GRASAS Y ACEITES 74 (4)
October-December 2023, e526
ISSN-L: 0017-3495
https://doi.org/10.3989/gya.0105221

The effect of harvest time on the volatile compounds and bioactive properties of the flowers, leaves, and stems of *Echinacea Pallida* and its utilization to improve the oxidative stability of vegetable oils

A. Kocacik[a,✉] and H. Yalcin[b]

[a]Ordu University, Central Research Laboratory, Ordu, Turkey
[b]Erciyes University, Engineering Faculty, Department of Food Engineering, Kayseri, Turkey
[✉]Corresponding author: aysesahan@odu.edu.tr

Submitted: 17 January 2022; Accepted: 2 May 2023; Published online: 11 January 2023

SUMMARY: The present study was undertaken to investigate the effect of harvest time on the bioactive properties of *Echinacea pallida* and to determine the antioxidant effect of its extract in vegetable oils. *E. pallida* was harvested in June, 2009, June, 2010 and August. 2010. Total phenolic content and antioxidant activity analyses of the plant extracts obtained with three different solvents were carried out using spectrophotometric methods. It was determined that harvest time and solvent type had significant effects on bioactive properties. In addition, the effect of *E. pallida* extract on the oxidative stability of vegetable oils was determined by the rancimat method. The extract (2000 ppm) obtained by ethanol (100%) showed similar oxidative stability on sunflower and canola oils compared to BHA (100 ppm). The GC-MS results revealed various volatile compounds such as bornyl acetate, caryophyllene E, musk ambrette, germacrene D, α-muurolol, musk ambrette, imidazo (1,2-a) pyrimidine, 1-pyrrolidino-1-cyclohexene, 2,3,5,6-tetrahydro-1H-pyrrolizine, pyrazine, and benzenaminium.

KEYWORDS: *Bioactive properties;* Echinacea pallida; *Harvest time; Rancimat; Volatile compounds*

RESUMEN: ***Efecto del tiempo de cosecha sobre los compuestos volátiles y las propiedades bioactivas de flores, hojas y tallos de*** **Echinacea Pallida** ***y su utilización para mejorar la estabilidad oxidativa de aceites vegetales.*** El presente estudio se realizó para determinar el efecto del tiempo de cosecha sobre las propiedades bioactivas de *Echinacea pallida* y el efecto antioxidante de su extracto en aceites vegetales. *E. pallida* se cosechó en junio de 2009, junio de 2010 y agosto de 2010. Los análisis de contenido fenólico total y actividad antioxidante de los extractos de plantas obtenidos con tres solventes diferentes se realizaron utilizando métodos espectrofotométricos. Se determinó que el tiempo de cosecha y el tipo de solvente tenían efectos significativos sobre las propiedades bioactivas. Además, se determinó el efecto del extracto de *E. pallida* sobre la estabilidad oxidativa de aceites vegetales mediante el método rancimat. El extracto (2000 ppm) obtenido con etanol (%100) mostró una estabilidad oxidativa similar en los aceites de girasol y canola en comparación con BHA (100 ppm). Los resultados de GC-MS mostraron la presencia de compuestos volátiles específicos, como el acetato de bornilo, cariofileno E, ambreta de almizcle, germacreno D, α-muurolol, ambreta de almizcle, imidazo (1,2-a) pirimidina, 1-pirrolidino-1-ciclohexeno, 2,3,5,6-tetrahidro-1H-pirrolizina, pirazina y bencenaminio.

PALABRAS CLAVE: *Compuestos volátiles;* Equinacea pallida; *Propiedades bioactivas; Rancimat; Tiempo de cosecha.*

Citation/Cómo citar este artículo: Kocacik A, Yalcin H. 2023. The effect of harvest time on the volatile compounds and bioactive properties of the flowers, leaves, and stems of *Echinacea Pallida* and its utilization to improve the oxidative stability of vegetable oils. *Grasas Aceites* **74** (4), e526. https://doi.org/10.3989/gya.0105221

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1. INTRODUCTION

The *Echinacea*, a member of the *Asteraceae* family, is an herbaceous perennial plant that is commonly called purple coneflower. For centuries, *Echinacea*, being native to North America, has been utilized externally in traditional cures for burns and wounds and internally for the treatment of colds, coughs, and headaches. *Echinacea* is mainly cultivated in America, Canada, Europe, Australia, and Russia. Moreover, many countries have carried out agricultural tests and research for the potential cultivation of *Echinacea* (Lin *et al.,* 2011). *Echinacea* consists of nine species, although only three species, namely *E. purpurea, E. pallida,* and *E. angustifolia,* possess medicinal properties. *Echinacea* species contain various bioactive components including alkamides, glycoproteins, polysaccharides, flavonoids, caffeic acid derivatives, and volatile compounds (Pellati *et al.,* 2004; Tsai *et al.,* 2012). Antioxidant (Pellati *et al.,* 2004; Lin *et al*., 2011; Erenler *et al.,* 2015), antimutagenic (Tsai *et al.,* 2012), antibacterial (Stanisavljević *et al.,* 2009), antiviral and immunostimulant activities (Aucoin *et al.,* 2020) of the species were proven in many studies.

Nowadays, there has been an increasing interest in the use of *Echinacea* and its preparations as an immune-modulator in COVID-19 treatment. Some reports have explained that *Echinacea* supplementation may alleviate the severity and interval of infection if taken during the first symptoms (Aucoin *et al.,* 2020). *Echinacea* is also one of the most preferred plants by cancer patients. In addition, some studies have revealed that the methanol extract from the roots of *Echinacea pallida* has antiproliferative activity against various cancer cells (Yaglioglu *et al.,* 2013), and its hexane extract has a higher cytotoxic effect on the tested cancer cells than the other two species (Chicca *et al.,* 2007).

While there are various studies about the utilization of *Echinacea* extracts for medicinal purposes, a recent study revealed that the silver nanoparticles of *E. purpurea* extract could be used as an effective antioxidant in food and pharmacological applications. Synthetic antioxidants are widely used as food supplements to avoid or retard lipid oxidation, which causes the formation of toxic compounds responsible for the bitter odor and taste which decrease food quality and safety. The utilization of synthetic antioxidants such as butylated hydroxyanisole (BHA) in foods raises concerns due to their carcinogenic risks. Thus, the identification of antioxidants from natural and safe sources is becoming increasingly more essential (Gecer *et al.,* 2022).

Echinacea is especially famous for its numerous volatile compounds which exhibit a wide range of beneficial effects. Recently, volatile compounds have attracted enormous attention for replacing synthetic substances in the food and pharmaceutic industries. They are drug candidates for treating various diseases due to their excellent biological effects. They have also gained importance as a non-toxic insecticide which is harmless to health in the fight against insects in stored products and agricultural production (Erenler *et. al.,* 2018; Karan *et.al.,* 2018).

The bioactive compounds in aromatic and medicinal plants largely rely on cultivation area, climate conditions, and harvest time (Thappa *et al.,* 2004). They also differ according to the species and parts of plant. Despite there being studies about the determination of the bioactive properties in *E. purpurea* for the whole plants, the bioactive properties of roots have only been determined for *E. pallida.* Therefore, comprehensive studies are required for assessing the bioactive compounds of the aerial parts of *E. pallida.*

The objectives of this study were: (1) to determine the effects of different harvest times on the bioactive compounds in the aerial parts of *E. pallida* cultivated in Turkey; (2) to assign a suitable solvent for the efficient extraction of phenolics from *E. pallida;* and (3) to determine the antioxidant effect of its extract on vegetable oils.

2. MATERIALS AND METHODS

2.1. Samples

The *E. pallida* plants used in this study were cultivated during the 2008 - 2010 growing seasons in the Experimental Horticulture area of Cumra Agricultural Vocational School in Konya, Turkey. Since Echinacea is a perennial plant, no harvest was done in the first year (2008), which is the planting year. It was harvested in two different developmental stages, at the beginning of flowering and full bloom, in the 2nd and 3rd years (2009-2010). The beginning of flowering is the period when 50% of the petals of the *Echinacea* come out. According to the development of aerial parts, the plants were harvested in June

2009 and 2010, at the beginning of flowering, and in August 2010, at the time of full flowering. The flower, leaf, and stem parts of the plants were brought to the laboratory and dried at room temperature.

2.2. Preparation of the extracts

Plant samples were ground in a laboratory-type grinder (Retsch MM400, Germany). Then the samples were extracted with different solvents, namely ethanol: water (80:20), methanol: water (80:20), and acetone: water mixtures (80:20). A weighed amount of ground samples was extracted with 25 ml of the solvent mixtures for 24 h at ambient temperature in the dark. The extracts were centrifuged at 4100 rpm for 15 min and then separated through a filter (Filter Discs No. 391). After that, the extracts were dried under a vacuum at 40 °C. After determining the extraction yield, the extracts were kept at -22 °C before the analyses of bioactive compounds.

2.3. Determination of total phenolic contents

The total phenolic content of the extracts was determined by the Folin-Ciocalteu colorimetric method (Tulukcu *et al.,* 2009). 40 µL of extract solution (1 mg/mL) were mixed with 2.4 mL distilled water and reacted with a 200-µL Folin-Ciocalteau reagent. After 5 min, 600 µL of a sodium carbonate solution (20% Na_2CO_3) and 760 µL of distilled water were added and the mixture was left for 2 h at ambient temperature in the dark. The absorbance of the samples was measured at 765 nm. The total phenolic content was expressed as mg gallic acid equivalent (GAE)/g of dry extract.

2.4. Determination of antioxidant properties

2.4.1. DPPH assay

The antiradical activity of the extracts was measured according to the 1,1- diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging assay used by Tulukcu *et al.* (2009) with some modifications. 50 µl of the extract were mixed with 3500 µL of a 0.1 mM methanol solution of DPPH and reacted for 30 min at ambient temperature in the dark. The absorbance of the samples was measured against a methanol blank at 517 nm. The percentage inhibitions of DPPH free radical (I%) was calculated according to the following equation:

$$I\ (\%) = 100 \times (1\text{-Absorbance of sample/ Absorbance of control})$$

As the reference substance, different concentrations of BHA were used (0-1 mg/mL). Then, the radical-scavenging activity of the samples was calculated as mg butylated hydroxyanisole equivalents (BHAE) /g of dry extract (Zulkafli *et al.,* 2014).

2.4.2. Phosphomolybdenum assay

The total antioxidant activity was measured by the phosphomolybdenum assay (Tulukcu *et al.,* 2009). For this purpose, 0.4 mL of extract were mixed with 4 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and then reacted in a water bath at 95 °C for 90 min. The absorbance of the samples was recorded at 695 nm. The antioxidant activity was expressed as mg ascorbic acid equivalents (AAE) /g of dry extract.

2.4.3. Rancimat assay

The antioxidant effect of *E. pallida* extract on the oxidative stability of vegetable oils was determined by a 743 Rancimat device. For this purpose, E. *pallida*'s powdered aerial part (4 g) was extracted by 100 ml of 100% ethanol at ambient temperature for 24 h in the dark. The extract was dried under vacuum at 40 ºC. 500, 1000, and 2000 ppm concentrations of the extracts and BHA (100 ppm) as the positive control were added to refined sunflower and canola oil. The induction period of these concentrations was measured at 120 ºC with 20 L/h air flow in duplicate.

2.5. Volatile compounds analysis

Volatile compounds were analyzed according to the method described by Yalcin *et al.* (2017) with GC-MS coupled to a mass selective detector (Agilent 7890A GC system) and HP-5MS column (60 m x 0.250 mm i.d.; film thickness 0.25 µm). About 3 g homogenized sample were extracted by Headspace (HS)-solid phase microextraction (SPME) at 60 °C for 40 min using a 75-µm carboxen-polydimethylsiloxane fiber (Supelco). GC oven temperature was held at 40 °C for 5 min, heated to 110 °C at 3 °C/min, from 110 °C to 150 °C at 4 °C/min, and from 150 °C to 210 °C at 10 °C/min and held for 15 min. Helium

was used as a carrier gas at 0.5 ml/min. The fiber was desorbed in the injection port for 20 min at 250 °C in the splitless mode. The mass spectrometer was scanned with an ionizing voltage of 70 eV and a scan range of *m*/*z* 35-450. The volatile compounds were identified by comparison with spectra from Flavor 2, NIST 05a, and Wiley7n libraries. The percentage of compounds was calculated from the TIC automated integrator.

2.6. Statistical analysis

The entire experiment was performed in triplicate. Data were analyzed with SAS statistical software. Comparative analyses between significant means were determined by using the analysis of variance and Tukey's multiple range test.

3. RESULTS AND DISCUSSION

The yield of extracts obtained with three different solvents, namely ethanol: water (80:20), methanol: water (80:20), and acetone: water mixtures (80:20), of the flower, leaf and stem parts of *E*. *pallida* harvested in June, 2009, June, 2010 and August 2010 are shown in Table 1. There was a significant difference ($P < 0.05$) in the extract yield of plant parts depending on harvest time. For flower and stem parts of the plant, while decreasing extraction yields were observed with late harvesting, the leaves of the plant did not show any definite tendency. Tulukcu *et al.* (2009) reported the extraction yield of clary sage as between 19.62 - 26.08%, depending on the harvest time. In this study, while the methanol extracts from the leaf parts had the highest extraction yield (15.79%), while the acetone extracts from the stem parts had the lowest extraction yield (4.19%).

The total phenolic content, free radical-scavenging activity, and antioxidant activity of the extracts obtained from different parts of *E. pallida* are provided in Table 2. The differences among the bioactive contents in the leaves, stems and flowers of *E. pallida* in the early and late harvest of the 2010 season were found to be significant ($p < 0.05$). Based on the plant's flowers, which contain the most bioactive substances, no significant difference ($p > 0.05$) was observed in the bioactive properties from June 2009 to June 2010. However, they exhibited higher bioactive properties with the late harvest in 2010. Complying with the present findings, Mistríková and Vaverková (2009) reported that the amount of hydrophilic and lipophilic compounds of *Echinacea*'s flower was higher during the third (ripening) developmental stage compared to the earlier stages, thus the third developmental stage was the best time for harvest. Chen *et al.* (2008) determined that the total phenolic contents in *E. purpurea* harvested in the spring were lower than that of the plants harvested in the autumn. Binns *et al.* (2002) reported increasing chicoric acid contents with age in E. *pallida* wildflowers, accompanied by decreasing values in roots because of developmental transport of this substance from the roots to the other plant parts or spatiotemporal shifts in phenolic pathways. The total phenolic contents in the *E. pallida*'s ethanol ex-

Table 1. Extraction yield of the Echinacea pallida (%)

Plant parts	Harvest Time	Acetone	Methanol	Ethanol
Flower	June 2009	10.29±1.24 ^{aA}	14.33±1.55 ^{aA}	12.61 ±1.86 ^{aA}
	June 2010	9.97±0.54 ^{aB}	14.01±1.00 ^{aA}	12.39±0.09 ^{aB}
	August 2010	7.72±1.05 ^{aB}	10.47±1.15 ^{bA}	7.93±0.79 ^{bB}
Leaf	June 2009	8.16 ±1.15 ^{aB}	15.79±1.82 ^{aA}	11.01 ±0.50 ^{bB}
	June 2010	9.38±0.62 ^{aB}	14.68±0.96 ^{aA}	12.51±0.90 ^{aBA}
	August 2010	9.33 ±1.04 ^{aB}	15.54±2.97 ^{aA}	12.24±0.75 ^{aBA}
Stem	June 2009	5.71±0.83 ^{bA}	7.51±0.85 ^{bA}	6.04 ±0.89 ^{cA}
	June 2010	5.22±1.10 ^{bB}	9.00±0.78 ^{bA}	6.55±0.89 ^{cBA}
	August 2010	4.19±0.41 ^{bB}	6.17±0.91 ^{cA}	4.80±0.77 ^{cB}

Each solvent type consists of a solvent: water mixture (80:20 v: v). Each value is expressed as mean± SD for three different harvest replications (n=3). Different lower-case letters in the same column and different upper-case letters in the same row indicate significant difference ($p < 0.05$), according to 2-way ANOVA with Tukey's test.

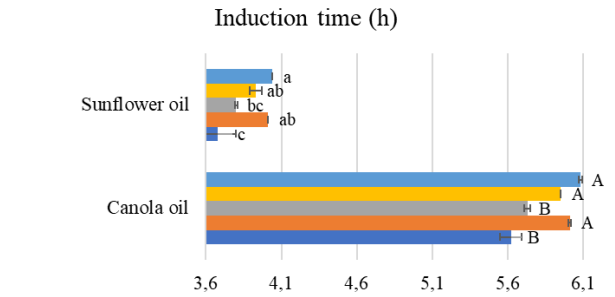
TABLE 2. Total phenolic contents, DPPH radical-scavenging activities and antioxidant activities of the *Echinacea pallida*

Plant parts	Harvest Time	Total phenolic contents (mg GAE/g dry extract)			DPPH radical-scavenging activities (mg BHAE/g dry extract)			Antioxidant activities (mg AAE/g dry extract)		
		Acetone	Methanol	Ethanol	Acetone	Methanol	Ethanol	Acetone	Methanol	Ethanol
Flower	June 2009	173.27±19.2 ^{bA}	131.80±7.1 ^{bB}	106.88±7.6 ^{bB}	317.51±27.23 ^{aA}	236.01±38.13 ^{bBA}	136.59±7.99 ^{bB}	151,23±9.69 ^{bA}	166.78±3.05 ^{bA}	145.00±9.42 ^{bA}
	June 2010	165.46±21.7 ^{bA}	109.37±8.96 ^{bB}	97.23±3.26 ^{bB}	239.08±41.70 ^{bA}	162.58±23.59 ^{cBA}	107.76±4.13 ^{bB}	149.69±3.68 ^{bBA}	156.47±4.06 ^{bA}	139.77±2.93 ^{bB}
	August 2010	225.07±28.74 ^{aA}	168.47±14.1 ^{aBA}	159.23±8.06 ^{aB}	370.33±9.65 ^{aA}	271.6±30.18 ^{aB}	252.67±29.79 ^{aB}	185.09±5.66 ^{aA}	194.99±5.22 ^{aA}	170.85±1.58 ^{aA}
Leaf	June 2009	71.30±7.02 ^{cA}	71.10±2.73 ^{cB}	45,31±7.07 ^{cB}	130.20±12.60 ^{cA}	155.07±4.48 ^{cA}	84.43±15.08 ^{bB}	136.97±14.36 ^{bA}	152.03±4.55 ^{bA}	130.65±6.81 ^{bA}
	June 2010	37.88±1.28 ^{dA}	31.34±0.89 ^{dB}	26.77±0,19 ^{dB}	44.17±4.30 ^{dA}	59.85±2.26 ^{dA}	16.77±1.75 ^{cB}	112.57±2.72 ^{cB}	123.105±0.06 ^{cA}	106.14±0.51 ^{cB}
	August 2010	45.95±1.95 ^{cA}	40.42±4.66 ^{dBA}	31.33±0.21 ^{dB}	63.07±5.28 ^{dA}	70.90±8.47 ^{dA}	38.03±2.87 ^{cB}	120.47±1.87 ^{cB}	131.42±2.08 ^{cA}	114.05±3.36 ^{cB}
Stem	June 2009	66.32±2.24 ^{cA}	55.34±4.22 ^{cB}	43.44±2.05 ^{cC}	95.81±8.20 ^{dA}	84.67±9.16 ^{dA}	47.78±3.16 ^{cB}	112.51±3.10 ^{cBA}	122.1±6.64 ^{cA}	103.06±7.29 ^{cB}
	June 2010	51.29±0.58 ^{cA}	37.10±3.01 ^{dB}	34.40±2.45 ^{dB}	55.04±4.88 ^{dA}	55.43±2.86 ^{dA}	27.25±1.31 ^{cB}	105.28±3.52 ^{cBA}	114.90±2.23 ^{cA}	97.77±5.66 ^{cB}
	August 2010	86.31±5.77 ^{cA}	65.34±4.64 ^{cB}	58.12±1.48 ^{cB}	117.45±6.33 ^{dA}	92.43±5.59 ^{dB}	70.67±3.66 ^{cC}	121.21±2.29 ^{cB}	131.72±2.89 ^{cA}	118.22±2.18 ^{cB}

Each value is expressed as mean± SD from three different harvest replicates x four measurements for each replicate (n=12). Different lower-case letters in the same column and different upper-case letters in the same row indicate significant difference ($p < 0.05$), according to 2-way ANOVA with Tukey's test.

tract were observed as 97.23-159.23, 26.77-31.33, and 34.40-58.12 mg GAE/g for flowers, leaves, and stems in the early and late harvest of the 2010 season, respectively. Stanisavljević *et al.* (2009) reported that the ethanol extract of the whole plant of *E. purpurea* contained 60.2 mg GAE/g dry extract of total phenolics. The highest bioactive contents were obtained from the flowers, complying with the results of Chen *et al.* (2008) and Lin *et al.* (2011). Erenler *et al.* (2015) reported the chicoric acid values, which is the main component of extracts using the LC-MS method, respectively, as 32.39 and 56.15 mg/100 g for flowers and 4.96 and 0.74 mg/100 g for leaves of *E. purpurea* and *E. pallida*. In general, the differences among the bioactive contents in the leaf and stem parts were not significant ($p > 0.05$), which revealed that the plant's stems contained important bioactive components.

Besides harvest times, the extraction solvents also had significant effects on the bioactive contents in the plant parts ($p < 0.05$). The highest total phenolic content was observed in the acetonic extracts, followed by the methanolic extracts; while the lowest total phenolic content was observed in the ethanolic extracts (Table 2). These results are coherent



	Canola oil	Sunflower oil
Echinacea (2000 ppm)	6,08	4,04
Echinacea (1000 ppm)	5,95	3,93
Echinacea (500 ppm)	5,73	3,8
BHA(100 ppm)	6,01	4,01
Control oil	5,62	3,68

FIGURE 1. Each value represents the mean of two replicates (n=2), bar errors show the standard deviations and significant differences ($p < 0.05$), according to one-way ANOVA with Tukey's test.

with those reported by Pellati *et al.* (2004) for the extraction of *E. pallida*. Although the methanol extracts gave the highest extract yields, they had lower total phenolic contents than the acetone extracts. Do *et al.* (2014) reported that proteins and carbohydrates had higher solubility in methanol than in ethanol and acetone. The components (polysaccharide and glycoprotein) of *E. pallida* other than the phenolics may

have increased the extract yield depending on solvent polarity.

The free radical-scavenging and total antioxidant activities of flowers were respectively identified as 185.04 mg BHAE/g and 370.33 mg AAE/g for acetone extracts; as 194.99 mg BHAE/g and 271.60 mg AAE/g for methanol extracts; as 170.85 mg BHAE/g and 252.67 mg AAE/g for ethanol extracts. It is clear from the results that the free radical-scavenging and antioxidant activities are well compatible with the total phenolic content. Tsai *et al.* (2012) observed that the ascorbic acid, BHA, and flower extract of *E. purpurea* exhibited the same highest radical scavenging ability at 100, 200, and 400 μg/ml, respectively. Pellati *et al.* (2004) and Erenler *et al.* (2015) reported that the radical scavenging activity of *Echinacea* species may be attributed to the caffeic acid derivatives, which have a high number of hydroxyl groups in their phenolic rings, which are proven to inhibit free radicals.

While the DPPH assay measures the free radical scavenging ability of the antioxidants found in the extract, the rancimat assay determines the oxidative stability of oils from a technological perspective. Rancimat quantifies the rise in the water conductivity resulting from the volatile degradation products of oil under accelerated oxidation conditions. The induction period represents the time required for the degradation of oxidation products which occurred with oil oxidation (Yalcin *et al.,* 2017).

The induction periods of canola and sunflower oils with or without added extract exposed to accelerated oxidation conditions are schematized in Fig. 1. The BHA (100 ppm) and *E. pallida* extracts (1000 and 2000 ppm) improved the oxidative stability of the oils, with similar induction periods. The *E. pallida* extract (2000 ppm) raised the induction periods of sunflower and canola oils from 3.68 h and 5.62 h to 4.04 h and 6.08 h, respectively ($p < 0.05$). Therefore, *E. pallida* extract exhibited high efficiency in delaying the oxidation of vegetable oils. This promising antioxidant effect for prolonging the shelf life of lipid-containing foods could be attributed to its bioactive components, which exhibit high free radical-scavenging capacity, such as volatile compounds and caffeic acid derivatives, especially cichoric and chlorogenic acid (Lin *et al.,* 2011; Erenler *et al.,* 2015).

Some studies evaluated comparing the antioxidant activity of *E. pallida* with synthetic antioxidants by spectrophotometric methods (Erenler *et al.,* 2015). However, no study was found on the effect of *E. pallida* on the oxidative stability of vegetable oils. Yalcin *et al.* (2017) reported that the IP of corn oil without and with 2000 ppm of grape seed extract changed from 3.18 to 3.31 - 3.41, depending on the seed variety. In another study, saffron extract (1000 ppm) had a similar effect ($p < 0.05$) with BHT (200 ppm) in preventing the oxidation of vegetable oils (Najafi *et al.,* 2022)

The volatile compounds in the flower, leaf, and stem parts of *E. Pallida* harvested in June, 2009, June, 2010 and August, 2010 are shown in Table 3. Bornyl acetate, caryophyllene E, musk ambrette, germacrene D, α-cubebene, α-copaene, α-humulene, α-muurolol, γ-cadinene, and caryophyllene oxide are some of the major volatile compounds which were identified in all the plant parts of *E. pallida.* Terpenes, which have many bioactive properties, exhibited the largest diversity and dominated all plant parts. Previous studies showed considerably higher proportions of terpenes in *Echinacea* species (Mazza and Cottrell, 1999; Thappa *et al.,* 2004; Mirjalili *et al.,* 2006; Lepojević *et al.,* 2017). In this study, bornyl acetate was determined to be one of the most abundant terpenes of all the plant parts. Although it was present in the essential oil of *E. pallida* in the study by Mirjalili *et al.* (2006), it was not identified in the essential oil of *E. purpurea* in some studies by Mazza and Cottrell (1999), Thappa *et al*. (2004), and Mirjalili *et al.* (2006). They reported that Bornyl acetate is the principal component in the above-ground parts of *E. pallida* and *E. angustifolia*, except for *E. purpurea*. There are reports that *E. purpurea* differs from other species due to its high monoterpene content, particularly α-phellandrene and myrcene, not detected for *E. pallida* in this study. The nitrogen-containing heterocycles, such as Imidazo (1,2-a) pyrimidine, 1-Pyrrolidinocyclohexene, 2,3,5,6-tetrahydro-1H-pyrrolizine, pyrazine, and benzenaminium, not detected as a major component in other studies, were present at high rates in the flower parts. They have a promising position in producing new bioactive compounds and in discovering drugs due to their excellent antiproliferative activity (Ivan *et. al.,* 2022). The musk ambrette (6.65-3.42%) was the heterocyclic flavor compound which dominated all the plant parts, but it was not identified in the other studies on *Echinacea* species.

Table 3. Volatile compounds in the flower, leaf, and stem parts of Echinacea pallida (%)

Volatile compounds	Flower			Leaf			Stem		
	June 2009	June 2010	August 2010	June 2009	June 2010	August 2010	June 2009	June 2010	August 2010
Monoterpene hydrocarbons	5.51	2.80	2.47	10.77	7.66	6.62	**9.95**	**12.82**	**9.91**
α -Pinene	0.36±0.00	0.26±0.01	-	0.31±0.03	0.60±0.14	0.62±0.07	0.84±0.06	0.92±0.07	0.45±0.09
β -Pinene	0.17±0.04	-	0.35±0.26	0.99±0.01	0.48±0.10	0.77±0.22	0.97±0.79	0.11±0.11	-
Limonene	1.52±0.02	0.79±0.05	0.33±0.03	4.26±1.11	2.27±0.48	2.00±0.03	1.52±0.23	2.21±0.49	1.13±0.09
Camphene	0.32±0.08	0.14±0.01	-	0.25±0.10	0.14±0.14	0.34±0.02	0.67±0.50	1.35±0.23	1.05±0.08
o- Cymene	0.50±0.03	0.34±0.03	0.32±0.01	2.22±0.38	0.99±0.01	0.93±0.00	0.24±0.01	0.26±0.06	0.18±0.01
Anethole	0.26±0.01	0.27±0.02	0.21±0.01	0.91±0.38	1.65±0.84	0.57±0.00	0.66±0.06	0.96±0.19	1.48±0.10
p -Cymenene	0.16±0.00	-	-	0.37±0.11	0.16±0.16	0.29±0.01	0.35±0.09	0.36±0.03	0.24±0.01
Thuja-2,4-diene	0.34±0.06	-	0.36±0.36	-	-	-	0.76±0.19	0.55±0.01	0.49±0.07
Carvone	1.88±0.01	1.00±0.04	0.90±0.05	1.46±0.16	1.37±0.02	1.10±0.09	2.69±0.10	2.54±0.31	2.50±0.18
Myrcene	-	-	-	-	-	-	1.25±0.04	3.56±0.01	2.39±0.27
Oxygenated Monoterpenes	**6.59**	**5.81**	**4.39**	11.09	10.63	10.51	**20.06**	**26.25**	**19.14**
Myrtenal	0.32±0.02	-	0.11±0.01	0.34±0.02	0.19±0.19	0.35±0.05	0.58±0.02	0.56±0.01	0.46±0.00
Bornyl acetate	3.97±0.56	3.67±0.03	2.51±0.07	6.29±1.22	3.18±0.02	6.11±0.20	16.17±0.61	20.79±0.21	14.35±0.63
Sabinol	-	0.73±0.04	1.06±0.15	0.62±0.12	0.28±0.28	0.54±0.18	-	-	-
Pinocarveol	0.21±0.02	0.11±0.00	0.13±0.02	-	-	0.22±0.04	0.31±0.31	0.46±0.02	0.43±0.02
Carveol	1.15±0.21	0.62±0.06	-	-	-	-	0.89±0.05	0.97±0.00	0.70±0.04
Carvacrol	0.94±0.05	0.68±0.04	0.58±0.00	3.84±0.05	4.63±0.43	3.29±0.50	1.20±0.15	1.18±0.01	1.00±0.08
α- Campholenal	-	-	-	-	2.35±2.35	-	0.53±0.04	0.82±0.15	0.61±0.06
Linalool	-	-	-	-	-	-	-	0. 98±0.09	1.01±0.05
Verbenol	-	-	-	-	-	-	0.38±0.38	0.49±0.00	0.58±0.02
Sesquiterpene hydrocarbons	**28.49**	**31.08**	**28.37**	25.95	22.99	23.29	**11.40**	**12.49**	**16.49**
α-Cubebene	2.09±0.05	1.99±0.19	2.42±0.13	1.15±0.05	1.09±0.06	1.28±0.00	0.97±0.08	1.09±0.12	1.32±0.07
α-Ylangene	0.64±0.05	0.57±0.01	0.59±0.07	0.63±0.02	0.37±0.37	0.57±0.02	0.55±0.00	0.54±0.00	0.53±0.03
α-Copaene	2.92±0.07	3.03±0.09	2.47±0.05	2.48±0.00	2.14±0.76	2.02±0.13	1.61±0.12	1.95±0.15	1.52±0.00
Caryophyllene E	5.54±0.17	6.24±0.13	7.57±0.92	5.11±0.52	5.10±0.62	5.85±0.31	2.48±0.24	2.14±0.11	2.46±0.43
α -Humulene	2.05±0.01	2.96±0.19	2.38±0.04	2.01±0.21	1.42±0.41	1.89±0.16	0.72±0.02	1.06±0.12	1.33±0.11
Germacrene D	3.52±0.04	5.16±0.42	1.58±0.11	3.35±0.12	3.84±3.84	2.27±0.03	0.94±0.94	0.58±0.58	1.05±0.66
γ-Muurolene	1.69±0.21	1.44±0.18	1.45±0.00	1.14±0.06	1.40±0.53	1.21±0.15	0.55±0.22	0.56±0.10	0.61±0.04
β-Cubebene	0.92±0.03	1.21±0.07	0.61±0.11	0.23±0.23	-	0.61±0.61	-	-	-
β-Bourbonene	1.05±0.04	-	1.11±0.04	0.29±0.29	-	1.78±0.54	-	0.93±0.05	1.52±0.25
β-Elemene	1.25±0.03	1.47±0.11	1.50±0.06	1.14±0.18	1.05±0.00	1.10±0.07	0.61±0.07	0.62±0.06	0.61±0.06
β- Copaene	0.88±0.05	0.87±0.01	1.08±0.08	0.71±0.21	1.13±0.87	0.70±0.05	0.43±0.03	0.48±0.04	0.54±0.00
α-Calacorene	-	-	-	0.65±0.02	0.33±0.33	0.24±0.24	-	-	-
β-Longipinene	0.76±0.76	1.08±0.00	1.16±1.16	0.70±0.70	-	-	0.63±0.01	0.72±0.72	1.02±0.46
Muurola-4,5-diene	0.69±0.69	0.75±0.75	0.66±0.03	0.81±0.04	-	-	0.49±0.23	-	0.58±0.11
γ-Cadinene	1.71±0.20	2.25±0.08	1.84±0.09	1.20±0.07	0.54±0.54	0.37±0.08	0.32±0.01	0.55±0.05	0.82±0.09
Calamenene	0.22±0.22	0.26±0.26	0.55±0.07	0.83±0.3	0.30±0.30	0.58±0.05	0.26±0.04	-	0.33±0.01
Murrola-4,5-diene	-	-	0,61±0.11	0.21±0.21	0.42±0.42	0.71±0.06	-	-	-
Cadina-1,4-diene	1.04±0.13	1.11±1.11	0.79±0.04	0.54±0.06	0.72±0.08	0.72±0.06	-	-	-
β-Gurjunene	0.51±0.16	0.69±0.03	-	0.39±0.39	0.47±0.47	0.87±0.14	-	0.25±0.25	0.92±0.19
β- Ylangene	1.01±1.01	-	-	0.62±0.62	0.94±0.94	0.27±0.27	0.34±0.34	0.22±0.22	0.29±0.29
Muurola- 3,5-diene	-	-	-	1.49±0.35	1.28±0.39	0.25±0.25	0.50±0.07	0.80±0.02	0.74±0.74
α- Clovene	-	-	-	0.27±0.27	0.45±0.45	-	-	-	0.30±0.01
Oxygenated Sesquiterpenes	**5.74**	**5.01**	**4.41**	2.20	1.47	3.94	1.86	**2.54**	**3.42**
α -Muurolol	3.47±0.29	3.37±0.16	2.94±0.08	1.66 ±1.66	1.47±1.47	2.68±0.98	-	0.46±0.46	0.86±0.16
Caryophyllene oxide	2.16±0.09	1.41±0.09	1.00±0.07	0.54±0.54	-	1.26 ±0.01	1.18±0.1	1.37±0.11	1.76±0.03
α-Cadinol	0.11±0.11	0.23±0.23	0.47±0.00	-	-	-	-	-	-
Humulene epoxide	-	-	-	-	-	-	0.68±0.09	0.71±0.16	0.80 ±0.05
Aldehyde	0.18	0.15	0.15	7.97	4.6	6.39	1.22	0.58	0.87
Nonanal	0.18±0.01	0.15±0.01	0.15±0.04	0.83±0.00	0.51±0.06	0.58±0.05	1.03±0.10	0.58±0.07	0.55±0.03
Benzaldehyde	-	-	-	2.73±0.77	0.56±0.56	0.99±0.99	-	-	-
2E.4E- hexadienal	-	-	-	0.85±0.11	0.41±0.41	0.74±0.05	-	-	-
2-Pentenal	-	-	-	0.38 ±0.38	0.39 ±0.39	0.75±0.11	-		--
2,4-Heptadienal	-	-	-	0.97±0.33	0.48±0.48	1.14±0.12	-	-	-
2-Hexenal	-	-	-	2.21±0.03	2.25±0.07	2.19±0.08	0.19±0.05	-	0.32±0.02
Ester	2.08	1.64	2.24	6.59	1.36	3.98	1.33	1.36	1.41
Methyl linoleate	2.08±0.76	1.64±0.20	2.24±0.16	2.64 ±0.26	-	-	1.33±0.11	1.36±0.23	1.41±0.03
Diisooctyl sulfate	-	-	-	3.95±1.2	1.36±1.36	3.98±0.53	-	-	-
Alcohol	1.99	1.56	2.64	1.55	1.28	1.77	0.68	0.54	-
n-Pentadecanol	1.52±0.02	1.56±0.04	2.26±0.08	0.88 ±0.88	0.87±0.87	1.41 ±0.01	0.68±0.68	0.54±0.54	-
Benzyl alcohol	0.47±0.02	-	0.38±0.01	0.67±0.06	0.41±0.41	0.36±0.06	-	-	-
Heterocyclic Compounds	**21.25**	**25.19**	**23.07**	12.29	10.98	8.45	**10.11**	**8.41**	**9.53**
Imidazo(1,2-a) pyrimidine	-	3.16±3.16	6.29±1.57	-	-	-	-	-	-
1-Pyrrolidino-1-cyclohexene	4.52±0.51	5.33±0.38	5.95±0.74	0.69±0.25	3.10±3.10	1.59±1.59	2.61±1.48	0.47±0.47	2.12±0.95
2,3,5,6-tetrahydro-1H-pyrrolizine	3.44±0.16	3.25±0.34	1.89±0.33	0.35±0.35	0.38±0.38	0.67±0.08	1.20±0.09	2.00±0.01	1.47±0.10
pyrazine	2.47±0.02	3.51±0.29	2.36±0.51	-	-	-	0.56±0.02	0.93±0.23	1.16±0.29
Musk ambrette	5.06±0.32	4.44±0.06	6.05±0.03	6.65±0.06	3.95±3.22	4.48±0.36	4.66±0.44	3.78±0.23	3.42±0.10

Volatile compounds	Flower			Leaf			Stem		
	June 2009	June 2010	August 2010	June 2009	June 2010	August 2010	June 2009	June 2010	August 2010
Benzenaminium	5.76±0.06	5.50±0.77	0.53±0.53	1.97±1.97	-	0.84±0.84	1.08±1.08	1.23±0.04	1.36±0.29
Benzothiazole	-	-	-	1.66±0.91	2.26±1.39	0.26±0.26	-	-	-
Dihydro actinidolide	-	-	-	0.97±0.29	1.29±0.46	0.61±0.01	-	-	-
Aromatic hydrocarbon	**3.87**	**4.58**	**2.98**	2.14	1.66	2.00	**2.80**	**3.30**	**3.58**
1,4-Dichlorobenzene	-	-	-	0.81±0.06	0.34±0.34	0.68±0.10	0.46±0.03	0.49±0.00	0.64±0.02
bicyclo[5.3.0]decan-2-one	3.87±0.01	4.58±0.31	2.98±0.80	-	-	-	1.39±0.58	0.34±0.34	0.94±0.94
Naphthalene	-	-	-	0.29±0.06	0.16±0.16	0.22±0.01	-	-	-
Tetracosane	-	-	--	0.59±0.19	0.44±0.44	0.56±0.08	-	-	-
Pentadecane	-	-	-	0.22±0.22	0.39±0.39	0.16±0.03	-	-	-
Tridecane	-	-	-	0.23±0.23	0.33±0.33	0.38±0.13	-	-	-
2-Undecene, 6-methyl	-	-	-	-	-	-	0.95±0.95	2.47±0.02	2.00±2.00
Ketone	-	-	-	1.46	2.21	2.00	0.14	0.12	0.14
β- Ionone	-	-	-	0.79±0.03	0.82±0.06	0.93±0.13	0.14±0.00	0.12±0.01	0.14±0.00
3,5-Octadien-2-one	-	-	-	0.67±0.03	1.39±1.39	1.07±0.12	-	-	-
Acid	-	-	-	-	-	-	2.81	1.88	1.12
Acetic acid	-	-	-	-	-	-	2.81±0.13	1.88 ±0.13	1.12±0.11
Total terpene compounds	**46.33**	**44.70**	**39.64**	50.01	42.75	44.36	43.27	54.10	48.96
Total volatile compounds	75.70	77.82	70.72	82.01	64.84	66.95	62.36	70.29	65.61

Each value is expressed as mean± SD for two different harvest replicates (n=2)

The large variations in volatile compounds were generally due to the plant parts rather than harvest time. Caryophyllene E, which is one of the major sesquiterpene compounds in all plant parts, reached its highest value (7.57%) for the late harvest. A sesquiterpene, Germacrene D were detected at 3.52 and 1.58% for flowers and 3.35 and 2.27% for leaves in the early and late harvest, respectively. The proportion of germacrene D was much lower than that reported for the flowerheads of *E. pallida* (Mirjalili *et al.,* 2006), and was like the one reported for *E. pallida* (Mazza and Cottrell, 1999). Thappa *et al.* (2004) observed that the amount of germacrene D, which is one of the most abundant components in Echinacea species, ranged from 7.2% (June) to 33.5% (December) and reported that weather conditions such as temperature and humidity had significant effects on the content and composition of the major terpene hydrocarbons in the flowers of *E. purpurea* during the growing season. Moreover, the percentage of α-copaene, which is one of the major sesquiterpenes compounds, was higher for all the plant parts from the early harvest than from the late harvest. Bornyl acetate, with known antioxidant activity (Karan *et al.,* 2018), showed the most abundant terpenoid component in stem parts and its level decreased from 20.79 to 14.35% with late harvesting. Among the volatile compounds, bornyl acetate is the most promising compound. Karan *et al.* (2018) observed that bornyl acetate showed significant antiproliferative activity against the tested cancer cells. In another study, it was found to have a neuroprotective effect on multiple sclerosis (MS), a neurological autoimmune disease (Lee *et al.,* 2023). Imidazo (1,2-a) pyrimidine compound, which is effective in treating anxiety disorders and ulcers (Goodacre *et al.,* 2006) and preventing unchecked cell growth (Aeluri *et al.,* 2015) was found at high rates in the flower parts of the plant, and its amount increased with late harvesting. Limonene, carveol, carvacrol, and carvone compounds, the major monoterpenes of *E. pallida,* decreased in all plant parts with late harvesting. The total monoterpene and oxygenated monoterpene hydrocarbon contents generally decreased with increasing temperature depending on late harvesting. The monoterpenes (C10) are smaller compounds to sesquiterpenes (C15), and thus they tend to evaporate more easily with the influence of high temperatures (Pirbalouti *et al.,* 2013).

4. CONCLUSIONS

The bioactive properties of *E. pallida* were significantly affected by harvest time. The highest total phenolic content was identified in the flowers of the plant with the late harvest of 2010. Among the solvents, acetone was the most suitable solvent for the efficient extraction of phenolics from *E. pallida*. While terpenes were detected as the dominant volatile compound in all the plant parts, the nitrogen-containing heterocycles were present at high rates in the flower parts. Total terpene content decreased with late harvesting for the flower and stem parts. *E. pallida* extracts exhibited high efficiency in delaying the oxidation of vegetable oils. These findings, which reveal the valuable volatile components and antioxidant activity of *E. pallida* also indicate that it is worth considering for further studies.

ACKNOWLEDGMENTS

We are grateful to Erciyes University Scientific Research Projects Unit (Project number is FBY-11-3477) for financial support and Associate Professor Dr. Eray Tulukcu (Cumra Agricultural Vocational School, Konya, Turkey) for providing the plant material (*Echinacea pallida*).

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