyrocline glumacea* DC.) (Boulos, 2002). *Helichrysum conglobatum* is distributed in the Mediterranean coastal belt from the border with Libya near El Sallum to Port Said (Boulos, 1995). Flowers of *H. italicum* are rich in Calcium, Potassium, Iron, Zinc, and other trace elements. Carbohydrates were the major compounds recorded in the flower (>80% of dry weight), followed by minerals (6.31% of dw), protein (5.44% of dw), and lipids were recorded 3.59% of dw (Primitivo et al., 2022). Five aromatic compounds, among them two glucosides and six flavonols were separated from the flowers and the aerial shoots of *Helichrysum conglobatum* (El-Ghazooly et al., 2003). Industrially, there are many products that use *Helichrysum* flowers oil as a sedative, rejuvenating, stimulating and for relaxation. Nevertheless, there are no previous studies reported on its primary products and nutritional composition. Therefore, this research aimed to consider the nutritional components of *Helichrysum conglobatum* growing on the western Mediterranean coastline of Egypt and the impact of seasonal environmental variations on plant growth and nutrient content, which support the use of the plant on a commercial scale.

Materials and methods

Plant materials

The fresh plants (2 kg) of *Helichrysum conglobatum* (Viv.) Steud. (Sys. *Gnaphalium conglobatum* Viv., *Helichrysum siculum* Boiss.) were collected seasonally (Autumn (19/10/2020), winter (22/1/2021), spring (18/4/2021) and summer (19/7/2021)) from Agiba 20 km far from west of Mersa Matrouh (N 31° 16' 55", E 027° 05' 11", Elevation 2M) on the Northwestern Mediterranean coast of Egypt (*Fig. 1*). The climate of Matrouh is classified as Arid Mediterranean which is characterized by a long, warm, dry summer and short, cool, rainy winter. Monthly temperatures typically ranged from 14.4 to 26.8 °C. August is the warmest month with the maximum average temperature of 29.5 °C, while January is the coolest month with a minimum average temperature of 8.7 °C (DRC staff, 2015). The lowest and highest

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2103_27472759

relative humidity varies between 66 to 75% in April and July, respectively. The average annual rainfall ranged from 100 to 190 mm (DRC staff, 2015). Notably in winter, Matrouh receives significant amounts of rainfall; the rainiest months are December and January, in summer, negligible drops of rain are recorded. Occasionally, heavy rains can occur in autumn and only about 10% falls during spring (Galal et al., 2019). The main source of irrigation water in the Northwestern Mediterranean coastal zone is rainfall. The plant parts (stem, leaves and flowers) were separated and air dried at room temperature until constant weight, then grounded to fine dry powder and reserved for further chemical analyses.

Figure 1. *Agiba habitat where Helichrysum conglobatum grows*

Proximate composition of Helichrysum conglobatum

Plant water content, ash, crude fiber, organic matter and lipid content were analysed according to the Association of Official Analytical Chemists (AOAC, 2000). The Kjeldahl method was applied to determine crude protein (AOAC, 2000). Carbohydrate determination was carried out by dissolving one gram of plant powder in 2-5 ml of 2M HCl in a closed tube, then heating at 100 °C for 5-6 h (Chaplin and Kennedy, 1994). The total carbohydrates were assessed using the general phenol-sulfuric acid assay according to Chaplin and Kennedy (1994). All values were calculated as a percentage of the studied sample.

Fundamental physical and chemical properties of lipids

The physical properties (color, odor and physical nature) of lipid fraction were studied. The solubility of lipids in petroleum ether, diethyl ether, benzene, chloroform, acetone, carbon tetrachloride and warm alcohol were also tested**.** Acid value (A.V), saponification value (S.V.) ester value (E.V.) and iodine value (I.V) were determined during spring season (flowering season) according to British Pharmacopoeia (1993) and Farag (1995).

Qualitative analysis

Investigation of free and protein-amino acids was attained during spring season according to Pellet and Young (1980), utilizing the amino acid analyzer SYKAM S 433 system High-Performance Analyzer. Column: Na High-Performance column (150*4.6) LC AKO6 Na -24050313, Injected volume: 0.1 ml, Flow Rate: 0.45, Detection: 440

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2103_27472759

+ 570 nm, Temperature: 57-74. Retention time and separated area were achieved using Hewlett Packard 3390 recording integrator. The determination of free and combined sugars was determined qualitatively during spring season by High-performance liquid chromatography (HPLC) Agilent 1050 series. The Detector is the Refractive Index (RI) Shodex RI-71 (Japan). The GLC analysis to identify unsaponifiable and saponifiable matters was investigated during spring season with a GC Pye-Unicam 204 series of gas chromatographic apparatus as mentioned by Farag et al. (1986).

Statistical analysis

All chemical analyses were performed in triplicate with blank samples. One-way analysis of variance (ANOVA) was used to measure the significant differences in nutritional composition due to seasonal variations using MINITAB software ver. 12, 21.

Results and discussion

Proximate composition of Helichrysum conglobatum

Moisture, ash, organic matter and crude fiber content

Water content, ash, organic matter, Crude fiber, carbohydrates, nitrogen, protein and lipids contents of flowers were estimated at their mature stage in spring, while those of stem and leaves were estimated during the four seasons. The seasonal variations in the proximate composition in different parts of *H. conglobatum* are summarized in *Table 1*.

The percentages of water content in different parts (stem, leaf and flower) of *Helichrysum conglobatum* reached their maximum values in winter, where it reached 32.6% in the stem and 23.5% in the leaf. The moisture content of the flower was detected only in spring (12.2%). Water content decreased gradually in spring and summer. The Ash content of *Helichrysum conglobatum* reached its maximum values of 7.81% and 8.77% for stem and leaf, respectively in summer. In addition, the minimum values of 3.80% and 4.81% for stem and leaf respectively were recorded in winter.

The maximum value of organic matter was recorded in winter. It reached 92.89% in the stem and 83.39% in the leaf. Organic matter was recorded at 79.13% in flowers during the spring season. This was followed by Crude fiber which attained 47.43% and 45.34% in the stem and leaves respectively in summer Crude fibers recorded 29.13% in the flowers in spring.

The steady and gradual increase in ash content, acid soluble, acid insoluble, water soluble, water insoluble, ash and crude fiber contents from winter, spring to summer was recorded. The percentage of pharmacopoeia constants and total ion accumulation increased as a result of an increase in soil moisture stress (Agboola and Adejumo, 2013).

Carbohydrates content

Determination of total, soluble and insoluble carbohydrates

Data in *Table 1* show that the percentage of total carbohydrates of *Helichrysum conglobatum* achieved its maximum values of 4.75% and 6.77% for stem and leaf, respectively in winter. The minimum values of 0.61% and 1.35% for stem and leaf, respectively in summer. The total carbohydrates of the flower were 4.32%. The percentage of soluble carbohydrates reached its maximum values of 1.84% and 2.69% for stem and leaf respectively in winter. The minimum values of 0.23% and 0.43% for stem

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2103_27472759

and leaf, respectively in summer. The percentage of soluble carbohydrates in the flower was 1.62%. The percentages of insoluble carbohydrates showed also the same trend as the soluble carbohydrates in the different parts (stem, leaf and flower) of *Helichrysum conglobatum* during the investigation. Water content, organic matter, total carbohydrates, soluble and insoluble carbs, total nitrogen, total protein and total lipids decreased gradually from winter, spring to summer. Drought stress decreased photosynthesis, which is correlated with the increase in respiration rate, which led to the reduction in the total carbohydrate content in the plant (Mielke and Schaffer, 2010). These results are in consistence with those achieved by Stocker (1960) and El-Monayeri (1982).

Table 1. *The average seasonal variations of proximate composition of Helichrysum conglobatum*

Variable	Season	Stem	Leaf	Flower
Plant water content (%)	Autumn	10.7^{c}	8.7^{c}	12.2
	Winter	32.6^{a}	23.5^{a}	
	Spring	24.2^{b}	15.1^{b}	
	Summer	7.8^{d}	6.4^{d}	
Total ash (%)	Autumn	6.63^{b}	7.61^{b}	6.98
	Winter	3.80^{d}	4.81^{c}	
	Spring	5.28^{c}	6.97^{b}	
	Summer	7.81^{a}	8.77^{a}	
Organic matter (%)	Autumn	86.43^{b}	77.74^{b}	79.13
	Winter	92.89^{a}	83.39^{a}	
	Spring	87.78^{b}	78.28^{ab}	
	Summer	85.25^{b}	76.58^{b}	
Acid soluble ash (%)	Autumn	4.60^{ab}	4.92^{a}	4.25
	Winter	2.65^{c}	2.83^{b}	
	Spring	3.84^{b}	4.76^{a}	
	Summer	5.03^{a}	5.52^{a}	
Acid insoluble ash (%)	Autumn	2.03^{b}	2.69^{ab}	2.73
	Winter	1.15^{d}	1.98^{c}	
	Spring	1.44^{c}	2.21^{bc}	
	Summer	2.78^{a}	3.25^{a}	
Water soluble ash (%)	Autumn	3.36^{b}	3.74^{b}	2.97
	Winter	1.30^{d}	2.16^{c}	
	Spring	2.71^{c}	3.81^{b}	
	Summer	4.40^{a}	4.57^{a}	
Water insoluble ash (%)	Autumn	3.27^{a}	3.87^{a}	4.01
	Winter	2.05^{c}	2.32^{c}	
	Spring	2.57^{b}	3.16^{b}	
	Summer	3.41^{a}	4.20^{a}	
Crude fibre (%)	Autumn	42.19^{b}	38.98^{b}	29.13
	Winter	36.20^{c}	33.57^{c}	
	Spring	40.26^{b}	37.55^{b}	
	Summer	47.43^{a}	45.34^{a}	

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2103_27472759

Total carbohydrates (%)	Autumn	2.59^{b}	3.86^{c}	4.32
	Winter	4.75^{a}	6.77^{a}	
	Spring	4.02^{a}	5.43^{b}	
	Summer	0.61^{c}	1.35^{d}	
Soluble carbohydrates (%)	Autumn	0.35^{b}	0.74^{c}	1.62
	Winter	1.84^{a}	2.69^{a}	
	Spring	1.48^{a}	1.87^{b}	
	Summer	0.39^{b}	0.43^{d}	
Insoluble carbohydrates (%)	Autumn	2.24^{b}	3.12^{c}	2.70
	Winter	2.91^{a}	4.08^{a}	
	Spring	2.54^{ab}	3.56^{b}	
	Summer	0.38^{c}	0.92^{d}	
Total nitrogen (%)	Autumn	0.70^{a}	1.22^{a}	1.39
	Winter	0.81^{a}	1.55^{a}	
	Spring	0.69^{ab}	1.32^{a}	
	Summer	0.52^{b}	0.76^{a}	
Total protein (%)	Autumn	4.37^{ab}	7.62^{c}	8.68
	Winter	5.06^{a}	9.68^{a}	
	Spring	4.31^{b}	8.25^{b}	
	Summer	3.25^{c}	4.75^{d}	
Total lipids (%)	Autumn	1.74^{c}	2.21^{c}	2.82
	Winter	5.56^{a}	7.07^{a}	
	Spring	4.34^{b}	4.44^{b}	
	Summer	0.44^{d}	1.22^{d}	

Values are expressed as a mean of three replicates. Different letters indicate significant variance at $p < 0.05$ following Tukey pairwise comparison of ANOVA test

Free and combined sugars of Helichrysum conglobatum using HPLC

Carbohydrates and their derivatives in living cells provide flexibility and structure, energy, and act as regulators and substrates for several specific biochemical processes (Bokov et al., 2017). The separation of the sugar's contents at different parts (stem, leaf and flower) of *Helichrysum conglobatum* were achieved using High Pressure Liquid Chromatography (HPLC), where the following sugars; rhamnose, arabinose, fructose, glucose and sucrose were detected as free sugars (*Fig. 2A*). Rhamnose, arabinose, ribose, fructose, glucose, galactose, sucrose, cellobiose and raffinose were detected as combined sugars (*Fig. 2B*). The concentration of glucose was the highest one of the separated free sugars in all different parts of *Helichrysum conglobatum*. Meanwhile, the glucose, ribose and rhamnose were the highest components of the separated combined sugars at the stem, leaf and flower, respectively. The non-structural carbohydrates (i.e., glucose, sucrose and rhamnose) serve as sources of energy for the cell, allocation of carbon in the plant cells and osmolytes (Makonya et al.,2019).

Nitrogen and protein content

The percentage of total nitrogen of *H. conglobatum* revealed its maximum values of 0.81% and 1.55% for stem and leaf respectively in winter. The minimum values of

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2103_27472759

0.52% and 0.76% for stem and leaf respectively were recorded in summer (*Table 1*). The percentage of total nitrogen in the flower was 1.39%. The percentages of total protein also showed the same trend of the total nitrogen in the different parts (stem, leaf and flower) of *H. conglobatum* during the investigation. The decrease in soil water resources in the summer may be linked to the buildup of some amino acids (like proline), which may help to regulate cell osmoregulation by changing total nitrogen and protein content. The observed results are consistent with those of Silva et al. (2020) and Coequal et al. (2017), who stated that under conditions of water stress, many plants exhibit a decline in protein levels along with a steady increase in the accumulation of amino acids in the tissue. The protein encourages the production of hormones that regulate a variety of bodily processes, including protein synthesis, repair, and maintenance (Mau et al., 1999).

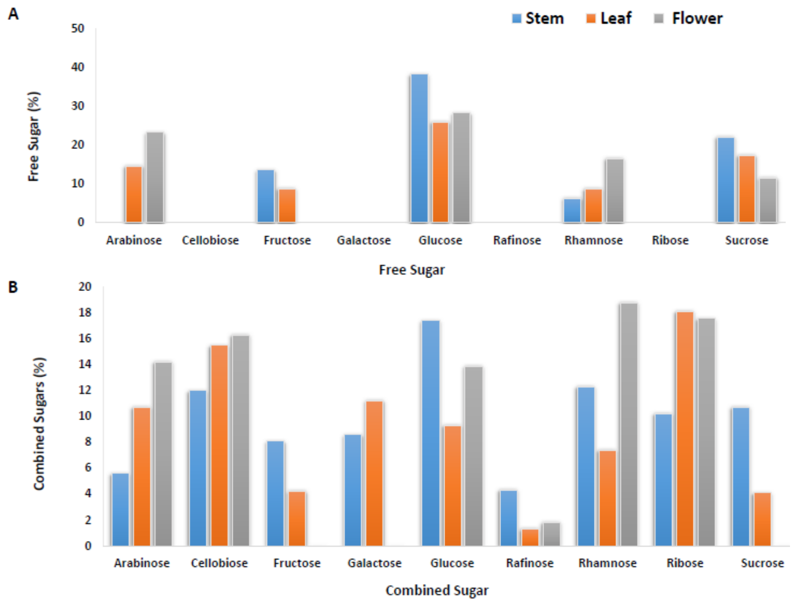

Figure 2. *Determination of free sugars (A) and combined sugars contents (B) in different parts (stem, leaf and flower) of Helichrysum conglobatum using HPLC*

Investigation of free and protein amino acids

The separation of free amino acids in the stem, leaf and flower of *H. conglobatum* was achieved using an amino acid analyzer as shown in *Table 2.* Nine free amino acids were identified in the stem. Twelve free amino acids were recorded in the leaf and thirteen free amino acids were recorded in the flower. The major free amino acid was serine in all different parts of the plant. The results of protein-amino acids indicated that fourteen protein amino acids with different concentrations were detected in the flower. Thirteen protein-amino acids were detected in the stem and leaf. Data in *Table 2* show that phenylalanine, aspartic and arginine were the major components of the separated protein amino acids in the stem, leaf and flower, respectively. Plants' protein production may have changed quantitatively and qualitatively because of a shortage of water (Pessarakali, 1995). Nine amino acids are crucial for an adult man's diet out of the 20 that the body typically needs to synthesize proteins and other substances (Lopez and Mohiuddin, 2023; Murray et al., 2006). Moreover, within the deficiency of any essential

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2103_27472759

amino acids in the body, maintaining nitrogen balance will not be possible since there will not be an adequate amount of this amino acid for protein synthesis nevertheless of the total protein intake (Murray et al., 2006). The human body uses various amino acids as building blocks for the creation of proteins and other nitrogen-containing substances.

Table 2. *Free amino acids and Protein amino acids in different parts (stem, leaf and flower) of Helichrysum conglobatum*

Amino acids	Protein amino acids content						Free amino acids content					
	Stem		Leaf		Flower		Stem		Leaf		Flower	
	R.T.	µg/ml	R.T.	µg/ml	R.T.	µg/ml	R.T.	µg/ml	R.T.	µg/ml	R.T.	µg/ml
Aspartic acid	11.03	235.18	10.97	65.30	65.30	65.30	11.54	232.22	12.27	487.09	11.93	266.80
Therionine	14.42	66.45	14.36	66.30	66.30	66.30	14.98	62.83	15.44	170.00	15.29	81.24
Serine	15.69	611.70	15.59	717.86	717.86	717.86	16.35	85.30	16.86	220.53	16.69	116.22
Glutamic acid	17.90	71.59	17.81	144.13	144.13	144.13	18.50	304.18	_	_	19.02	395.87
Glycine	24.89	35.70	24.80	454.00	454.00	454.00	25.58	95.74	26.06	265.39	25.91	126.14
Alanine	26.11	89.98	26.00	152.92	152.92	152.92	26.90	108.78	27.33	240.00	27.22	167.70
Valine	29.95	111.80	29.90	198.02	198.02	198.02	31.77	111.53	31.96	241.22	31.98	180.35
Isoleucine	33.96	53.84	33.93	90.61	90.61	90.61	36.08	55.40	36.19	122.38	36.25	94.34
Arginine	62.64	218.15	62.63	145.30	145.30	145.30	60.25	34.15	60.61	112.88	60.62	1559.45
Phenylalanine	_	_	42.15	81.97	81.97	81.97	44.25	527.61	44.48	117.67	44.51	64.25
Histidine	_	_	50.41	56.40	56.40	56.40	52.07	60.58	52.40	92.40	52.24	106.57
Lysine	_	_	_	_	_	_	54.94	60.20	55.31	158.22	55.25	110.6
Leucine	_	_	35.15	40.45	40.45	40.45	37.31	89.58	37.38	232.97	37.45	138.86
Tyrosine	_	_	_	_	_	_	_	_	41.64	88.50	41.62	49.66

R.T. = Retention time/minute

Lipid content

The percentages of total lipids of different parts (stem, leaf and flower) of *Helichrysum conglobatum* reached their highest values of 5.56% and 7.07% for the stem and leaf, respectively in winter and their lowest values of 0.44% and 1.22% for stem and leaf, respectively in summer (*Table 1*). The percentage of total lipids in the flower was 2.82%. Scarcity of water in summer results in metabolic variations in plants. Among them are the stimulation of lipolytic, and peroxidative activities and the inhibition of lipid biosynthesis which lowers the content of membrane lipids (Labusch et al., 2013). Drought also reduces the accumulation of lipids in peas, sunflowers, maize, lupine, and wheat (Silva, et al., 2020). The highest content of total lipids in winter may be a result of the enhancement in the metabolic rate of the plant that leads to an increase in carbohydrate content, which converts to lipids by oxidation reaction (Stocker, 1960).

Fundamental physical and chemical properties

The obtained lipid was yellowish green in color, semi-solid having a faint odor and disagreeable taste. It was soluble in benzene, petroleum ether, diethyl ether, chloroform, acetone, warm methyl and ethyl alcohol. The fundamental chemical properties of the extracted lipids of different parts (stem, leaf and flower) of *H. conglobatum* are presented in *Figure 3*. The acid value (A.V.) reached 7.15, 4.93 and 4.05 at the stem, leaf and flower, respectively. Iodine value (I.V.) reached 68.90, 55.82 and 32.91 at the stem, leaf and flower, respectively. Ester value (E.V.) reached 227.70, 187.64 and 144.10 at the stem, leaf and flower, respectively. Saponification value (S.V.) reached

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2103_27472759

234.85, 192.57 and 148.15 at the stem, leaf and flower, respectively. The observed higher iodine value in the plant indicates that they are likely to be healthier for consumption. Studies have recommended to switch from saturated to unsaturated fats because of the risk of cardiovascular disease associated with high consumption of saturated fatty-acids (Li et al., 2015).

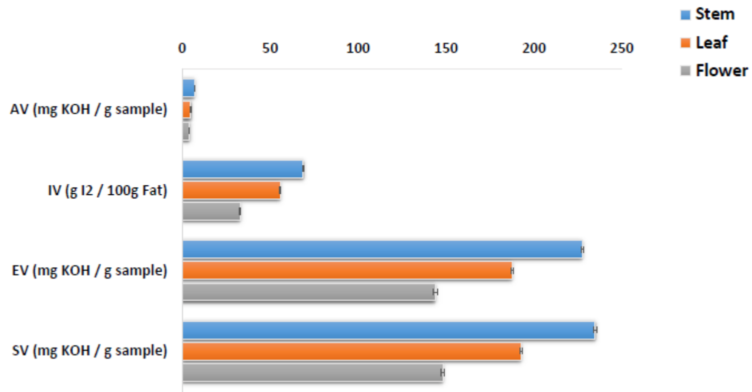

Figure 3. *Determination of acid value (AV), iodine value (IV), ester value (EV) and saponification value (SV) of lipids in different parts (stem, leaf and flower) of Helichrysum conglobatum*

Investigation of unsaponifiable matter fraction (hydrocarbons and sterols)

The unsaponifiable matter content of different parts (stem, leaf and flower) of *H. conglobatum* was determined using GLC technique (*Table 3*). The results revealed that stem of *H. conglobatum* contained tetradecane, eicosane, heneicasane, hexadecane, docosane, tetracosane, hexacosane, triacosane, campesterol and ß-sitosterol. The leaf of *H. conglobatum* contained dodecane, tetradecane, octadecane, eicosane, heneicasane, docosane, tricosane, tetracosane, pentacosane, hexacosane, octacosane, squalane, triacontane, dotriacontane, cholesterol, campesterol and ß-sitosterol. The flower of *H. conglobatum* contained dodecane, tetradecane, octadecane, heneicasane, tetracosane, hexacosane, triacontane, dotriacontane and ß-sitosterol with different concentrations. The plant sterols lie in their potential to act as a natural preventive dietary product (Piironen et al., 2000).

Investigation of saponifiable matter (fatty acids)

The fatty acid contents of different parts (stem, leaf and flower) of *Helichrysum conglobatum* were determined using the GLC technique (*Table 4*). The results indicated that the saturated fatty acids; pelargonic acid, capric acid, lauric acid, myristic acid, palmitic acid, arachidic acid and stearic acid along with the unsaturated fatty acids; myristoleic acid, palmitoleic acid, oleic acid, linoleic acid, linolenic acid and arachidonic acid were detected in the stem, leaf and flower with different concentrations. Lauric acid was not detected in the stem and flower, arachidic acid was not found in the leaf and flower. Arachidonic acid was not detected in the stem. It is clear from *Table 4* that Palmitoleic acid (25.96%, 29.72% and 36.01%) presented the highest percentages of fatty acids in the stem, leaf and flower, respectively. Palmitic acid was the lowest percentage (0.26%) at the stem. Pelargonic acid was the lowest

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2103_27472759

percentages 0.74% and 0.12% at the leaf and flower. Palmitoleic acid was described as a lipokine able to regulate different metabolic processes such as an increase in insulin sensitivity in muscle, β cell proliferation, prevention of endoplasmic reticulum stress and lipogenic activity in white adipocytes (Bermúdez et al., 2022). Linoleic acid has long been accepted as having a hypocholesterolemic effect and inhibitory properties against colon cancer metastatic cells (El-Beltagi, et al., 2019). Linoleic acid also found to prevent cardiovascular diseases (El-Beltagi, et al., 2018). Fatty acids play a natural preventative role in cardiovascular diseases and the improvement of some other health problems (Faremi and Ekanem, 2011). Poly-unsaturated fats promote the reduction of both total and LDL cholesterol and a significant small decrease in HDL cholesterol (Chougui, et al., 2013).

Table 3. *Gas-liquid chromatography of hydrocarbons and sterols at different parts (stem, leaf and flower) of Helichrysum conglobatum*

Item	No. of carbon atom	Hydrocarbons and sterols (%)		
		Stem	Leaf	Flower
Dodecane	12	-	9.63	17.04
Tetradecane	14	5.09	6.21	1.16
Octadecane	18	-	6.52	14.49
Eicosane	20	5.24	10.34	-
Heneicasane	21	16.40	4.11	7.15
Docosane	22	3.76	6.41	-
Tricosane	23	-	3.10	-
Tetracosane	24	7.29	1.89	4.49
Pentacosane	25	-	4.28	-
Hexacosane	26	8.63	1..34	1.65
Octacosane	28	-	0.71	-
Squalane	29	-	1.04	-
Triacontane	30	5.92	1.40	4.63
Dotriacontane	32	-	7.03	9.27
Cholesterol	27	-	7.81	-
Campesterol	27	8.72	3.56	-
B- sitosterol	27	26.04	10..33	17.85

Table 4. *Gas-liquid chromatography of fatty acids at different parts of Helichrysum conglobatum*

Fatty acids	No. of carbon atom	Fatty acids (%)		
		Stem	Leaf	Flower
Pelargonic	9:0	1.57	0.74	0.12
Capric	10:0	0.31	2.35	7.49
Lauric	12:0	-	5.47	-
Myristic	14:0	8.59	6.84	5.83
Palmitic	16:0	0.26	3.07	3.90
Arachidic	20:0	15.63	-	-
Stearic	18:0	20.02	5.30	3.51
Myristoleic	14:1	3.74	4.95	-
Palmitoleic	16:1	25.96	29.72	36.01
Oliec	18:1	11.08	15.61	14.07
Linoleic	18:2	3.75	8.62	7.25
Linolenic	18:3	1.68	1.13	0.47
Arachidonic	20:4	-	10.50	3.61

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2103_27472759

Conclusion

This study investigated the seasonal variation in the proximate composition of different parts (stem, leaves and flowers) of the *Helichrysum conglobatum* plant. Water content, organic matter, total carbohydrates, soluble and insoluble carbs, total nitrogen, total protein, and total lipids decreased gradually from winter, spring to summer. Ash and crude fiber contents reached their maximum values in summer. The separation of the sugar's contents was achieved using (HPLC). Five free sugars and nine combined were recorded. Nine free amino acids were detected in the stem, while 12 and 13 were recorded in the leaf and flower respectively. Fourteen protein amino acids were detected in the flowers, while 13 were detected in both the stem and leaf. Saturated and unsaturated fatty acid contents of different parts (stem, leaf and flower) of *Helichrysum conglobatum* were determined using the GLC technique.

Acknowledgements. This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Grant No. 3322].

REFERENCES

[1] Abd El-Moaty, H. I., Gouda, H. M., Sorour, W. A., Metwally, A., Hassan, L. M., Youssef, A. K., ... Galal, T. M. (2022): Nutritional composition of the edible wild plant *Opuntia littoralis* (Engelm.) in relation to different seasons and edaphic factors. – Applied Ecology and Environmental Research 20(4): 3655-3669.
[2] Abdel-Rahman, A. M. Migahid, A. M. (2019): The autecological characteristics of endangered medicinal plant *Thymus capitatus*, in the western Mediterranean region of Egypt. – Egypt. Journal of Botany 59(2): 387-398.
[3] Agboola, O. S., Adejumo, A. L. (2013): Nutritional composition of the fruit of the Nigerian wild date palm, Phoenix dactylifera. – World Journal of Dairy and Food Sciences 8(2): 196-200.
[4] AOAC (2000): Official Methods of Analysis of AOAC International. 17th Ed. – Association of Official Analytical Chemists, Washington DC.
[5] Bermúdez MA, Pereira L, Fraile C, Valerio L, Balboa MA, Balsinde, J. (2022): Roles of palmitoleic acid and its positional isomers, hypogeic and sapienic acids, in inflammation, metabolic diseases and cancer. – Cells 11(14): 2146.
[6] Bokov, D. O., Samylina, I. A., Nikolov, S. D. (2017): HPLC/RI and HPLC/PDA phytochemical analysis of free and bound carbohydrates in Galanthus woronowii Losinsk. and G. nivalis L. homeopathic matrix tinctures. – Pharmaceutical Chemistry Journal 50(12): 810-813.
[7] Boulos, L. (1995): Flora of Egypt Checklist. – Al-Hadara Publishing, Cairo.
[8] Boulos, L. (2002): Flora of Egypt. 1 Vol. 3 (Verbenaceae-Compositae). – Al Hadra Publishing. Cairo, pp. 209-210.
[9] British Pharmacopoeia (1993): International Edition 2: 146A. – Published on the recommendation of the Medicines Commission. H.M. Stationery Office, London.
[10] Cauquil, A. S., Ory-Magne, F., Jardiné, V., Galitzky, M., Rosito, M., Brefel-Courbon, C., Celebrini, S. (2017): Parkinson's patients can rely on perspective cues to perceive 3D space. – Brain Research 1663: 161-165.
[11] Chaplin, M. F., Kennedy, J. F. (1994): Carbohydrate Analysis - A Practical Approach. 2nd Ed. – Oxford University Press, Oxford.
[12] Chougui, N., Tamendjari, A., Hamidj, W., Hallal, S., Barras, A., Richard, T., Larbat, R. (2013): Oil composition and characterisation of phenolic compounds of Opuntia ficus-indica seeds. – Food Chemistry 139(1-4): 796-803.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2103_27472759

[13] DRC Staff (2007-2015): Integrated Agriculture Development of Northwestern Coastal Wadies. Annual Report. – Desert Research Center Publications, Cairo.
[14] Ebifa-Othieno, E., Kabasa, J. D., Nyeko, P., Nakimbugwe, D., Mugisha, A. (2020): Nutritional potential of tamarind (Tamarindus indica L.) from semi-arid and subhumid zones of Uganda. – Journal of Food Measurement and Characterization 14(2): 1125-1134.
[15] El-Beltagi, H., Afify, A. E. M., Fayed, S., Alansary, A. (2018): Enhancing effect of olive leaves extract on lipid profile and enzymes activity in streptozotocin induced diabetic rats. – Fresenius Environmental Bulletin 27(3).
[16] El-Beltagi, H. S., Mohamed, H. I., Elmelegy, A. A., Eldesoky, S. E., Safwat, G. (2019): Phytochemical screening, antimicrobial, antiaxidant, anticancer activities and nutritional values of cactus (Opuntia ficus indicia) pulp and peel. – Fresenius Environmental Bulletin 28(2A): 1534-1551.
[17] El-Ghazooly, M. G., El-Lakany, A. M., Abou-Shoer, M. I., Aly, A. H. (2003): Chemical constituents of *Helichrysum conglobatum* growing in Egypt. – Natural Product Sciences 9(4): 213-219.
[18] El-Monayeri, M. O., Fawzia, A. E., Youssef, M. M., Hanna, M. S. (1982): Effect of soil Moisture stress on carbohydrates, proteins and Mineral composition of three barley varieties. – Faculty of Agric. Ain Shams Univ. Research Bull. 2098.
[19] Faremi, A. Y., Ekanem, J. T. (2011): Haematological parameters and enzyme studies in Trypanosoma brucei-infected rats reared on Nigella sativa oil-based diet. – Asian Journal of Biochemistry 6: 90-97.
[20] Farag, R. S. (1995): Chemical and Physical Analysis of Oils and Lipids. – Published by Academic Library.
[21] Farag, R. S., Ahmed, A. I., Rashad, S. E., Ewies, M. W. (1986): Unsaponifiable matter of six pollens collected by honey bees in Egypt. – J. Agric. Res. 19(4): 52-58.
[22] Galal, T., Farahat, E., Mohsen, L. (2019): Phytosociology of rainfed barely along the western Mediterranean Coast, Egypt. – Taeckholmia 39(1): 18-33.
[23] Galbany-Casals, M., Sáez, L., Benedí, C. (2006): A taxonomic revision of *Helichrysum* sect. Stoechadina (Asteraceae, Gnaphalieae). – Botany 84(8): 1203-1232.
[24] Hendawy, S. F., Abouziena, H. F., Abd El-Razik, T. M., Amer, H. M., Hussein, M. S. (2019): Winter weeds and its control in the medicinal plants in Egypt: a survey study. – Egyptian Pharmaceutical Journal 18(1): 16.
[25] Labusch, C., Shishova, M., Effendi, Y., Li, M., Wang, X., Scherer, G. F. (2013): Patterns and timing in expression of early auxin- induced genes imply involvement of phospholipases. A (pPLAs) in the regulation of auxin responses. – Molecular Plant 6(5): 1473-1486.
[26] Li, Y., Hruby, A., Bernstein, A. M., Ley, S. H., Wang, D. D., Chiuve, S. E., ... Hu, F. B. (2015): Saturated fats compared with unsaturated fats and sources of carbohydrates in relation to risk of coronary heart disease: a prospective cohort study. – Journal of the American College of Cardiology 66(14): 1538-1548.
[27] Lopez, M. J., Mohiuddin, S. S. (2023): Biochemistry, essential amino acids. – StatPearls Publishing LLC.
[28] Makonya, G. M., Ogola, J. B. O., Muthama Muasya, A., Crespo, O., Maseko, S., Valentine, A. J., Ottosen, C. O. (2019): Chlorophyll fluorescence and carbohydrate concentration as field selection traits for heat tolerant chickpea genotypes. – Plant Physiol. Biochem. 141: 172–182.
[29] Mau, J. L., Miklus, M. B., Beelman, R. B. (1999): Shelf life studies of foods and Beverages. – In: Charalambous, G. (ed) Chemical, Biological, Physical and Nutritional Aspect. Elsevier Science publishers, Amsterdam.
[30] Meigs, P. (1973): World Distribution of Coastal Deserts, Their Natural and Human Environments. – In: Amiran, D. H. K., Wilson, A. W. (eds.) Coastal Deserts; Their Natural and Human Environments. University of Arizona Press, Tucson, pp. 3-13.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2103_27472759

[31] Mielke, M. S., Schaffer, B. (2010): Leaf gas exchange, chlorophyll fluorescence and pigment indexes of Eugenia uniflora L. in response to changes in light intensity and soil flooding. – Tree Physiology 30(1): 45-55.
[32] Murray, R. K., Granner, D. K., Rodwell, V. W. (2006): Harper's Illustrated Biochemistry. – McGraw-Hill Medical, New York.
[33] Pellet, P. L., Young, V. R. (1980): Nutritional evaluation of protein foods. – The United Nation University, Tokyo.
[34] Pessarakali, M. (1995): Hand Book of Plant Physiology. – Marc E-Dekker. Inc., New York.
[35] Piironen, V., Lindsay, D. G., Miettinen, T. A., Toivo, J., Lampi, A. M. (2000): Plant sterols: biosynthesis, biological function and their importance to human nutrition. – Journal of the Science of Food and Agriculture 80(7): 939-966.
[36] Primitivo, M. J., Neves, M., Pires, C. L., Cruz, P. F., Brito, C., Rodrigues, A. C., ... Ribeiro, V. S. (2022): Edible flowers of *Helichrysum italicum*: Composition, nutritive value, and bioactivities. – Food Research International 157: 111399.
[37] Silva, A. N., Ramos, M. L. G., Júnior, W. Q. R., de Alencar, E. R., da Silva, P. C., de Lima, C. A., ... Silva, M. A. V. (2020): Water stress alters physical and chemical quality in grains of common bean, triticale and wheat. – Agricultural Water Management 231: 106023.
[38] Stocker, O. (1960): Physiological and morphological changes in plants due to water deficiency. – Arid Zone Research 15: 63–104.
[39] UNESCO (1977): Map of the world distribution of arid regions. – MAB Technical Notes 7. UNESCO, Paris.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2103_27472759