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A Comparative Study on Fatty Acid Compositions, Total Phenolic Contents, and Antioxidant Potentials of Various Extracts from Different Parts of *Euphorbia chamaesyce* L.

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Abstract

In this study, fatty acid compositions, total phenolic contents, and antioxidant effects of the stem and seed extracts from *Euphorbia chamaesyce* L. (Euphorbiaceae) were investigated. Linolenic and linoleic acids were found as the main fatty acid components in the seed and stem hexane extracts by using GC/MS. In the seed hexane extracts, linolenic and linoleic acids were found as 32.42% and 15.04%, respectively. On the other hand, in the stem hexane extracts, they were found as 22.31% and 14.25%, respectively. EC/SEM (seed methanolic extract, IC_{50} = 5.35 ± 0.01 µg/mL), EC/SEA (seed acetone extract, IC_{50} = 8.61 ± 0.11 µg/mL), and EC/SEE (seed ethyl acetate extract; IC_{50} = 20.6 ± 1.12 µg/mL) showed higher radical scavenging effect than synthetic antioxidant BHT. The results showed that the seeds of *E. chamaesyce* had a high amount of polyunsaturated fatty acids and remarkable antioxidant activities.

1. INTRODUCTION

Euphorbia L. (Euphorbiaceae) is one of the largest genus, with about 2160 species, divided into several subgenera. These species are known to produce irriating and toxic milky latex [1]. Euphorbia is represented by two subgenera (subg. Chamaesyce, subg. Esula) with a total of 111 taxa (except for cultivated ones) in Turkey [2]. *Euphorbia chamaesyce* L. belongs to subgenus Chamaesyce and is known as "şebrem" and "alçak boylu sütleğen" in Turkey. In Europe, an infusion of this species is used as eye drops for the infections of the eyes [3]. Several compounds isolated from *E. chamaesyce* exhibited strong inhibitory effects on Epstein Barr virus [4] and cancer panel including 39 human cell lines [5].

The species belonging to Euphorbiaceae family are suggested as a source of unsaturated [6] and rare fatty acids [7]. The essential fatty acids cannot be produced by human cells and thus must be obtained from food sources [8]. The lack of these fatty acids causes many abnormalities and diseases such as breast cancer [8], cardiovascular disorders [9], and inflammatory and immunological responses [10]. Antioxidant compounds are very important for the human body to defend against such diseases, including cancers. Antioxidant compounds can be introduced into the human body naturally and synthetically. However, synthetic antioxidant compounds may not be useful or safe for the human body, so most of the researchers have focused on natural antioxidants obtained from plants [11].

There are no available data in the literature on the analysis of fatty acid composition, total phenolics, and antioxidant effect of *E. chamaesyce*. Therefore, the goal of the present study is to evaluate the fatty acid composition and antioxidant activity of the stem and seed parts of *E. chamaesyce* extracted by different solvent systems.

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2. MATERIAL AND METHODS

2.1. Chemicals and Solvents

Ethylene diamine tetraacetic acid (EDTA), dimethyl sulphoxide, gallic acid, ethyl alcohol, ferrozine, $DPPH^{\cdot}$, iron (II) chloride, and BHT were purchased from Sigma-Aldrich GmbH (Taufkirchen/Germany). *n*-Hexane, anhydrous sodium carbonate, methanol, acetone, chloroform, and ethyl acetate were purchased from Merck (Darmstadt/Germany).

2.2. Plant Material

E. chamaesyce materials were collected from Elazig (Turkey) in September 2012. The plant materials were identified by a taxonomist from Bingol University. A voucher herbarium specimen (*L behcet* 7461b, herbarium No:312) was deposited at the Bingol University Herbarium (BIN).

2.3. Preparation of the Extracts

The dried stem (57 g) and seed materials (5 g) were crushed and respectively extracted with n-hexane, ethyl acetate, chloroform, acetone, and methanol at room temperature for 7 days in each solvent. The obtained extracts were dried using a rotary evaporator under vacuum.

2.4. Determination of Fatty acids and Steroids

A solution of the hexane extracts was mixed with 1 mol/L KOH/MeOH solution (5 mL) and rigorously vortexed. The filtered hexane phase analyzed in a GC system (Agilent 7890A) coupled with an triple-axis MS detector (Agilent 5975C) and an column (Agilent HP-5MS; 30 m x 0.25 mm, 0.25 μm). The carrier gas (hellium) with flow rate of 1 mL/min was used. The temperature program was as follows: 120 ºC for 4 min; from 120 to 200 ºC at 3 ºC/min, 10 min hold; from 200 to 280 ºC at 15 ºC/min, 15 min hold.

2.5. Antioxidant Activity

The radical scavenging effects of *E. chamaesyce* were determined using the DPPH assay [12]. The following formula: Radical scavenging % = $[(A_{control} - A_{sample}) / A_{control}] \times 100$ was used. Metal chelating activity of *E. chamaesyce* extracts was determined according to the ferrozine method [13], with some modifications [14].

2.6. Total Phenolic Content

Total phenolic contents of *E. chamaesyce* extracts were determined using the method given in detail references [15, 16].

2.7. Statistical Analysis

Statistical analyses of the data were done by one way ANOVA test and the averages were compared by Tukey's test using the SPSS 11.5. The level of statistical significance was taken at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. The Yields of the Extracts

The yields, amounts, and the codes of the extracts are given at Table 1. The percentage extractive yield was affected by type of solvents. The highest yield was obtained from the seed methanolic extract (13.31%, (w/w)). This may be attributed to the polar protic nature and high dielectric constant of methanol. The dielectric constant of the methanol solvent is 33 and has a polar character, while the other

solvents are apolar solvents with low dielectric constants [17]. Therefore, the yield of the obtained extracts depends on the type of solvents having variable polarities [18].

Table 1. *The yields, amounts, and codes of the extracts from E. chamaesyce*

Parts	Solvent	Code	Amount (g)	Yield % (w/w)
Stem	Chloroform	EC/STC	0.19	1.94
	Ethyl acetate	EC/STE	0.06	0.61
	Acetone	EC/STA	0.01	0.05
	Methanol	EC/STM	0.73	7.30
Seed	Chloroform	EC/SEC	2.13	3.74
	Ethyl acetate	EC/SEE	0.66	1.15
	Acetone	EC/SEA	0.69	1.22
	Methanol	EC/SEM	7.59	13.31

3.2. Fatty Acid Composition

The major fatty acids obtained from the seed (Table 2) and stem (Table 3) hexane extracts were determined as linolenic acid (32.42% and 22.31%, respectively) and linoleic acid (15.04% and 14.25%, respectively). Palmitic acid and oleic acid were found as other major constituents found in the hexane extracts from *E. chamaesyce*. In addition to fatty acids, some alkanes (17-pentatriacontene, hentriacontane) and phytol were also identified as other constituents of these extracts (Table 2 and 3). Fatty acid chromatograms of the seed and stem hexane extracts are given in Figure 1.

Table 2. *Fatty acid composition of the seed hexane extract of E. chamaesyce*

Retention Time (min)	Compound	Composition (%)
30.37	Palmitic acid	8.89
35.74	Stearic acid	2.59
36.24	Oleic acid	9.93
37.29	Linoleic acid	15.04
38.77	Linolenic acid	32.42
39.69	Phytol	7.46
40.20	Arachidic acid	2.29
45.53	Behenic acid	3.03
47.19	Nonacosane	2.06
55.09	Hentriacontane	3.10
63.05	17-Pentatriacontene	11.06

Table 3*. Fatty acid composition of the stem hexane extract of E. chamaesyce*

Retention Time (min)	Compound	Composition (%)
20.30	Lauric acid	1.73
24.66	Myristic acid	1.91
30.38	Palmitic acid	10.72
35.75	Stearic acid	2.20
36.25	Oleic acid	7.64
37.29	Linoleic acid	14.25
38.77	Linolenic acid	22.31
39.70	Phytol	5.81
45.53	Behenic acid	6.92
55.09	Hentriacontane	2.87
63.08	17-Pentatriacontene	23.62

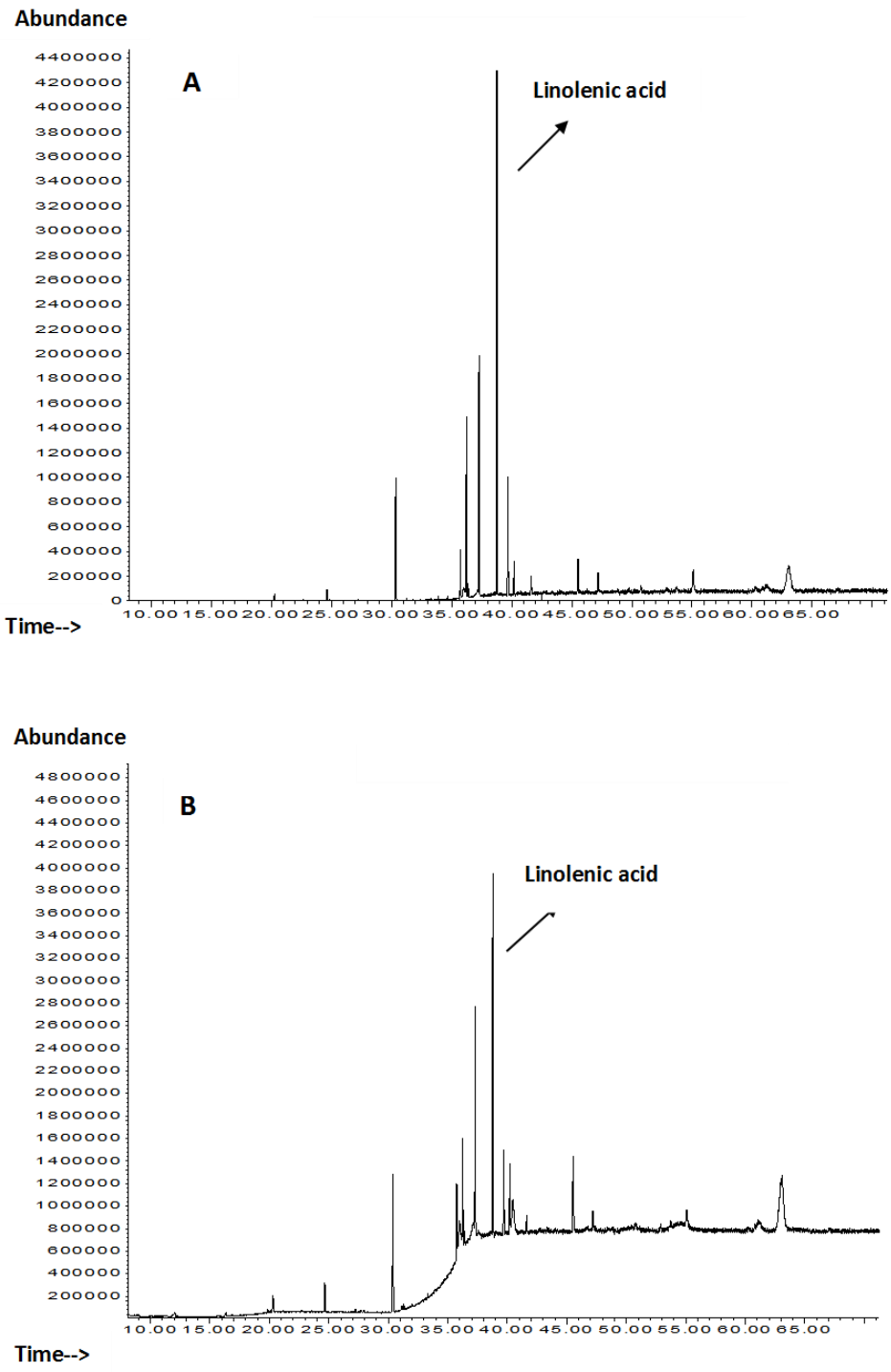

Figure 1. *Fatty acid chromatograms of the seed (A) and stem (B) hexane extracts*

Similar to our study, several researchers have reported that the linolenic acid was the major fatty acid of some Euphorbia species [19]. Bruni et al. [19] reported that three Sardinian wild Euphorbia species; *Euphorbia pithyusa* subsp. *cupanii* (59.0%), *Euphorbia dendroides* (46.38%), and *Euphorbia characias* (52.02%) seeds were rich in linolenic acid. On the other hand, Ertas et al. [20] reported that the major constituent of fatty acids from *Euphorbia gaillardotii* and *Euphorbia macroclada* species was palmitic acid, in contrast to the results of our study. The human body cannot produce essential fatty acid and so it has to be supplemented [21]. Polyunsaturated fatty acids are very important for the normal growth of many organ systems, especially for the eye and the brain. Docosahexaenoic, which can be synthesized from linolenic acid, is a crucial component of cell membranes in the retina and brain, where neurotransmitter metabolism as well as visual and neural function are involved [22]. According to these

results, the hexane extracts of *E. chamaesyce* may be a good source of polyunsaturated fatty acids especially linolenic acid.

3.3. DPPH˙ Radical Scavenging Activities of the Extracts

IC_{50} values and the percentages of DPPH radical scavenging activities of the extracts from *E. chamaesyce* at tested concentrations are presented in Table 4 and Figure 2. *E. chamaesyce* seed methanolic extract (EC/SEM) showed the highest radical scavenging effect with IC_{50} of 5.35 ± 0.01 µg/mL. EC/SEA (seed acetone extract, IC_{50} = 8.61 ± 0.11 µg/mL), EC/SEE (seed ethyl acetate extract; IC_{50} = 20.6 ± 1.12 µg/mL), and EC/SEM showed higher radical scavenging effect than the synthetic antioxidant BHT.

Table 4. *DPPH scavenging effects of E. chamaesyce extracts* [1]

Material	Scavenging effect (%) at tested concentrations							IC_{50} (µg/mL)
	5 µg/mL	10 µg/mL	15 µg/mL	20 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	
EC/SEM	45.5 ± 2.2	79.1 ± 1.1	94.4 ± 0.5	96.0 ± 2.7	96.3 ± 0.5	96.5 ± 0.9	97.1 ± 0.5	5.35 ± 0.01[a]
EC/SEA	28.5 ± 1.0	57.6 ± 0.9	77.8 ± 0.6	90.4 ± 0.9	93.4 ± 1.5	93.6 ± 1.2	94.8 ± 0.7	8.61 ± 0.11[b]
EC/SEE	9.6 ± 0.7	23.0 ± 1.4	34.9 ± 0.1	50.0 ± 1.8	60.1 ± 2.4	89.4 ± 0.8	94.0 ± 0.6	20.60 ± 1.12[c]
EC/SEC	0.3 ± 0.1	0.6 ± 0.1	1.0 ± 0.2	1.3 ± 0.0	2.5 ± 0.4	3.5 ± 0.6	4.8 ± 0.7	>100
EC/STM	4.8 ± 2.1	9.5 ± 2.2	14.3 ± 1.8	18.9 ± 1.5	22.2 ± 1.6	42.1 ± 1.2	75.1 ± 3.9	56.72 ± 0.70[f]
EC/STA	0.2 ± 0.0	4.8 ± 1.2	7.3 ± 0.8	9.8 ± 0.8	10.5 ± 0.7	20.4 ± 0.4	40.0 ± 0.3	>100
EC/STE	4.5 ± 0.8	9.1 ± 1.2	14.0 ± 1.6	18.3 ± 1.4	21.6 ± 1.2	41.8 ± 0.8	73.1 ± 0.7	53.21 ± 0.08[e]
EC/STC	0.2 ± 0.0	0.5 ± 0.2	0.9 ± 0.2	1.1 ± 0.5	1.5 ± 0.3	2.8 ± 0.7	3.7 ± 1.0	>100
BHT	12.5 ± 0.1	25.2 ± 0.0	36.5 ± 0.2	47.4 ± 0.1	58.8 ± 0.3	93.2 ±0.5	94.1±0.7	23.08 ± 1.42[d]

[1] *IC_{50} values with different small letters are significantly ($p < 0.05$) different.*

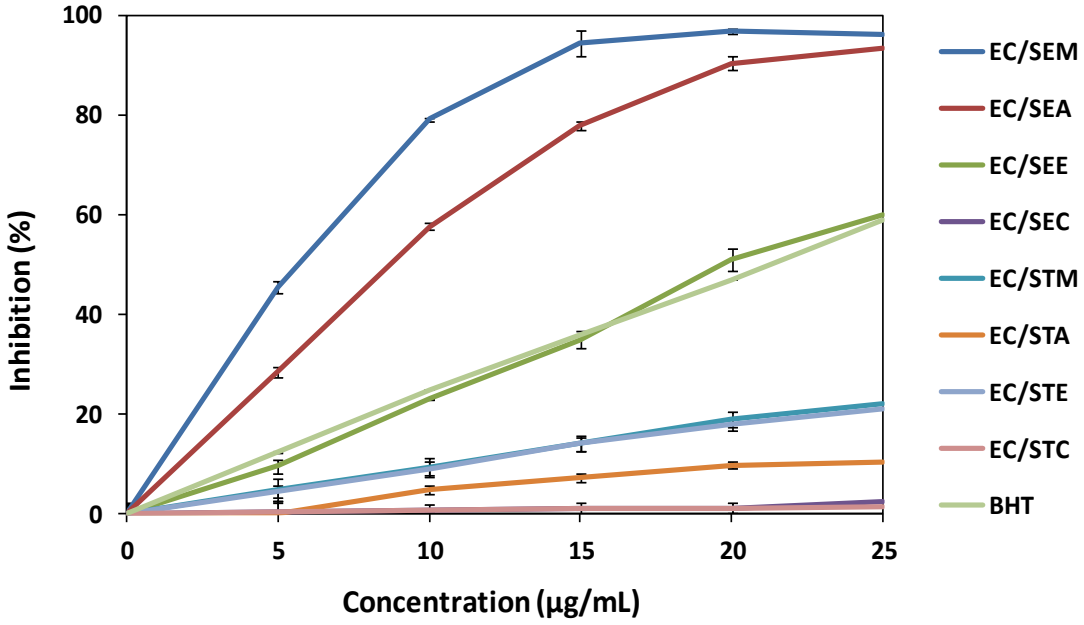

Figure 2. *DPPH scavenging activitiy of the extracts at tested concentrations (EC/SEM: E. chamaesyce seed methanol extract, EC/SEA: E chamaesyce seed acetone extract, EC/SEE: E. chamaesyce seed ethyl acetate extract, EC/SEC: E. chamaesyce seed chloroform extract, EC/STM: E chamaesyce stem methanol extract, EC/STA: E. chamaesyce stem acetone extract, EC/STE: E. chamaesyce stem ethyl acetate extract, EC/STC: E. chamaesyce stem chloroform extract)*

In general, the seed extracts showed higher radical scavenging effect than the stem extracts. The extracts of *E. chamaesyce* exhibited the radical scavenging effect in a concentration-dependent manner. According to Figure 1, $DPPH^{\cdot}$ radical scavenging abilities of the extracts increased with the increased concentration. EC/SEM exhibited 94.4% scavenging activity at the concentration of 15 µg/mL, whereas BHT showed 36.5% at the same concentration.

To the our knowledge, in the literature, there is no study on *E. chamaesyce* about its antioxidant activity. However, several studies have been reported about antioxidant activity of other Euphorbia species. Similar to our study, in the study of Barla et al. [23], the acetone and ethanol extracts of *Euphorbia acanthothamnos* Heldr. et Sart. Ex Boiss. and *Euphorbia macroclada* Boiss. exhibited higher activity than BHT.

3.4. Metal Chelating Activities of the Extracts

Metal chelating has key role to inhibition of pro-oxidant metal ions, such as Fe^{2+}, Cu^{2+} [24]. The chelating abilities (%) of the extracts and EDTA are presented in Figure 3. EC/SEC extract exhibited higher chelating effect than the other extracts.

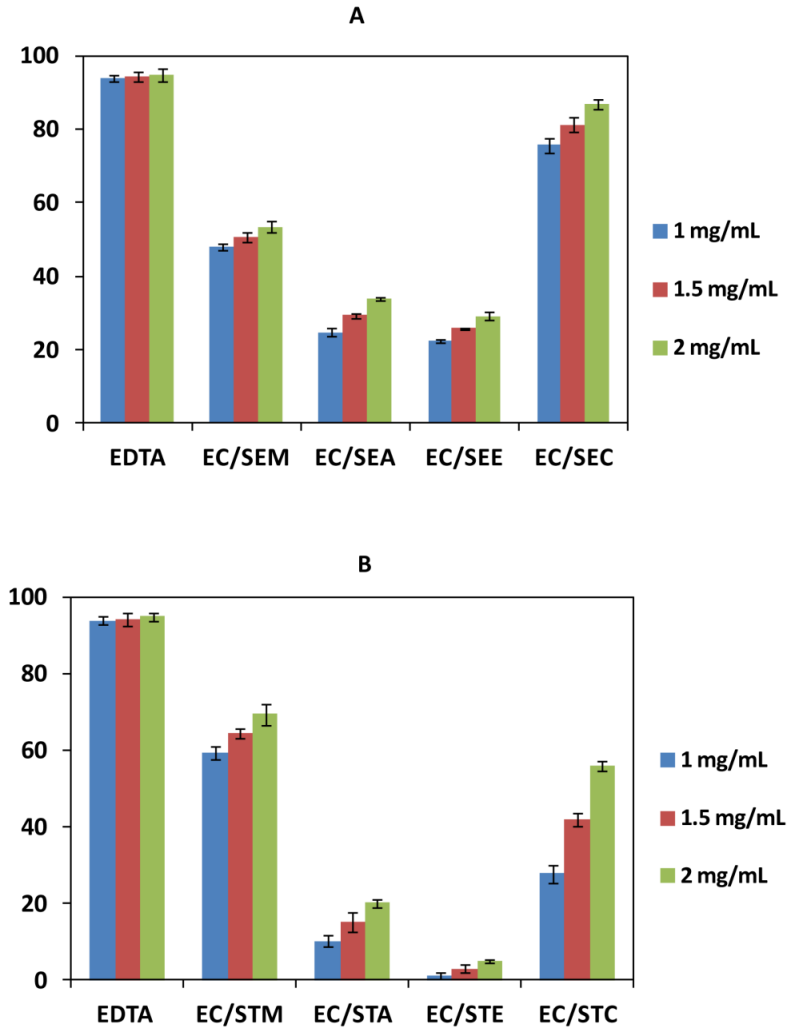

Figure 3*. Metal chelating effects of the seed (A) and stem (B) extracts at the tested concentrations (EC/SEM: E. chamaesyce seed methanol extract, EC/SEA: E chamaesyce seed acetone extract, EC/SEE: E. chamaesyce seed ethyl acetate extract, EC/SEC: E. chamaesyce seed chloroform extract, EC/STM: E chamaesyce stem methanol extract, EC/STA: E. chamaesyce stem acetone extract, EC/STE: E. chamaesyce stem ethyl acetate extract, EC/STC: E. chamaesyce stem chloroform extract)*

3.5. Total Phenolic Contents

Total phenolic contents of the extracts were estimated by the Folin-Ciocalteu procedure, and the amount of polyphenols in the extracts was expressed in micrograms per milligram. Total phenolic contents of the extracts were found in a range of 24.2 ± 2.6 to 277.6 ± 2.1 µg/mg (Table 5). EC/SEM, which exhibited the highest DPPH effect (IC_{50} = 5.35 ± 0.01 µg/mL), had the highest total phenolic content (277.6 ± 2.1 µg/mg).
Total phenolic contents of the extracts were determined in the order of EC/SEM > EC/SEA > EC/SEE > EC/STE > EC/STM > EC/STA > EC/SEC > EC/STC. Similar to our study, Ashraf et al. [26] found positive correlation between total phenolic content and DPPH scavenging activity of another species of Euphorbia *(E. royleana).*

Table 5. *Total phenolic contents of the extracts*[1]

Total phenolic content (µg GAE/mg extract)		
Material	Seed	Stem
Methanol extract	277.6 ± 2.1[a]	78.0 ± 0.8[b]
Acetone extract	251.2 ± 1.2[b]	28.9± 0.7[c]
Ethyl acetate extract	141.7 ± 3.7[c]	82.3 ± 5.2[a]
Chloroform extract	24.2 ± 2.6[d]	25.9 ± 1.9[d]

[1]*Values in the same column with different small letters are significantly ($p < 0.05$) different*.

4. CONCLUSION

In this study, fatty acid compositions and antioxidant activities of the extracts obtained from seed and stem parts of *E. chamaesyce* were evaluated. Linolenic and linoleic acids were found as the major fatty acids of this plant. Therefore, *E. chamaesyce* can be considered as a source of these polyunsaturated essential fatty acids. On the other hand, the seed methanol extract exhibited the highest scavenging ability on DPPH radicals. This property is probably due to high polyphenol contents of the methanolic extracts. The results showed that *E. chamaesyce* could be used as a natural antioxidant agent in food and pharmaceutical industries.

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CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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