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(Original Article)

Variability of Essential Oil Content, Composition and Antioxidants of Sweet Marjoram Grown at Different Locations in Egypt

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Abstract

The present study was carried out to evaluate the essential oil content and chemical composition of sweet marjoram (*Majorana hortensis* Mnch.) plants grown at seven locations in Egypt (Giza, Beni-Suef, Faiyum, Minia, Assiut, Sohag and east Elowainat). The plant samples were collected from such regions, their volatile oils were extracted and oil content (%) in air-dry herbage was calculated. The essential oil composition was determined by using GC-MS and the percentages of its main components from the studied regions were compared. Moreover, antioxidant potential of total phenolic content (TPC) and total flavonoid content (TFC) in plant extracts were determined. Results showed that the essential oil content and its percentages of volatile oil's major components were changed also TPC and TFC differed among the collected plant samples grown at the studied locations as a result of variations in the geographical regions, soil characteristics and climatic conditions. Thus, the essential oil percentage in such sites varied from 1.2 to 3.0 %. The main components of oil; 4-terpineol, γ-terpinene, α-thujene, (+) trans-4-thujanol, sabinene, α-terpinene, linalool, α-phellandrene, β-thujene, (-)-B-caryophyllene, β-pinene, α-pinene and carvacrol can be interpreted as a factor connected to oil composition which is characteristics for commercial marjoram. The difference variability between the maximum and minimum values of each constituent among the locations gave an account in descending order: 27.57, 11.76, 10.56, 10.46, 8.81, 8.66, 6.01, 4.01, 3.39, 2.16, 0.91, 0.89 and 0.21%, respectively. However, TPC ranged from 128.43 to 140.71 mg PEs /g dry wt., and TFC from 86.61 to 117.22 mg/RU g dry wt. According to this investigation, it is recommended to study the environmental factors of the location where marjoram plants will be cultivated since marjoram is considered to be a promising essential oil crop.

Keywords: *Marjoram, Essential oil composition, Antioxidants, environmental conditions.*

Introduction

Sweet marjoram (*Majorana hortensis* Mnch.) is an annual herb, natively grown in the tropical and warm temperate climates. It belongs to the Lamiaceae

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and is cultivated around the world in a range of ecological conditions (Weiss, 1997). It is widely used in culinary, pharmaceutical, cosmetic and aromatherapy industries as a source of flavor and fragrance. Marjoram extract and essential oil have been shown to possess antioxidant activity (Rameilah, 2009). Steam distillation has been traditionally used to obtain marjoram essential oil from complete aboveground herbage (leaves and stems) (Guenther, 1972). The beginning of flowering is considered to be the optimal harvesting stage for essential oil production as the oil concentration and preferred composition are at their peak (Amna *et al*., 2010).

Variations in the yield and chemical composition of the essential oil of Lamiaceae herbs with respect of geographical regions and altitude were apprised by many authors (Vokou *et al*., 1993; Özek *et al*., 1995; D'Antuono *et al*., 2000; Angioni *et al*., 2004; Telci *et al*., 2006; Celiktas *et al*., 2007; Zheljazkov *et al*., 2008; Verma *et al*., 2010; Bazaid *et al*., 2013; Gong *et al*., 2014 and Napoli *et al*., 2020). Since the oil mainly contains monoterpenes and sesquiterpenes which easily change by environmental conditions, other studies revealed that the volatile oil composition may vary according to localities, climatic regions and seasonal variations (Milos *et al*., 2001; Atti-Santos *et al*., 2004; Jordán *et al*., 2006; Amna *et al*., 2010; Abu-Darwish *et al*., 2012 and Mohiuddin, 2019). Furthermore, a direct relationship between soil nutrients and essential oil yield was found that related to enhance the amount of biomass production per unit area (Sangwan *et al*., 2001), also soil nutrients has beneficial effects on production and diversity of volatile terpenoids from plants (Ormeño and Fernandez, 2012).

Despite its popularity and its numerous uses, there is little published research on sweet marjoram growing criteria. Furthermore, there is a lack in the information concerning the comparison of essential oil composition among different productive locations in Egypt despite of the suitable conditions for volatile oil production. Therefore, the study aims to investigate the variation in essential oil content of sweet marjoram grown at different locations in Egypt.

Materials and Methods

Plant material

Dry samples of sweet marjoram herb and soil (3 samples of each presented as 3 replicates) were collected at the end of the growth season in August 2019, from seven productive locations; each one was taken from Giza (El-Ayyat), Beni-Suef (Samasta), Faiyum (Ibsheway), Minia (Maghagha), Assiut (Abnub), Sohag (Tahta), and east Elowainat governorates in Egypt (Figure. 1). Samples were transferred to laboratories of Floriculture Department, Faculty of Agriculture, Assiut University, for estimating various active constituents.

The climatic conditions (ambient temperature and relative humidity) of the collection sites during the growth season from September 2018 to June 2019 are presented in Table 1.

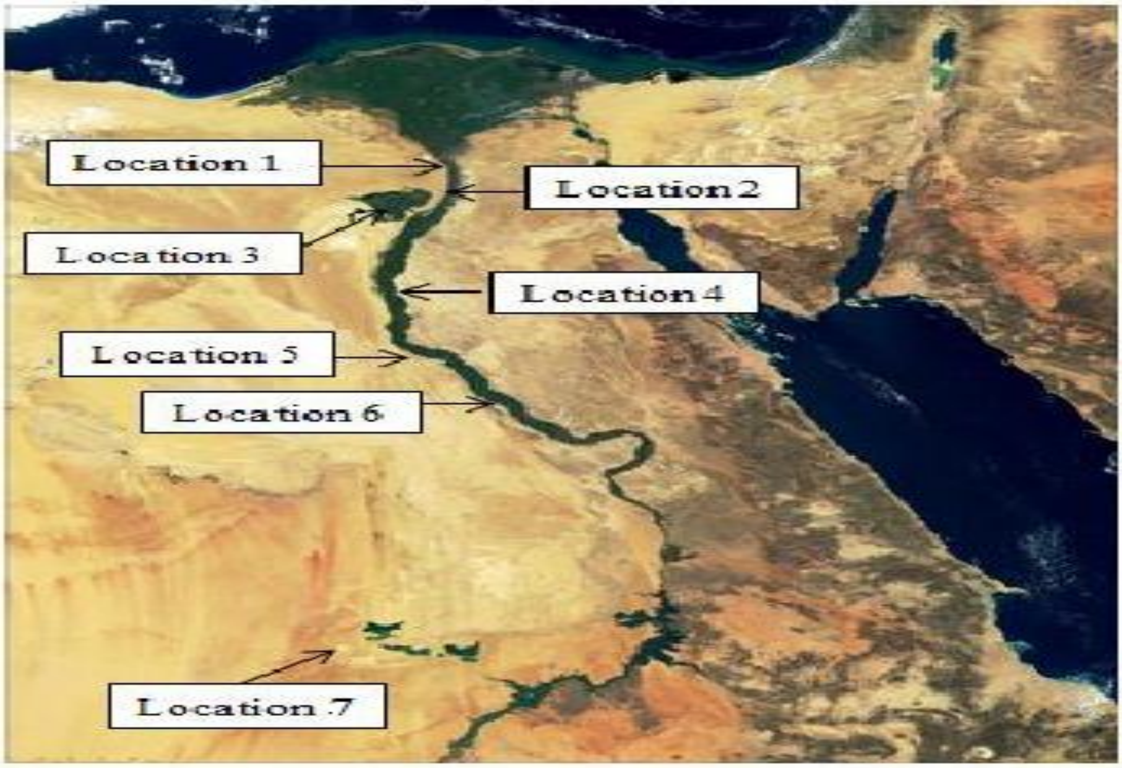

Figure 1. Map shows the seven productive locations of the current study where marjoram plant grew and collected from: Location 1: Giza (GPS coordinate 29°37'12.84"N, 31°15'34.21"E); Location 2: Beni-Suef (28°55'7.31"N, 30°48'55.57"E); Location 3: Faiyum (29°22'17.83"N, 30°38'34.8"E); Location 4: Minia (28°38'54.49"N, 30°50'14.97"E); Location 5: Assiut (27°16'2.65"N, 31°9'6.94"E) Location 6: Sohag (26°45'50.38"N, 31°29'53.56"E), and Location7: east Elowainat (22° 34' 58.44" N, 28° 42' 26.28" E).

Essential oil isolation

Air-dried marjoram samples (leaves and stems) from the seven locations (50g each, n= 3) were subjected to hydro- distillation (Guenther, 1972) in a Clevenger-type apparatus for 3 h. At the end of distillation, the oils were collected, dried with anhydrous sodium sulfate (Na_2SO_4) prior to analyses, measured, and transferred to glass tubes and stored at 4°C.

The essential oil percentage was calculated according to the following equation:

$$\text{Essential oil \%} = \frac{\text{oil volume in the graduated tube}}{\text{dry weight of sample}} \times 100$$

Table 1. The average monthly maximum and minimum temperatures and relative humidity (R.H) of the seven locations in Egypt during sweet marjoram growth from September 2018 to June 2019

Month	Temperature (C°)		R.H (%)	Temperature (C°)		R.H (%)	Temperature (C°)		R.H (%)	Temperature (C°)		R.H (%)
	Max	Min		Max	Min		Max	Min		Max	Min	
	Giza			Beni-Suef			Fayium			Minia		
September	33.3	20.6	53.24	34.1	19.5	39.40	34.7	19.8	56.26	33.9	19.0	50.75
October	30.2	17.4	55.23	30.7	16.5	36.40	31.4	17.0	58.30	30.8	15.9	53.43
November	25.1	13.4	58.28	26.9	13.2	32.50	26.1	12.3	64.85	25.5	10.7	60.78
December	20.7	9.2	64.05	20.9	7.41	60.40	21.5	7.7	67.65	20.7	6.0	63.15
January	19.4	7.9	63.1	19.7	5.70	57.30	20.3	6.0	66.08	19.8	4.8	62.09
February	20.8	8.6	59.01	21.8	6.95	50.10	22.1	6.7	59.76	21.7	5.7	55.41
March	23.7	10.7	57.26	25.8	10.20	34.40	25.2	9.5	55.10	25.2	8.5	50.98
April	28.2	14.0	49.35	31.2	14.30	40.90	30.2	13.1	47.95	30.3	12.6	42.22
May	32.0	17.3	47.11	35.1	18.10	43.30	33.7	16.8	43.78	34.1	16.7	36.65
June	34.7	20.7	45.1	37.0	20.4	43.50	36.8	19.8	42.13	36.2	19.6	39.32
Mean	**26.81**	**13.98**	**55.17**	**28.32**	**13.22**	**43.82**	**28.20**	**12.87**	**56.19**	**27.82**	**11.95**	**51.48**

Month	Assiut			Sohag			E.Elowainat		
	Temperature (C°)		R.H (%)	Temperature (C°)		R.H (%)	Temperature (C°)		R.H (%)
	Max	Min		Max	Min		Max	Min	
September	34.0	19.7	38.87	36.0	20.3	43.24	38.5	23.5	21.49
October	31.0	16.5	40.76	33.7	18.0	42.05	34.5	19.9	25.28
November	25.3	11.2	47.59	27.9	13.1	50.32	28.7	13.9	33.25
December	20.4	6.9	50.73	23.1	8.7	56.13	24.3	9.4	38.93
January	19.0	5.5	49.60	21.8	7.2	55.71	23.1	7.5	38.17
February	21.2	6.7	41.32	23.6	8.3	50.78	25.7	9.6	29.50
March	24.8	9.8	35.20	27.5	11.4	43.26	29.6	12.8	22.44
April	32.2	14.3	27.40	33.2	16.0	34.92	34.5	17.5	17.67
May	33.8	18.1	25.05	37.3	20.3	30.11	37.9	21.8	15.75
June	36.7	21.1	27.75	39.4	22.7	32.19	40.7	24.9	15.30
Mean	**27.84**	**12.98**	**38.43**	**30.35**	**14.60**	**43.87**	**31.75**	**16.08**	**25.79**

* Data was obtained from Egyptian Meteorological Authority (EMA) stations of the concerned locations.

Analysis of essential oil composition

Quantitative deter environmental conditions, Lamiaceae, mination of the main oil fractions of the dry herb was analyzed by GC/MS at National Research Center, Giza, Egypt. The GC analysis was carried out using HP 6890 Series/ GC system equipped with HP5973/Mass selective detector operating by electron ionization (EI) at 70 eV, and TR-FAME capillary column (30 m X 0.25 mm i.d, 0.25 µm film thickness). The multi-step temperature program was increased from 80ºC (held for 2 min) to 230 ºC (held for 2 min) with the rate of 3ºC min^{-1}. The carrier gas was helium at the flow rate of 2 ml min^{-1} and the sample size was 1 µl of diluted samples (5µl oil / 2 ml chloroform, v/v). Injector temperature was 250ºC and a spectral range of 35-500 *m/z* analysis was used. Identification of essential oil constituents was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC-MS data system, other published mass spectra and CAS number. Retention index was calculated for each compound using the retention times of a homologous series of C_6-C_{26} n-alkanes as outlined by Schmidt *et al.* (2008).

Preparation of marjoram samples extracts

Air-dried samples of marjoram herb (leaves and stems) were grounded and extracted by 70% ethanol according to the method described by Ahmed *et al.* (2019)

Determination of total phenolic content (TPC)

The content of total phenolic of marjoram sample extract was determined colorimetrically based on the Folin-Ciocalteau method of Kang *et al.* (2010). The TPC content was expressed as mg pyrocatechol equivalents (PEs) per g plant dry weight.

Determination of total flavonoid contents (TFC)

Total flavonoid content of marjoram sample extract was determined using colorimetric method according to the method described by Zhu *et al.* (2010). The TFC content was expressed as mg rutin equivalents (REs) per g plant dry weight.

Soil sampling

In early September 2018, before land preparation, three surface soil samples were collected at a depth of 0-30 cm from each location will be cultivated with sweet marjoram for soil analysis. Samples were transported to Soils and Water Department laboratory, Faculty of Agric., Assiut Univ., Egypt in plastic bags for soil analysis. The collected soil samples were air- dried, crushed, sieved through a 2 mm sieve and then stored in air-tight polyethylene bottles for analysis. Some chemical and available nutrients of the samples collected from the investigated locations are present in Table 2.

Table 2. Soil characteristics (0-30 depth) and the concentrations of extractable nutrients of the seven locations cultivated with sweet marjoram in Egypt in September 2018

Locations	Soil Texture	pH	EC (dS/m)	OM (%)	Available nutrient (mg/kg)						
					N	P	K	Fe	Mn	Zn	Cu
Giza	Sandy	8.37	0.51	1.68	323	4.11	140	3.76	2.41	0.22	0.67
Beni-Suef	Sandy loam	7.85	1.48	2.81	625	4.55	940	3.44	2.36	0.34	1.41
Faiyum	Sandy loam	7.91	0.69	2.55	429	3.67	280	3.11	2.27	0.36	1.29
Minia	Loamy	7.72	0.77	2.14	385	6.39	540	2.42	2.34	0.69	1.49
Assiut	Loamy	7.71	0.85	2.48	511	4.96	766	2.36	2.19	0.74	1.02
Sohag	Sandy loam	7.55	5.54	1.87	410	3.76	240	2.44	2.06	0.38	0.89
East Elowainat	Loamy sand	8.80	0.36	0.27	307	2.88	160	2.07	2.00	0.30	0.32

Soil analysis

The following soil characteristics were performed on the tested soil samples:

- Particle size distribution, Mechanical analysis was determined using the pipette method (Jackson, 1973).
- Soil pH was measured in (1:1) soil to water suspension using Beckman pH meter (Page *et al*., 1982).
- Total soluble salts, the electrical conductivity (E.C) was measured in1:1 soil to water extract using conductivity meter according to (Jackson, 1973).
- Organic matter (O.M.) content was determined according to Walkely and Black’s wet oxidation method (Jackson, 1973).

Available soil nutrients

Available nitrogen was extracted with 1% K_2SO_4 at a ratio of 1:5. Then, 20 ml of the extract were distilled with the addition of 1 g Devarda's alloy using a micro Kjeldahl’s distilling unit into a flask containing 10 ml boric acid-mixed indicator solution until about 50 ml distillate in each flask was collected. After the distillation, the available nitrogen content was determined in the distillate by titrating with standardized 0.01 N sulphuric acid (Jackson, 1973). Available soil phosphorus was extracted using 0.5 M $NaHCO_3$ solution, at pH 8.5 ratio 1:10, then the extracted P was measured by spectrophotometer using stannous chloride phosphomolybdic-sulfuric acid system as outlined by Olsen *et al*. (1954) and (Jackson, 1973). Available soil potassium was extracted from 5 g of soil sample using 50 ml of 1 M ammonium acetate at pH 7.0 and measured by flame photometer (Jackson, 1973). The available micronutrients (Fe, Mn, Zn and Cu) were extracted using 0.005 M diethlene triamine penta-acetic acid (DTPA) at pH 7.3 ratio 1:2 according to Lindsay and Norvell (1978). DTPA-extractable Fe, Mn, Zn and Cu were determined by atomic absorption spectrophotometer (Perken Elmer A Analyst 400).

Statistical analysis

The obtained data were statistically analyzed using Statistix 8.1 analytical software, and the mean were compared using the least significant difference (L.S.D.) test according to Dowdy and Wearden (1983).

Results and Discussion

Essential oil content (%)

It is clear from data in Table 3 that the growing location was very important for marjoram essential oil. Obviously, the oil percentage greatly varied by the location of samples. The maximum content (3.0%) was obtained from east Elowainat although it was the most southern location with higher temperatures (Table 3), as well as soil characteristics had higher pH, lower in organic matter and E.C, and relatively lower concentrations of available nutrients than other locations. Minia region resulted in the next high oil content (2.3%). In contrast, Giza site showed the minimal oil content (1.2%), but Faiyum, Assiut and Sohag recorded moderately oil percentages were 1.5, 2.0 and 1.8%, respectively. These findings indicated that essential oil of marjoram cultivation guidelines stressing geographic, climatic conditions and soil characteristics needs have been devised. Clearly, environmental factors of east Elowainat is favourable for the physiological dynamics and regulating the essential oil production that may be due to soil type and warm climate have important effects on the biogeochemical cycling nutrients in agroecosystems (Marschner *et al.,* 1997 and Newton *et al.,* 2007) reflecting directly on adequate nutrients requirement and plant quality and supposed the increase oil content as indicated by previous research on medicinal plants (Ncube *et al.,* 2012). The obtained results support previous observations on oregano and marjoram grown in different regions of many countries by Vokou *et al.* (1993), D'Antuono *et al.* (2000), Amna *et al.* (2010), Bazaid *et al.* (2013), Gong *et al.* (2014) and Napoli *et al.* (2020). These results are parallel to the results of Milos *et al.* (2001) on *Satureja montana* L.; and Zheljazkov *et al.* (2008) on *Ocimum basilicum* L., and *O.sanatum*. These authors concluded that the essential oils were differently depending on microclimatic conditions and soil characteristics of the habitat in which the plants grew.

Table 3. Essential oil percentage of sweet marjoram plants cultivated at the seven locations in Egypt

Essential oil (%)	Locations						
	Giza	**Beni-Suef**	**Faiyum**	**Minia**	**Assiut**	**Sohag**	**E. Elowainat**
	1.2	1.6	1.5	2.3	2.0	1.8	3.0

In this regard, Sangwan (2001) reported that essential oil production is highly integrated with the physiology of the whole plant and so depends on the metabolic state and preset developmental differentiation programme of the synthesizing tissue. Essential oil productivity is considered ecophysiologically and environmentally friendly.

It is important to keep in mind that marjoram samples of the different locations in Egypt have potential as a new high-value essential oil content in comparison with other countries in the world such as India (Amna *et al.,* 2010), Saudi Arabia (Bazaid *et al.*, 2013), China and Pakistan (Gong *et al.*, 2014) and Italy (D'Antuono *et al.* 2000 and Napoli *et al.,* 2020). Apparently, marjoram could develop well under environmental conditions of Egypt especially under east Elowainat region which characterized by more suitable climate and soil properties that provide with large amounts of essential oil with typical composition which would facilitate marketability since marjoram is considered to be a promising essential oil crop in Egypt.

Essential oil composition

Many investigations on oregano's essential oils have been done, revealing a large deal of its intraspecific variability. The occurrence of three well-defined chemo-groups is generally acknowledged, namely: 1) the linalool, terpinene-4-ol and sabinene hydrate group; 2) the carvacrol and/or thymol group; and 3) the sesquiterpenes group (Skoula and Harborne 2002; Bazaid *et al.,* 2013 and Napoli *et al.,* 2020). Since separated biosynthetic routes seem responsible for the in-plant production of these major chemical groups it is reasonably deduced that the prevalence of one of them (hence the outcoming chemotype) is basically genetically determined (Crocoll *et al., 2*010 and Novak *et al.,* 2010). Inside each chemotype, significant variations may occur leading to a wide variability in the measured contents of certain chemical constituents. Many factors may play roles in these variations, and therefore in assessing the final chemical characteristics of Origanum essential oils. The field of study is huge and much research has been conducted worldwide to explore the effects exerted on essential oil yield and quality by a number of environmental factors such as altitude (Giuliani *et al.*, 2013), temperature (Novak *et al.,* 2010), harvest season and geographical position (Kokkini *et al.,* 1997; D'Antuono *et al.*, 2000 and Verma *et al.,* 2010). It is well recognized that cropping technique is also a crucial factor in assessing, within each chemotype, important variations of essential oil components (Sangwan *et al.,* 2001, and Carrubba and Catalano, 2009).

Table 4. Essential oil constituents (%) of marjoram cultivated plants at the seven locations in Egypt

Locations Compound%	Giza	Beni-Suef	Fayium	Minia	Assiut	Sohag	E. Elowainat	CAS #
Sabinene	12.73	10.28	5.13	11.63	3.92	5.45	7.51	3387-41
γ-Terpinene	12.81	11.23	10.82	22.53	10.81	11.72	10.77	099-85
α-Terpinene	6.35	6.79	0.16	6.39	8.82	0.56	0.76	99-86-5
4-terpineol	19.53	12.48	35.43	18.46	26.61	40.05	35.87	562-74-3
(+)-trans-4-Thujanol	9.75	16.77	tr	tr	9.07	tr	6.31	17699-16-0
Linalool	7.28	1.68	tr	1.69	1.27	3.31	tr	078-70-6
α-Pinene	tr	tr	0.59	1.31	0.42	0.47	tr	07785-26-4
β- Pinene	tr	1.03	tr	0.12	0.70	0.12	0.13	0127-91-3
α-Thujene	tr	1.46	2.85	2.46	4.16	12.02	4.14	02867-05-2
β-Thujene	tr	3.26	6.48	5.56	3.38	6.17	3.09	28634-89-1
α –Phellandrene	1.76	0.88	1.50	1.32	1.27	tr	4.89	0555-10-2
Carvacrol	0.44	0.45	0.24	0.37	0.41	0.34	0.31	0499-75-2
(-)-β-caryophyllene	5.12	4.03	6.19	5.62	5.37	5.14	5.86	0087-44-5
Other compounds	24.22	29.69	30.61	22.54	23.81	14.67	20.37	
Total	99.99	100.00	100.00	100.00	99.99	99.99	100.00	

Values are mean of three samples, tr= component present less than ≥ 0.001

The obtained results in Figure 2 and Table 4 showed the qualitative and quantitative GC-MS analysis of marjoram volatile oil in the present study identified the main components in all growing sites. The known constituents of oil grouped into three items; the major components (more than 10 %), minor components (less than 10% and more than 1%) and trace ones (less than 1%). The analysis allowed for full characterization of the extracted essential oils. More than 50 essential oil components were fully identified, but for easier comparison the different chemotypes of marjoram oil were identified based on main compounds of essential oils such as 4-terpineol, sabinene, γ-terpinene, linalool, α-terpinene, trans-4- thujanol and β-caryophyllene. Clearly, the main component of 4-terpineol was the largest percentage ranging from 12.48 to 40.05%

depending on the different locations which following the descending order: Sohag > east Elowainat> Fayium > Assiut > Giza > Minia > Beni- Suef. The next high component in the essential oil was γ-terpinene ranged from 10.77 to 22.53% since the studied locations followed the descending order: Minia > Giza>Beni-Suef> Sohag> Fayium > Assiut > east Elowainat. However, sabinene showed considerable variation ranged from 3.92 to 12.73% and the locations decreased in the sequence of Giza> Minia> Beni-Suef>east Elowainat>Sohag> Fayium> Assiut. The component of α- terpinene was ranged from 0.16 to 8.82% and the locations following the descending order: Assiut> Beni-suef> Minia> Giza> east Elowainat> Sohag > Fayium. The component of linalool in the essential oil was ranged from 1.27 to 7.28% and the regions decreased in the sequence of: Giza> Sohag > Minia> Beni-Suef> Assiut, meanwhile, it was trace in oil samples of Fayium and east Elowainat.

The component of α-pinene was ranged from 0.42 to 1.31% and the locations following the descending order: Minia > Fayium> Sohag> Assiut, but it was in trace in Giza, Beni-Suef and east Elowainat sites. The component of β-pinene was ranged from 0.12 to 1.03% and the locations following the descending order: Beni-suef > Assiut > east Elowainat > Minia> Sohag, while trace amount was observed in Giza and Fayium locations. The component of (+) trans-4-thujanol was ranged 6.31 to 16.77% and the sites following the descending order: Beni-Suef > Giza> Assiut> east Elowainat, but trace content was found in Fayium, Minia and Sohag locations. The component of α-thujene was ranged from 1.46 to 12.02% and the locations following the descending order: Sohag> Assiut> east Elowainat> Fayium> Minia> Beni-Suef, while trace amount was found in Giza site. The component of β-thujene was ranged from 3.09 to 6.48% and the locations following the descending order: Fayium > Sohag > Minia>Assiut> Beni-Suef> east Elowainat, but it was in trace content Giza site.

The component of α-phellandrene was ranged from 0.88 to 4.89% and the locations following the descending order: east Elowainat> Giza> Fayium> Minia>Assiut> Beni-Suef, meanwhile, it was in trace level in Sohag location. The component of carvacrol was ranged from 0.24 to 0.45% and the sites following the descending order: Beni-Suef>Giza> Assiut>Minia> Sohag> east Elowainat > Fayium. The component of (-)-B-caryophyllene was ranged from 4.03 to 6.19% and the sites following the descending order: Fayium> east Elowainat> Minia> Assiut> Sohag> Giza > Beni-Suef.

As evidenced, in the studied locations the composition of marjoram essential oil showed considerable variations, such variations in the essential oil content might be attributed to the varied geographical and climatic regions because the oil mainly contains monoterpenes and sesquiterpenes which easily change by environmental conditions and geographic origin. These results are coincided with those obtained on *Origanum vulgare* by D'Antuono *et al.* (2000), Verma *et al.* (2010), Bazaid *et al.* (2013) and Gong *et al.* (2014). They concluded that the chemical composition of oregano essential oil was varied according to geographical regions, altitude and climatic factors such as temperature and drought. Also, the chemical composition varied according to the plant growing location and some components were disappeared in some locations and presented in the other whereas the climate of the zone plays an important role in the essential oil formation. Thus, oil yield and composition were significantly differed depending on the locations where the plants grew.

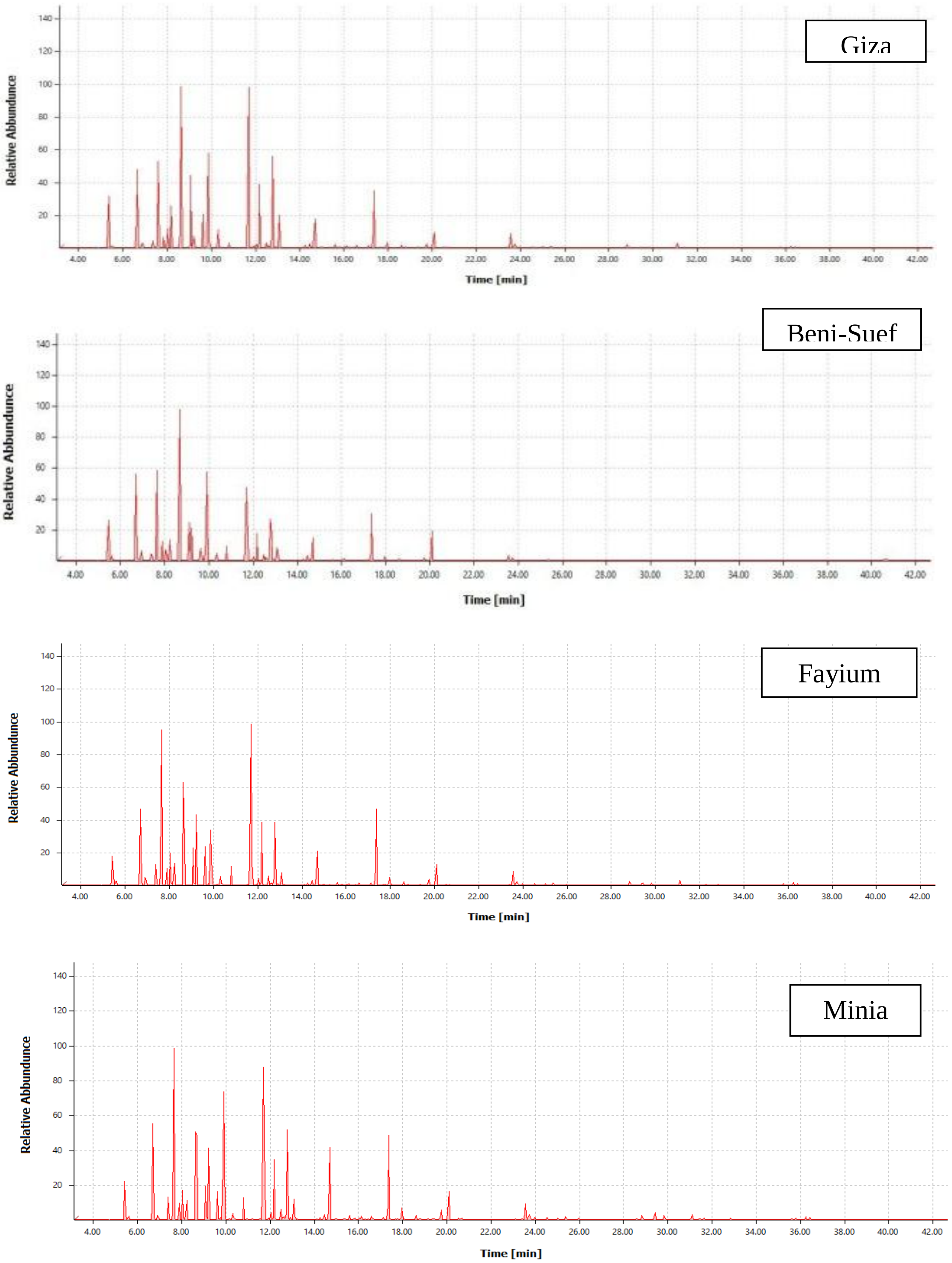

Figure 2. Total GC analysis of marjoram's essential oil composition collected from seven locations in Egypt

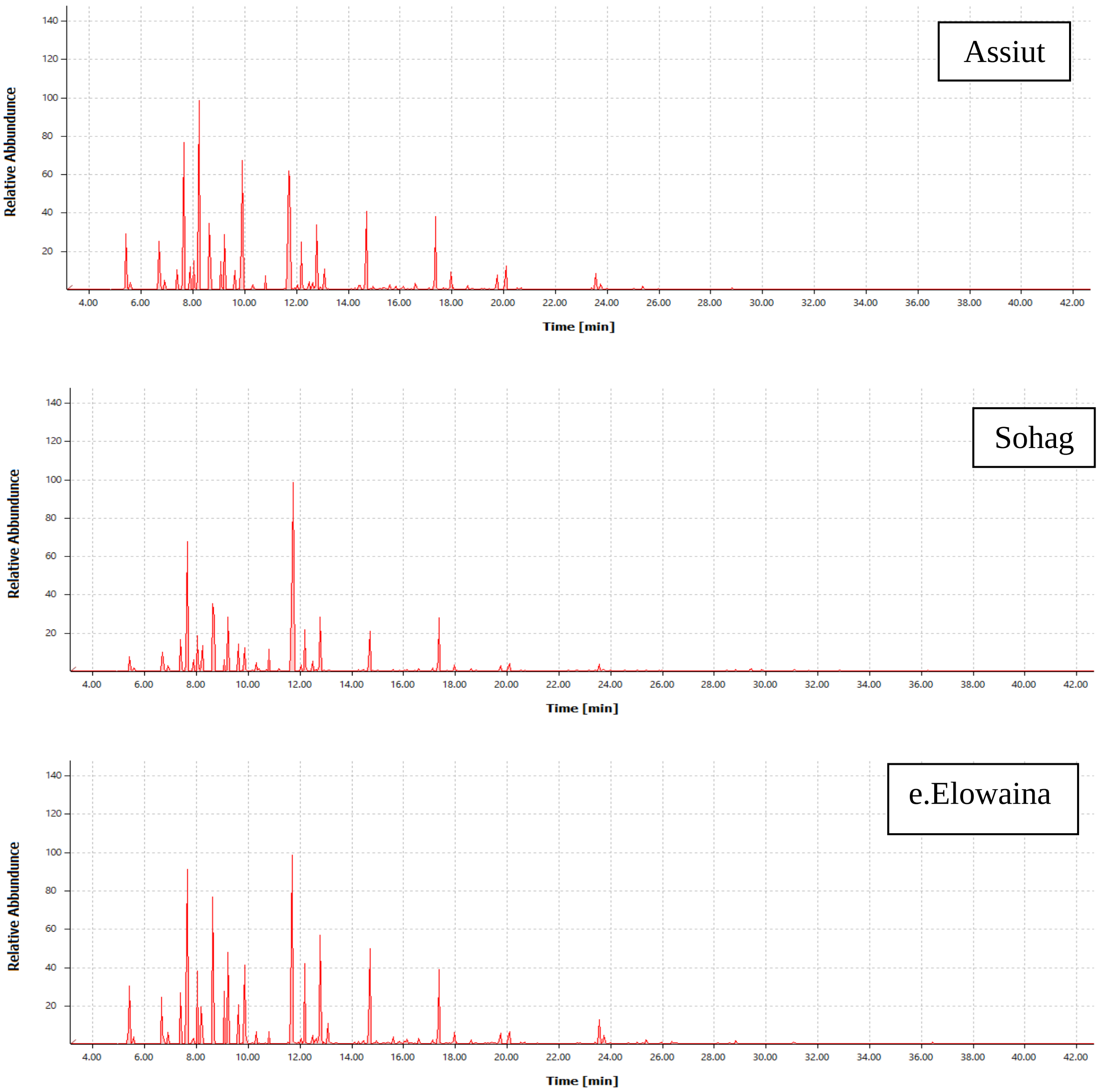

Figure 2. Continue. Total GC analysis of marjoram's essential oil composition collected from seven locations in Egypt

Different studies can be found in the scientific literature regarding marjoram essential oil yield and how it changes depending on the geographical origin (D'Antuono *et al* ., 2000; Verma *et al* ., 2010, and Bazaid *et al* ., 2013). In this case, the essential oil yield for typicus variety was higher in upper semi-arid zones than that obtained from sub-humid regions. From these results it is cleared that marjoram essential oil is influenced by the habitat in which the plants grow. Not only the variations in the composition of the essential oils related to geographical and climatic regions, but also changes in the essential oil content and composition was varied during annual growth. Such observations were found in marjoram (Verma *et al.*, 2010), oregano (Amna *et al.*, 2010), and thyme (Atti-Santos *et al.*, 2004 and Jordán *et al.*, 2006).

The differences in the essential oil composition among the different locations may be due to the development or variations in cultivation conditions of the plant or as a result of structural or physiological modification of the plant cause by specific environmental factors (phenotypic plasticity). Taking into consideration the previous results, it could be noticed that the growing locations were affected the oil percentage and oil chemical composition. The main components obtained from oil analysis support the others obtained previously by numerous researchers as stated above.

Generally, the results discussed above concluded that it will be useful to study the conditions of the location where medicinal and aromatic plants will be cultivated since the geographical regions, even in the same country, affected not only the essential oil yield, but also its composition. Thus, this investigation is recommended to explain exactly how the environmental conditions, as well as altitude can affect the essential oil content and chemotype depending on the bioclimatic area.

Table 5. Total phenolic and flavonoid content in marjoram extracts as affected by different locations in Egypt

Phytochemicals	Locations						
	Giza	Beni-Suef	Faiyum	Minia	Assiut	Sohag	E.Elowainat
TPC (mg PE/ g DW)	138.69	138.38	138.81	140.71	132.94	128.43	137.00
TFC (mg RU/ g DW)	101.53	115.31	111.90	117.22	102.96	86.61	113.58
L.S.D		**TPC**		**TFC**			
0.05		2.11				2.54	
0.01		2.93				3.52	

Total phenolic content (TPC) and total flavonoid content (TFC)

It is interesting to note a close biosynthetic relationship of the two main antioxidants in marjoram extracts (TPC and TFC) under the studied locations (Table 5). Apparently, TPC and TFC were greatly influenced by environmental factors and soil characteristics of the different sites. The highest TPC and TFC were induced under Minia region, in contrast to this, Sohag recorded the lowest levels. However, the other locations showed intermediate results. Mostly, the differences among the different locations were significantly, particularly regarding TFC.

Since nutrition quality of medicinal plants can affect their antioxidant content. Which also depend on genetic, environmental factors (temperature, light and nutrient availability), the agricultural techniques used (fertilization and date of harvest etc.), and post-harvest storage conditions (Mohiuddin, 2019). Moreover, Dumas *et al.,* (2003) demonstrated that leaf polyphenol content of young greenhouse tomato plants was considerably increased in response to low N availability, but there is no available information about the effect of P or K supply on the fruit phenolic compounds. Temperatures below 12°C strongly inhibit phenols biosynthesis and temperatures above 32°C stop this process.

Aćimović *et al* (2015) reported that climate conditions during the growth season of caraway plant as daily temperatures, altitude and longitude, as well as soil properties (pH, E.C and O.M content) at three locations of cultivation had a neutral reaction to antioxidants and controlling TPC and TFC in seed quality. Gad (2019) revealed that total soluble phenolic compounds in leaves of *Salvia coccinea* was markedly affected by soil moisture content, nutrient element availability, salinity level and the ambient temperatures. Omar (2020) found that the addition of organic matter to soil significantly increased TPC and TFC in caraway seeds.

Conclusions

The present study offers research basis for contributing primary knowledge of sweet marjoram plants from seven locations in Egypt regarding the essential oil composition and antioxidants content of phenolics and flavonoids.

In fact, samples of marjoram plants from such locations showed great variability in essential oils content and their range of composition included all chemotypes already described in this work. Furthermore, phenolics and flavonoids synthesis are subject to continuous variation within the examined locations according to the geographical site, soil characteristics and climatic conditions which limitation of essential oil quality and quantity of sweet marjoram which would facilities marketability.

As far as utilizing new regions for cultivation of marjoram in Egypt, this paper could represent a starting point for further analyses aimed at selecting high-value essential oil yields.

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تباين محتوى وتركيب الزيت الطيار ومضادات الأكسدة في البردقوش الحلو النامي في مواقع مختلفة بمصر

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الملخص

أجريت هذه الدراسة بهدف تقييم محتوى الزيت الطيار وتركيبه الكيميائي لنباتات البردقوش الحلو المنزرعة في سبعة مواقع بمصر (الجيزة، بني سويف ، الفيوم ، المنيا ، أسيوط ، سوهاج ، شرق العوينات) حيث جمعت منها عينات نباتية لاستخلاص الزيت الطيار وتحديد نسبته المئوية في العشب الجاف وأيضاً تحليل مكوناته الكيمائية باستعمال جهاز GC-MS ، وكذلك تم تقدير المحتوى الكلى لكل من الفينولات و الفلافونيدات كمضادات للأكسدة في المستخلصات النباتية للمقارنة بين المواقع.

وقد أوضحت النتائج ما يلي

- كان هناك اختلافات ملحوظة في نسبة الزيت الطيار ومكوناته الرئيسية وأيضاً المحتوى الكلى لكل من الفينولات والفلافونيدات بين عينات المواقع المختلفة.
- اتضح جلياً أن الاختلافات السابقة قد ارتبطت بشدة بتغيرات المناطق الجغرافية، خصائص التربة وتأثيرات الظروف المناخية في المواقع المدروسة.
- تراوحت نسبة الزيت الطيار في العشب الجاف من 2.1 الى 3.0% في هذه المواقع.
- أظهر التحليل الكيماوي أن المكونات الرئيسية للزيت الطيار والتي لها علاقة وثيقة بالمواصفات التجارية للبردقوش هي 4- تربينول، جاما- تربينين، ألفا- ثيوجين، (+) ترانس-4-ثوجانول، سابينين، ألفا- تربينين، لينالول، ألفا-فيلاندرين، بيتا- ثيوجين، (-) - بيتا- كاروفيللين، بيتا-بينين، ألفا- بينين وكارفاكرول. وقد أوضحت تباين الفروق بين القيمتين الدنيا والقصوى على الترتيب لكل مركب بين هذه المناطق تنازلياً كالاتي:27.57 و11.76 و 10.56و10.46 و8.81 و8.66 و6.01 و4.01 و3.39 و2.16 و0.91 و 0.89 و0.21%.
- أظهر تقدير محتوى الفينولات الكلية أنها تراوحت ما بين 128.43 إلى 140,71 مجم / مكافئ PEs لكل جم جاف، وكذلك تراوح محتوى الفلافونيدات الكلية من 86,61 إلى 117,22 مجم / مكافئ RU لكل جم جاف.

تشير نتائج هذه الدراسة لأهمية معرفة ودراسة الظروف البيئية للموقع الذي سوف يزرع بنبات البردقوش والذي يعتبر من محاصيل الزيوت الطيارة الواعدة.