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Effect of seasonal variations on the yield of essential oil and antioxidant of *Achillea fragrantissima* (Forssk) Sch. Bip

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Temperature stress is becoming the major concern for plant scientists worldwide due to climate change. Temperature stress has devastating effects on plant growth and metabolism. The aim of the study was to investigate the effect of climatic seasonal change on the yield and composition of essential oil of the plant, *Achillea fragrantissima*. The gas chromatography-mass spectrometry (GC-MS) used to analyze the essential oil collected during dry and wet seasons revealed it has 25 compounds. The major compounds were santolina alcohol (5.31), camphor (4.3), and cedrene (9.01) during winter months, while the percentages of α-cubebene (17.1%), spathulenol (1.54) and globulol (5.2) were highest during summer season. The analysis of essential oil in the two seasons revealed that there are different amounts and composition of essential oils. The antioxidant activity of the essential oil of the plant shows higher activity of IC_{50} 0.11±0.01g/L and Ec_{50} 0.25±0.02 g/L during winter than in summer. However, the reducing capacity of standard substances used (ascorbic acid and α-Tecopherol) were 0.033±0.001 and 0.93±0.07 g/L for DPPH and 0.091±0.002 and 0.026±0.002 g/L for FRAP method, respectively. These results showed that *A. fragrantissima* is a natural source of active compounds, and antioxidant properties, and the difference in chemical composition leads to changes in the antioxidant activity of the plant, which contributes to seasonal change.

Key words: *Achillea fragrantissima*, antioxidant, seasonal vibration, essential oil.

INTRODUCTION

Gebel Elba is a mountain of south-eastern Egypt. This mountain range is considered a continuation of the granitic formation of the Red Sea highland complex between Egypt and Sudan. It is situated between 36 and 37° of the eastern longitudes and about 22° of the northern latitude. The flora of this area comprises hundreds of species of plants.

Gebel Elba receives a vast majority of its precipitation as mists that form the Oasis on the upper areas of the mountains. The mountain tops get 400 mm of rainfall during the year and the surrounding area gets notably less. To cope with this problem some of the plants have evolved to uptake water from their leaves from the mists that lay over the area. There is no real watershed in

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Gebel Elba because any water that escapes the oasis quickly evaporates or soaks into the sands. In aromatic crops photoperiod, intensity of light, temperature and season of harvesting have profound influence on terpenoid composition of those crops (Voirin et al., 1990; McGimpse et al., 1994)

According to Brant et al. (2008), plant species belonging to the same botanical family do not present a similar behavior based on the environmental conditions. As one of the many factors that may influence the characteristics of essential oils, climatic variations that occur in a year have been the focus of many researchers attempting to identify the most appropriate time of the year for optimal extractions in terms of yield and/or compound concentration. When the set of climatic factors in seasonal climates with two well-defined seasons is modified, these variations act on the plants and generally alter their metabolism (Scherer, 2007).

Lawlor (2002) reported that the growth and metabolism of plants have optimum temperature limits for every species. The growth, development and productivity of plants that are continuously exposed to environmental stimuli are affected. High temperature, insufficient light, and water deficiency all are factors that negotiate plants' productivity (Lawlor, 2002).

El Zalabani et al. (2017) reported that the essential oil yield of *Artemisia monosperma* during summer was higher than that detected in plants grown in Libya during winter, where the essential oil yield was 0.16% v/w. This higher yield of essential oil is related to plant growth and its ability to survive under drought condition. Plants which grow under this condition can adapt due to their ability to accumulate chemical compounds (El Zalabani et al., 2017).

Achillea fragrantissima, Asteraceae family is a common plant in the Mediterranean region and easily found growing in fields and on roadsides. It contains a high percentage of flavonoids, tannins, volatile oils, sterols and triterpenes; it contains unsaturated amides and sesquiterpene lactones. *Achillea* was highly valued as a medicinal plant for its antiseptic properties. It was used to cover cuts and sores and hasten scar tissue formation. Its clinical uses are not described (Nemeth and Bernath, 2008).

The present work attempts to study the effects of seasonal variation on biomass, essential oil yield, chemical composition, and antioxidant of essential oil of the plant, *A. fragrantissima* which grows widely in Gebel Elba, in 2017.

MATERIALS AND METHODS

Area of study

The Gebel Elba mountainous group is one of 3 coastal mountains in the south-east corner of Egypt that faces the Red Sea, extending between latitude 24° 50'N and 22° N on the Sudano-Egyptian border. A wide coastal desert plain separates the Gebel Elba mountain range from the Red Sea coast. Although not the highest in its group, Gebel Elba is nearest to the sea (20-25 km), as described by Monier and Kadry (2006).

Climate of the study area

Monier and Kadry (2006) reported that the area of the study lies in the arid climatic province; its rainfall ranges between 50 and 10 mm year^{-1} in spring; it has mild winter (18-22°C) and hot summer (28-33°C). As for its geographical position and peculiar set of environmental conditions, Gebel Elba receives greater water revenue from orographic precipitation than the other northern blocks.

Plant material

Flowering aerial parts of *A. fragmentissima* (Figure 1) were collected from wild population in Gebel Elba, Hlayeb Region, in 2017, during two seasons: dry season (summer) and wet season (winter). The plants were identified in Desert Research Center, Cairo Egypt; voucher specimens were deposited in the Herbarium of Desert Research Center.

Sample preparation

The fresh aerial parts of *A. fragmentissima* (50 g plant powder) were extracted by percolation with a mixture of n hexane- ether (1:1, v/v). The solvents were removed subsequently under reduced pressure (Elsharkawy et al., 2013).

Fresh and dry weight analysis

For fresh weight the plants were uprooted and washed to remove surface adhered soil particles and wrapped in blotting papers. Dry weight of the plants was recorded after drying them at 80°C for 24 h in hot air oven.

GC-MS analysis

The constituents of the volatile oils obtained from the n-hexane-ether extracts were analyzed by GLC and GC-MS as reported previously (El-Shazly et al., 2002). Compounds were identified by comparison of their retention indices (RI) (C9 to C24 n-alkane mixture) and mass spectra with those reported in the literature (Merfort et al., 1994; Cavalieri et al., 2004; Adams, 1995).

Antioxidant activity

To evaluate the antioxidant activity of essential oil of *A. fragmrntissima* in the two seasons (summer and winter), its scavenging activities on DPPH radicals were tested. DPPH test is a direct and reliable method for determining radical scavenging action. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515 to 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, that can be quantitatively measured from the changes in absorbance. According to Abdallah et al. (2016):

$$\%\ \text{Inhibition} = \frac{(\text{A blank} - \text{A sample})}{\text{A blank}} \times 100$$

Figure 1. *Achillea fragrantissima* plant.

Ascorbic acid and α-tocopherol were used as positive control and the concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted.

Antioxidant capacity: Fe (III) to Fe (II) reduction capacity

One milliliter of each concentration was mixed with 2.5 mL of potassium hexacyanoferrate $K_3Fe(CN)_6$ solution and 2.5 mL of phosphate buffer (0.2 mol/L, pH 7.0). It was incubated at 50°C for 30 min. Later, 2.5 mL of trichloroacetic acid (10%) was added to the mixture. Then, 2.5 mL of this solution was homogenized with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%). The absorbance was measured at 700 nm and the concentration of the samples at the absorbance of 0.5 (EC_{50}) was determined. Ascorbic acid and α-tocopherol were used as positive control for comparison.

RESULTS

Seasonal climatic changes

Annual climatic variation for the last years in Gabel Elba region was described by Monier and Kadry (2006). The study area has arid climate, its rainfall ranges between 50 and 10 mm year^{-1} in spring, has relatively low temperatures in winter season (18-22°C); its sky is cloudy and sunshine hours are minimal during these seasons. Temperature rises from spring and reaches maximum during summer (28-35) in some days of the month; in May, June and finally August. For the previous three years, there has been increase in temperature during summer month and decrease in rainfall. These drastic conditions (hot and dry summer and cold winter) change the flora of the region and lead to low vegetation. Some plants adapt to these conditions by increasing the concentration of some bioactive compound, like essential oils.

Effect of climate changes on plant growth and physiological attributes

It is evident from the results that *A. fragrantissima* plant,

Table 1. Essential oil composition % of Achelia fragmentissima during different seasons.

S/N	Chemical compound	Summer	Winter	RI
1	Eucalyptol	0.21	0.31	883
2	Thujone	**9.93**	9.98+	928
3	Benzyl alcohol	0.12	0.31	1026
4	limonene	1.52	-	1029
5	Camphor	2.5	4.3	1123
6	limonene oxide	2.4	0.23	1137
7	α-Terpineol	-	0.4	1169
8	trans-p-Mentha-2,8-dienol	0.3	0.25	1160
9	Gerinol	**0.79**	0.09	1253
10	Lavandulyl-acetate	0.25	0.15	1264
11	B-Caryophyllene	0.41	0.30	1408
12	Eugenol	0.02	0.06	1440
13	Cedrene	3.34	9.01	1421
14	Caryophyllene oxide	-	0.51	1568
15	Isocalamendiol	-	0.05	1545
16	Globulol	5.2	3.2	1578
17	Cis-Farnesene	-	0.74	1697
18	Santolina Alcohol	0.46	5.31	1690
19	α –Tocopherol	**0.08**	0.6	
20	Cederan-diol	-	0.14	1597
21	α-Cubebene	17.1	0.11	1560
22	Spathulenol	1.54	0.50	1573
23	beta-Sesquiphellandrene	1.48	0.56	1525
24	Santanol acetate	-	0.18	1771
25	Farnesol	0.27	-	1722
	Total identified (%)	98	93	
	Monoterpene (%)	4.76	4.34	
	Oxygenated monoterpene (%)	33.33	34.78	
	Sesquiterpenoids (%)	23.80	21.73	
	Oxygenated sesquiterpenoids (%)	19.04	26.08	
	Other (%)	14.85	13.04	

collected in two different seasons, exhibited diverse pattern of growth parameter and metabolite. Plants collected in winter season have highest values for plant height (50.32 cm); significant reduction in plant height was recorded in summer (45.35 cm); highest fresh weight (40.54 g) per plant was recorded in winter, but was lowest (25.67g) in summer.

Plants exhibited maximum (23.63 g) dry matter accumulation and minimum (17.61 g) during April and July, respectively. Winter season has high rainfall, short photoperiods, increased metabolic process and fresh weight.

Essential oil composition

Results of statistical analysis indicate that environmental factors significantly affect the concentration and the essential oil yield *of A. fragmrntissima* ranging from 0.02 to 0.4% during summer and winter season, respectively. The difference in essential oil yield and composition of *A. fragmentissima* in response to seasonal changes was investigated under semi-arid tropical climatic conditions of Gabel Elba region. The results showed that essential oil concentration during the winter months is higher than that of summer season in some compounds (Table 1). Evaluation of terpenoid compositions showed minimum concentrations of mentha-2,8-dienol, geraniol and maximum concentrations of santolina alcohol (5.31), camphor (4.3), and cedrene (9.01) during winter months, while percentages of α-cubebene (17.1 %), spathulenol (1.54) and globulol (5.2) were highest during summer season. The plants grown under these hard conditions are tolerant to this abiotic stress due to their accumulation of some compounds or by accumulation of some compounds like acetate or alcohol, santanol acetate and

Table 2. Antioxidant activity of essential oil during different seasons.

Antioxidant assay	Summer	Winter	Ascorbic acid	α-Tocopherol
IC $_{50}$(g/L)	0.95±0.03	0.11±0.01	0.033±0.001	0.93±0,07
EC_{50} (g/L)	2.11±0.05	0.25±0.02	0.091±0.002	0.026±0.002

lavandulyl acetate, cis-farnesene found in winter season and converted to farnesol in summer season.

Antioxidants activity

In the present study, the FRAP method and DPPH scavenging capacity were used to determine the antioxidant capacity of *A. fragmentissma* essential oil, by reducing ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}), and reduction of DPPH. The results of this study show that *Achillea* has higher antioxidant activity of IC_{50} 0.11±0.01 and Ec_{50} 0.25±0.02 during winter than summer (Table 2). However, the standard substances used in this study (Ascorbic acid and Tecopherol) present an antioxidant activity of 0.033±0.001 and 0.93±0.07 g/L for DPPH and 0.091±0.002 and 0.026±0.002 g/L for FRAP method, respectively.

DISCUSSION

In this research we studied the growth, physiological and biochemical attributes of *A. fragmentissima* widely distributed in Gabel Elba, Egypt in response to varying temperature conditions of Gabel Elba. The plant has optimum plant height, fresh weight, dry weight, yield of essential oil accumulation during winter season, while the same parameters significantly decrease in the plant samples investigated in July and January (summer season). This agrees with the study of Prakash et al., (2011, 2011), who observed an increase in the morphological characteristics of plants in suitable environmental conditions and a decrease in this parameter observed beyond a certain limit. Sudden and extreme increase in temperature is accompanied with more stressful conditions which affect growth and development of plant species.

While some compounds of essential oil show opposite trend, the major compounds, camphor (4.5%), α-cubebene (17.1%) and globulol (6.51%) had more concentration during dry season. This is because plants grown under stressful condition can adapt due to their ability to accumulate secondary metabolites.

The results agree with those of Mozaffari et al. (2000) that myrcene and alpha-pinene are synthesized and released in higher amounts under stress. Drought stress causes induction of essential oil of monoterpene carvacrol in *Neigella sativa* (Mozaffari et al., 2000; Costa et al., 2010).

The results revealed that essential oil compositions are sensitive to seasonal climatic changes. Many studies support our results (Castelo et al., 2012; Hazzoumi et al., 2015). The yield of essential oils changes in a major way during the growth period since they are found between January and early February, a period that corresponds to dormancy phase; levels of essential oils decrease from 0.8% to 0.4% and then increase slightly during the growth phase between March and April (0.6%). However, the yield increases very well from mid-April until the end of June and reaches a maximum value of 1.2%. This period corresponds to the blooming phase of the plant. Hazzoumi et al., (2015) studied *Pelargonm graveolens; when* the plant was subjected to moisture stress, essential oil was rich with citronellol which was accumulated as a mechanism to adjust the thermal stress (Hazzoumi et al., 2015)

Antioxidant of essential oil at different seasons of DPPH shows the essential oil exhibits highest radical scavenging activity during winter season than in summer. Essential oil containing cederan-diol, santolina alcohol, caryophllene oxide and cederen with different chemical compositions had the highest levels of yield during winter. The results are in agreement with those of other studies, where essential oil of the leaf of *Artemisia absinthium* showed radical scavenging activity of DPPH in flowering stage during winter season (Canadanovic-Brunet et al., 2005; Mohammadi et al., 2015).

Conclusion

The results of antioxidant and chemical composition during two seasons (wet season and dry season) reflect the effect of seasonal change on the growth and behavior of plant to adapt to dry and harsh condition in Gabel Elba region. The analysis of the essential oil in the two seasons revealed there are differences in the composition and amount of essential oil. Some compounds are accumulated in high concentration in dry season, which reflects the method of tolerance to stress. By accumulation of these compounds plant can grow and survive. The results of antioxidant activity are compatible to the composition of essential oil. This study has shown that some compounds such as santolina alcohol, camphor, and cedrene were responsible for antioxidant activity in essential oil in preflowering stage during winter season.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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