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Fractionation, Phytochemical Screening and Antioxidant Activity of Different Sub-Fractions from Leaves and Flowers of *Erica arborea* L.

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ABSTRACT

The purpose of this study was to prepare eight sub-fractions from leaves and flowers of *Erica arborea* L., characterize their phytochemicals constituents and investigate their potential antioxidant, in order to validate the beneficial medicinal properties of this shrub in Algeria folk medicine. Total polyphenols, flavonoids and condensed tannins contents were determined using Folin-Ciocalteu's, aluminum chloride and vanillin reagents, respectively. The *in vitro* antioxidant activity was evaluated by using 2,2-diphenyl-l-picrylhydrazyl and reducing power assay. *E. arborea* L. contains various compounds such as polyphenols, flavonoids, tannins, quinones, anthraquinones, saponins and terpenoids in different sub-fractions. All the tested extracts showed an appreciable total phenolic, flavonoids and condensed tannins contents as well as strong antioxidant capacity. The highest total phenolic and flavonoids content was found in the ethyl acetate extracts ranging from 649.38 to 944.55 mg gallic acid equivalent/g dry extract and 65.31 to 67.15 mg quercetin equivalent/g dry extract, respectively. Whereas, the highest condensed tannins content was found in the crude extract for leaves and aqueous extracts for flowers. The ethyl acetate extract of the flowers and the crude extract of leaves exhibited the better antioxidant activity by DPPH assay (IC_{50} =17.72 µg/mL) and reducing power assays (IC_{50} = 2.91 µg/mL), respectively. Our findings indicate that leaves and flowers extracts are rich in natural antioxidant substances and have good qualities in antioxidant properties and may be beneficial against diver's disorders related to free radicals.

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Introduction

Free radicals are highly reactive molecules, promising to the initiation of multiple chain reactions with other oxidants particles, which contain one or more unpaired electrons involve some degree of oxidative stress (Köroğlu et al., 2018; Akgül et al., 2022; Krupodorova et al., 2022). When the chain reaction of these radicals takes place in a cell, it can cause damage or death to the cell (Selamoglu, 2018). The oxidative stress is the major leading cause of many pathological disorders including neurodegenerative and cardiovascular diseases **(**Selamoglu et al., 2020). Many of synthetic antioxidants were used such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are casted to be responsible for some carcinogenesis problems and toxicological effect in liver (Chaouche et al., 2018; Kına et al., 2021). Since very old times, plants have been generated much attention and used in the folk medicine by several indigenous herbal practices in human health to cure several diseases because their nontoxic implications (Phuyal et al., 2020; Mohammed et al., 2022). Due to the use of various plant species as a resource of naturally occurring products, the study of their antioxidant ability and biological activities augmented in recent years (Selamoglu et al., 2017; Chaves et al., 2020; Phuyal et al., 2020, Karbab et al., 2021; Pehlivan et al., 2021). Thus, these studies could contribute to establishing the value of these species as a source of new antioxidant drugs (Chaves et al., 2012; Sevindik et al., 2017). Antioxidants are tremendously important substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress (Almada-Taylor et al., 2018; Unal et al., 2022). Phenols and flavonoids form different origin include fruits, vegetables, and medicinal and aromatic plants, are phytoconstituents gaining reputation for their antioxidant abilities (Karbab et al., 2019; Phuyal et al., 2020; Uysal et al., 2021). These components are well known for a variety of therapeutic properties on health of human (Selamoglu, 2017a; Selamoglu, 2017b; Jiang et al., 2019; Selamoglu and

Akalin, 2019). The genus Erica (Ericaceae) contains more species spread all over the world, three of which are that found abundantly in the flora of Algeria (Guendouze et al., 2015). *Erica arborea* grows commonly in Algeria and its medicinal properties is exploited by it enter in herbal tea composition (Guendouze et al., 2015; Suna et al., 2018). This shrub is known for the treatment of several health benefits such as digestive disorders include constipation and have excellent diuretic, anti-inflammatory and anti-lithiatic importance (Suna et al., 2018; Amroun et al., 2021). Several phytocomponents such as polyphenols, flavonoids, alkaloids been described from different parts of *E. arborea* (Luis et al., 2011) and biological activities of these natural antioxidants are responsible for several antioxidant properties to prevent diseases by scavenging free radicals and delaying or preventing oxidation of biological molecules (Afsar et al., 2018). Some experiments have been carried out in *E. arborea* aerial parts or their leaves alone regarding their antioxidant properties (Ay et al., 2007; Nazemiyeh et al., 2008; Köroğlu et al. 2018; Amroun et al., 2021). However, only one study has investigated its antioxidant activities of their separated vegetal parts (Luis et al., 2011). Therefore, the comparative study from different parts of the plants is still insufficient and no work done the effect of solvents in extracting phytochemicals from the leaves and flowers of the plant. So, the main objective of this study was conducted to carry out a study of different solvent extracts (methanol, chloroform, ethyl acetate, aqueous extracts) prepared for separated vegetal parts (leaves and flowers) of Algeria *E. arborea* used in medicine as natural medicines, on their antioxidant capacities as well as on total phenolic, flavonoids condensed tannins content. The in vitro antioxidant capacity was performed by 2,2-diphenyl-1-picrilhidrazil (DPPH) radical scavenging and reducing power activity.

Materials and Methods

Plant Material

Different vegetal parts of *E. arborea* were collected from the mountain of Djebel of Tadergount, Bejaia, North of Algeria. The plant was identified by Pr H. Laouer (Laboratory of Valorization of Natural Biological Resources, University of Setif, Algeria) under voucher specimen (015/DBEV/UFA/19). The dried material was powdered and stored in darkness until use.

Bioactivity Guided Fractionation

The separated parts *E. arborea* were prepared by using solvents with different polarities (Karbab et al., 2020). Leaves and flowers was extracted by methanol using a ratio of 1:10 at room temperature, and then stirred during 24 hours. The methanolic extract was partitioned sequentially by fractionation with organic solvents (hexane, chloroform, ethyl acetate) in order of increasing polarity (Figure 1). All the four fractions of different vegetal parts were dried by evaporating respective solvent using rotary evaporator. All extracts were stored at 4°C till further analysis.

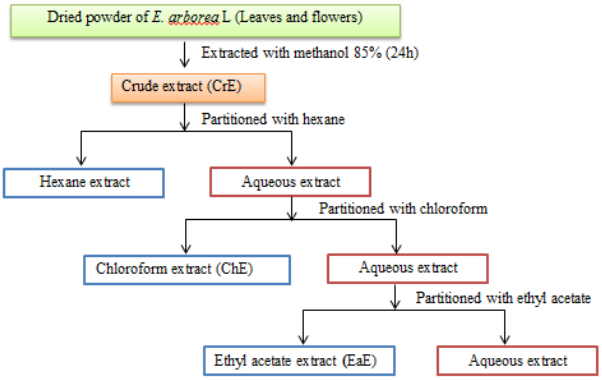

Figure 1 Preparation of different sub-fractions of *E. arborea* different parts. CrE: crude extract; ChE: chloroform extract; EaE: ethyl acetate extract.

Phytochemical Screening

Qualitative tests for the presence of different phytochemical compounds include: polyphenols, flavonoids, hydrolyzables tannins, free quinones, anthraquinones, anthocyanins, coumarins, alkaloids, terpenoids, and saponins according to published procedure (Karbab, 2020). These are qualitative analyses based on coloring and/or precipitation reactions.

Determination of Total Phenolic Flavonoid and Condensed Tannins Contents

The total phenolic contents in the different extracts were assessed by the Folin-Ciocalteau's method, in according to the method outlined by our previous publication (Karbab et al., 2020). Briefly, an aliquot of 100 µL of the extract was mixed with 500 µL of Folin-Ciocalteu's diluted reagent for 4 min, followed by the addition of 400 µL of a 7.5% Na_2CO_3 solution. After 1.5 h of incubation, the absorbance was measured at 765 nm. The total flavonoids content was determined by the colorimetric method described by (Karbab et al., 2020). One mL of each sample was added to 1 mL of aluminum chloride ($AlCl_3$) solution (2%). After 10 min of incubation, the absorbance of the mixture was measured at 430 nm. The condensed tannins in the extracts were determined by the vanillin method according to (Ali-rachedi et al., 2018). One mL of extract was mixed with 1.5 mL of 4% vanillin/methanol solution. Then, 750 µL of concentrated hydrochloric acid (HCl) was added. The mixture was allowed to stand in the dark at 20 °C for 20 min, and absorbance was determined at 500 nm. Different calibration curve was prepared. The polyphenol, flavonoids and condensed tannins content of the extract was expressed as µg Gallic Acid Equivalent/mg dry weight (DW), µg of Quercetin Equivalent/mg DW and µg Catechin Equivalent/mg DW, respectively. All determinations were done in triplicate (n = 3).

Antioxidant Capacity

DPPH scavenging assay

The antioxidant ability of extracts of *E. arborea* was performed by quenching DPPH (Charef et al., 2015). Briefly, freshly prepared DPPH solution (0.4 mM) was prepared in methanol, stored in an amber color bottle.

All the *E. arborea* extracts were dissolved in methanol and make various concentrations of an extract by applying serial dilution method. Add 1.25 mL of DPPH solution to 50µl of each serial dilution of extract. Additionally, 1.25 mL of DPPH solution is mixed with 50 µL of methanol as control without extract. The mixture was mixed well and incubated in darkness for 30 min. The assay was performed in triplicate, and the mean absorbance was calculated and noted at 517 nm. The butylated hydroxytoluene (BHT) is used as positive control. The percent of inhibition of DPPH radical was calculated using the formula: Radical scavenging activity (%) = $[(A_c - A_s)/A_c]\times100$; where A_c: the absorbance of the solution except the tested sample and A_s: the absorbance of extract or standard.

Reducing power assay

The reducing power assay was performed to estimate the ability of various extracts to reduce Fe^{+3} to Fe^{+2} (Bouaziz et al., 2015), with some modifications. According to this procedure, an aliquot of 400 µL of extract was mixed with an identical volume of both phosphate buffer (0.2 M, pH= 6.6) and potassium ferricyanide (1%). This mixture was then incubated for 20 min at 50°C in a water bath. The reaction was terminated by adding 400 µL of trichloroacetic acid (TCA) (10%), and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (400 µL) was added to distilled water (400 µL) and 80 µL of 0.1% ferric acid. The color intensity of the mixture was measured at 700 nm after 10 min of incubation. Reducing power assay (%) = $[(A_c - A_s)/A_c]\times100$; where A_c: the absorbance of the solution except the tested sample and A_s: the absorbance of extract or standard.

Statistical Analysis

Statistical analysis was performed by using the Graph Pad Prism (version 5.03 for Windows). In this study, statistical analysis was analyzed by one-way analysis of ANOVA. All determinations were carried in triplicate, and all results were estimated as the mean ± standard deviation (SD). Tests of significant differences were determined by multiple range tests at $P<0.05$.

Results

Extraction Yield

The yields of various extracts are shown in Table 1. The highest yield was noted with crude extract (CrE) in all used parts compared to other extraction by various solvents. Their yields decreased in the following order: CrE> AqE > EaE> ChE in *E. arborea;* which ranged from 21.28 to 1.2% for flowers, 21.21 to 1.36% for leaves Auxiliary, it was observed that the CrE extract (CrE) of flowers and leaves produced an almost similar maximum yield of phytochemicals about 21.28 and 21.21%, respectively. Although, chloroform extracts (ChE) of leaves, flowers displayed a lower yields with range from 1.2 to 1.36%. Color and consistency of extract varied also according to the extraction solvents, a mirrored powdery are recorded for Crude (CrE), ethyl acetate (EaE) and aqueous extracts (AqE), while the chloroform extract (ChE) of leaves and flowers has a matte powdery appearance.

Phytochemical Screening

The preliminary results from the phytochemical study of the studied extracts are shown in Table 2. Phytochemical investigation of the *E. arborea* extracts obtained from different parts of *E. arborea* (leaves and flowers) reveals the presence of metabolites such as polyphenols, terpenoids and quinones in all solvents extract. Whereas, in all the extracts, anthocyanins, coumarins, and alkaloids substances were found to be absents. The flavonoids, hydrolyzable tannins, anthraquinones and saponins were not found in all sub-fractions.

Total Phenolic Content, Flavonoids and Condensed Tannins Determination

The amounts of total phenolic (TPC), flavonoids (TFC) and condensed tannins (TC) were detected in tested extracts of all part of *E. arborea* and their results are given in Table 3. Firstly, in all sub-fractions, the highest level of polyphenols compounds were recorded in leaves (LE) followed by flowers (FE), which ranged from 80.45 µg GAE/mg dry extract for chloroform of FE to 944.55 µg GAE/mg extract for ethyl acetate of LE. The TPC decrease in the following order: EaE > CrE > AqE > ChE for LE and EaE>AqE > CrE> ChE for FE. Secondly, the flavonoids content are varying from 6.02 µg QE/mg extract for aqueous of FE to 67.15 µg QE/mg extract for ethyl acetate of LE. The largest amount of flavonoids was obtained via LE followed by FE. The TFC decrease in the following order: EaE> CrE> ChE> AqE for LE and FE. Furthermore, the condensed tannins content ranged from 14.77 (µg CE/mg extract) for chloroform of FE to 337.53 (µg CE/mg extract) for crude of LE. The TC decrease in the following order CrE> AqE >EaE > ChE for LE and AqE > CrE > EaE > ChE for FE.

Antioxidant Capacity

DPPH scavenging assay

The results revealed that all extracts scavenged the DPPH radical with an IC_{50} values varying from 38.18 to 60.16 µg/mL for leaves and from 17.72 to 65.29 µg/mL for flowers. All these values are higher than synthetic drug BHT with an IC_{50} value 87.65 µg/mL. The highest DPPH radical scavenging activity was exhibited with EaE of flowers with IC_{50} values of 17.72± 0.00 µg/mL, following by CrE of flowers with IC_{50} values of 24.81± 0.00 µg/mL, respectively. The lowest value was obtained by ChE of leaves.

Table 1. Yield, color and consistency of *E. arborea* extracts.

Extracts	Extraction yield (%)		Color and Consistency	
	LE	FE	LE	FE
CrE	21.21	21.28	Dark brownish mirrored powder	Dark brownish mirrored powder
ChE	1.36	1.2	Dark greenish mate powder	Clair greenish mate powder
EaE	6.84	5.32	Clair orangish mirrored powder	Clair orangish mirrored powder
AqE	15.81	12.20	Dark brownish mirrored powder	Dark brownish mirrored powder

CrE: crud extract; ChE: chloroform extract; EaE: ethyl acetate extract; LE: leaves extracts; FE: flowers extracts.

Table 2. Phytochemical screening of different extracts from *E. arborea* parts

Phytochemicals		Extracts			
		CrE	ChE	EaE	AqE
Polyphenols	LE	+++	+	+++	++
	FE	+++	+	++	++
Flavonoids	LE	++	-	+++	++
	FE	++	-	+++	-
Hydrolysable tannins	LE	+++	-	-	+++
	FE	+++	-	-	+++
Saponins	LE	-	+++	-	++
	FE	-	+++	-	+
Anthraquinones	LE	+	-	+++	+
	FE	+	-	+++	+
Quinones	LE	++	+	++	++
	FE	++	+	++	++
Coumarins	LE	-	-	-	-
	FE	-	-	-	-
Anthocyanins	LE	-	-	-	-
	FE	-	-	-	-
Terpenoids	LE	++	++	++	+
	FE	++	++	++	+
Alkaloids	LE	-	-	-	-
	FE	-	-	-	-

Key: += less presence, ++ =middle presence, +++=fort presence, -=absence. CrE: crud extract; ChE: chloroform extract; EaE: ethyl acetate extract; LE: leaves extracts; FE: flowers extracts.

Table 3 Total polyphenols, flavonoids and condensed tannins of various part extracts of *E. arborea*. The data represent the mean ± SD of three determinants.

Extracts		TPC (µg GAE/mg dry extract	TFC (µg QE/mg dry extract	TC (µg CE/mg dry extract
CrE	LE	591.58 ± 0.97	51.12 ± 1.42	337.53 ± 1.88
	FE	416.07 ± 1.46	25.25 ± 1.68	212.36 ± 0.23
ChE	LE	109.24 ± 0.49	18.53 ± 0.32	30.60 ± 1.41
	FE	80.45 ± 0.24	14.58 ± 1.99	14.77 ± 0.23
EaE	LE	944.55 ± 1.95	67.15 ± 0.04	173.53 ± 0.47
	FE	649.38 ± 1.95	65.31 ± 0.56	267.12 ± 1.06
AqE	LE	489.86 ± 1.46	9.37 ± 0.04	262.62 ± 0.12
	FE	481.24 ± 1.95	6.02 ± 0.11	280.37 ± 1.65

CrE: crude extract; ChE: chloroform extract; EaE: ethyl acetate extract; LE: leaves extracts; FE: flowers extracts.

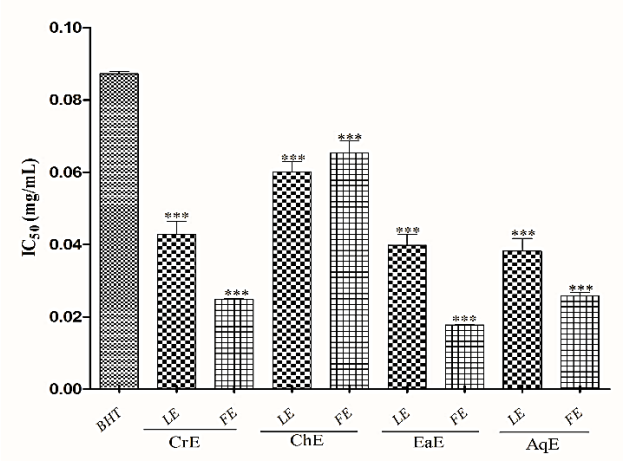

Figure1 DPPH radical scavenging activity of leaves, flowers and stems extracts of *E. arborea*.

CrE: crude extract; ChE: chloroform extract; EaE: ethyl acetate extract; LE: leaves extracts; FE: flowers extracts. Data are presented as IC_{50} values. Each value represents the mean ± SD (n = 3). ***: P≤ 0.01.

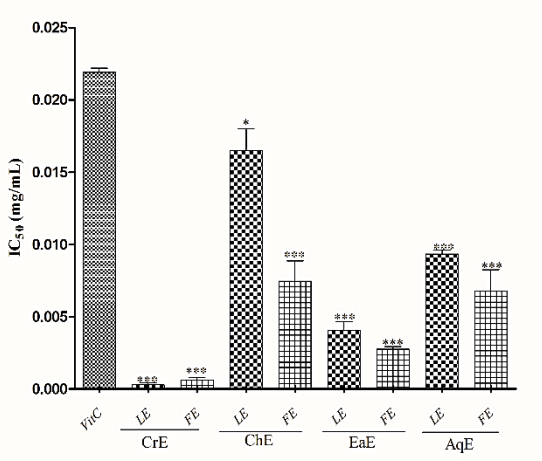

Figure 2 Reducing power of leaves, flowers and stems extracts of *E. arborea*.

CrE: crude extract; ChE: chloroform extract; EaE: ethyl acetate extract; LE: leaves extracts; FE: flowers extracts. Data are presented as IC_{50} values. Each value represents the mean ± SD (n = 3). ns: no significant difference, *: P≤ 0.05, ***:P≤ 0.001.

Reducing power assay

In the current test, the reducing power ability was determined for all extracts of *E. arborea* (Figure 2). All the extracts except chloroform extract displayed good reducing power by the reduction of Fe^{3+}/ferricyanide to Fe^{2+}-ferrozine complex by electron donation capacity. The reducing power of the leaves is ranged from values of 2.91 to 151.21 µg/mL, whereas, the IC_{50} of flowers is ranged from values of 6.22 to 74.71 µg/mL. In comparison to ascorbic acid, the greatest reducing antioxidant power was recorded for CrE of leaves, followed by CrE of flowers with an IC_{50} = 2.91 µg/mL and 6.22 µg/mL, respectively. Whereas, ChE of leaves displayed weak chelating properties. The trend of the total reducing power of the all extracts was; CrE> EaE > AqE > ChE >.

Discussions

Extraction is the first step for polyphenolic analysis, which consists in their isolation from plant materials (Chaouche et al., 2018). The variability of extraction yield depends on the plant part used as well as the solvent used. These results are in agreement with those obtained by Luis et al. (2011), who shown that the crude of *E. arborea* from the LE and FE recovered a better yield (Luis et al., 2011). Extraction yield of bioactive compounds from plant materials depends on the polarity of the extracting solvent (Do et al., 2013; Masoko, 2017; Chaouche et al., 2018). The difference in polarities of the extraction solvents might influence the nature of phytochemicals extracted in a sample (Dewi et al., 2020).

In the context to gain preliminary knowledge on the nature of chemicals compounds presents in various extracts of *E. arborea* systematic phytochemical investigation is accomplished. In the present study, polyphenols, hydrolyzable tannins, terpenoids, quinones, and anthraquinones detected in *E. arborea* of LE were in agreement with those obtained by Amezouar et al. (2013) from the dried leaves of *E. arborea* in which the presence of saponins was also reported. Flavonoids and phenolics are the main compounds isolated from Ericaceae species and that also contains terpenoids (Guendouze et al., 2015). Polyphenols, flavonoids, Tannins, saponins, quinones, anthraquinones, and terpenoids are widely known for their essential medicinal properties (Jiang et al., 2019).

The content of phenolic, flavonoids and condensed tannins was largely influenced by extraction solvents and its polarity, as well as by the vegetal parts. The quantitative determination these compounds are widespread assays (Luis et al., 2011). Firstly, some auteurs investigated the phenolic content of *Erica arborea* in several parts of the shrub (Luis et al., 2011; Suna et al., 2018). In general, LE has the highest amount of phenolic compounds. This result is similar to those obtained by Lius et al (2011). Suna et al. (2018) studied methanol extract of dried leaves and found value at 749.48 ± 34.46 mg gallic acid equivalent/g extract) of total phenolic content. However, this amount is higher than found in crude extract of LE (591.58 ± 0.97mg GAE/g of dry weight). Luis et al. (2011) examined different parts of methanol extracts from *E. arborea* such as leaves and flowers for their phenolic contents with 260.2 ± 1.9 (mg gallic acid equivalent/g extract) for leaves and 178.1 ± 0.2 (mg gallic acid equivalent/g extract) for flowers. These values obtained by this study were lower to those of present study.

The EaE of *E. arborea* was found to have high content of total phenols especially in flowers extracts. These results are in a good agreement with the literature (Ay et al., 2007; Köroğlu et al. 2018) for aerial part of *E. arborea*. Additionally, Köroğlu et al. (2018) were conducted the study of several *Ericaceae* species of Turkish, and concluded that their richness on phenolic compounds, and that ethyl acetate is one of the best solvents for their extraction (Köroğlu et al. 2018). Maximum total phenolic content found in ethyl acetate extracts of aerial part of *E. arborea* was 315.52±3.81 (µg pyrocatechol equivalents/mg extract) and 875.5 (mg gallic acid equivalent/g extract) in previous studies of Ay et al. (2007) and Köroğlu et al. (2018), respectively (Ay et al., 2007; Köroğlu et al. 2018). It was observed the effect of ethyl acetate solvent on TFC is similar to that on TPC which their content increased with solvent polarity; this is in agreement with the polar nature of flavonoids (Rebaya et al., 2015; Kohoume et al., 2017). Thus, Polar extract (EaE and CrE) showed more flavonoids than apolar extract (ChE) (Rebaya et al., 2014; Dewi et al., 2020).These results accorded to study of Dewi et al. (2020), which has the highest flavonoids content on ethyl acetate extract of *Scorodocarpus borneensis* Becc bark. Additionally, the ethyl acetate extract of mixed parts of *E. arborea* was found to be richest in terms of flavonoids (150.42±1.63 µg quercetin equivalents/mg extract) contents. Flavonoid content of leaves was higher than flowers. These results are similar to those reported by Rebaya et al. (2015). The distribution of condensed tannins across all the used parts using different solvents exhibited also a very great difference. There are no literature data for condensed tannins contents of leaves and flowers extract, but in this work they were shown to be rich in these compounds.

Regarding to the obtained phenolic, flavonoids and condensed tannins compounds values, it is difficult to establish a correct comparison between our results and those of literature data. The geographical and climatic conditions can lead to significant difference in both the concentration of bioactive compounds in plant and their bioactivity for human health (Guendouze et al., 2015). Medini et al. (2014) found that the flowering stage of plant *Limonium delicatulum* had a higher level of phenols compound than the vegetative stage (Medini et al., 2014). It was also known the phenolic content was affected by solvent polarity and a particular part of the plant (Dewi et al., 2020). This large difference in this content can be explained by other factors, among these the origin, the harvested period (Dewi et al., 2020). These amounts may be also affected by the presence of different amounts of other compounds (Aryal et al., 2019).

Due to the complex nature of phytochemicals and their interactions, the importance of using various methods based on different mechanisms for a comprehensive study of the antioxidant properties of plant extracts has been argued (Bekkai et al., 2022). The effect of extracts at different concentrations was studied for their ability of hydrogen or electron transferring ability measured using DPPH and FRAPS tests. The estimation of reducing power by chelating of ferrous ion Fe (III) or the utilization of free DPPH reagent in antioxidant assay among other methods

are very suitable and widely used for determining the antioxidant potency of plant extracts (Chaouche et al., 2018; Aryal et al., 2019; Phuyal et al., 2020). The purple - colored DPPH radical is capable turning to the colored-yellow as well as transformed into DPPH-H when interacted with antioxidant components presents in extracts (Aryal et al., 2019; Trinh et al., 2020; Baliyen et al., 2022). The formation of yellow colorless, diphenylpicrylhydrazine in solution can be quantified spectrophotometrically, which DPPH radical showed a maximum absorption at 515-528 nm (Selamoglu et al., 2017; Karbab, 2020). The reduction ability of reductants or extracts under acidic conditions indicated by the transformation of Fe (III)/ferricyanide complex to it Fe (II)/ferrous colored form by giving away an electron. The yellow color test solution changes to green and blue depending on the can be monitored by measurement of blue color at 700 nm (Selamoglu et al., 2017**;** Aryal et al., 2019). Some researchers investigated the antioxidant capacity of *Erica arborea* (Amezouar et al., 2013; Suna et al., 2018) for dried leaves, (Ay et al., 2007; Guendouze et al., 2015; Köroğlu et al. 2018) for aerial parts and (Luis et al.,2011) for separated aerial parts.

All the examined extracts were able to reduce DPPH and ferrous ion Fe (III) by donation of a hydrogen atom or electron. The scavenging activity against DPPH of all extracts with different polarities of the aerial parts of *E. arborea* native to Turkey were investigated in study of Köroğlu et al. (2018) in the same following order obtained in our study: EaE> AqE> CrE> ChE. All extracts exhibited strong antioxidant activities except the chloroform extract (Köroğlu et al. 2018). In FRAP assay, CrE presents the highest result and the ChE presents the lowest one (Aadesariya et al., 2017). These results were in agreement with those found by Chaouche et al. (2018) on aerial part from *Teucrium polium* which reported that methanolic extract exhibited highest reducing power ability when compared to other extracts. In comparison to the previous study of Amezouar et al. (2013), which studied the leaves of *E. arborea* macerated by methanol, it IC_{50} value is lower than the present study (Amezouar et al., 2013). Also, another investigation on the dried leaves macerated by methanol showed higher IC_{50} about 154.73 ± 1.59 µg/mL (Suna et al., 2018).

Hence, the strong antioxidant activity of *E. arborea* extracts of different vegetal parts could be due to their richness on natural antioxidant substances (Luis et al., 201; Köroğlu et al., 2018). Shrubs of the family of Ericaceae are known as natural sources of bioactive compounds associated to their phenolic compounds composition (Bekkai et al., 2022; Köroğlu et al., 2018). The high phenolic content found in these species is thought to be linked to their strong free-radical scavenging effects and potential health related to therapeutic functions (Ay et al., 2007; Marquez- Garcia et al., 2009; Guendouze et al., 2015; Köroğlu et al. 2018). Some researchers reported that there is a strong correlation between total phenolic contents and the antiradical effectiveness of extracts (Köroğlu et al., 2018; Hmaidosh et al., 2020). This correlation is consistent with the current study, particularly in the EaE and ChE for polyphenols content. This following trend shown in the concentrations of condensed tannins of the leaves extracts. This may imply that the reducing power of leaves had a direct relationship with the concentration of condensed tannins they contain.

The data obtained from this study reveal that EaE of flowers exhibited the most powerful antiradical effect than that of other extracts in different solvent extraction. This may be suggested by the kind of antioxidant phytochemicals such as flavonoid and phenolic compound present in these sub-fraction as well as the presence of other natural products. Also, these same extracts marked high amount of total phenols and flavonoids (Table1). Acoording to Sannigrahi et al. (2008), the low IC_{50} value of ethyl acetate fraction of *Enhydra fluctuans* Lour is due to presence of high polyphenolics and flavonoids (Sannigrahi et al., 2008). Moreover, in the study of Ay et al. (2007), the ethyl acetate extract of mixed parts (leaves and flowers) of *E. arborea* showed the highest phenolic compounds and antioxidant capacity than other extracts in the DPPH assay even higher than BHT, used as reference compound (Ay et al., 2007). In other study, the ethanolic extract of *E. arborea* exhibited notable antioxidant activity in FRAP and DPPH free radical scavenging with the high total polyphenols and tannins values (Pavlovic et al., 2009). Although, the effect of the antioxidant activity of the samples differs rendering of the nature of the solvent used which could be due to different antioxidant compounds extractable (Chaouche et al., 2018; Köroğlu et al. 2018).

In recent years the powerful antioxidant capacity of the flavonoids has been attracting much attention (Köroğlu et al. 2018). The ethyl acetate extract of aerial parts of *E. arborea* was found to be richest in terms of phenolic especially flavonoids contents which exhibited the highest DPPH antioxidant activity (Ay et al., 2007; Köroğlu et al. 2018). Furthermore, in plates sprayed with 1% vanillin-H_2SO_4 analysis detected mainly flavonoids such as kaempferol and luteolin in ethyl acetate extracts of aerial part of *Erica* species (Köroğlu et al. 2018). In aqueous extract among compounds found of aerial part of this shrub are as follow chlorogenic acid and five glycosylated flavonoids (Amroun et al., 2021). In previous study of Nazemiyeh et al. (2008) the methanol extract of the leaves of *E. arborea* afforded five flavonoids and exhibited a higher antioxidant activity than the propyl gallate used as standard (Nazemiyeh et al., 2008). Pavlović et al. explained the antioxidant capacity of *Erica* species, macerated in ethanol by the presence phenolic compounds and kaempferol-3-O-β-D-galactoside and quercetin (Pavlović et al., 2014). Flavonoids in the middle of natural phenolic compounds were known to possess the most potent radical-scavenging and to act in different mechanisms in the regulation of oxidative stress (Alrawaiq and Abdullah, 2014; Baliyen et al., 2022; Dewi et al., 2020; Masriani et al., 2020). Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Adaramola et al., 2017).

According to multiple reports in the literature, the structural characteristics of polyphenols is certainly the most important parameter, for free radical scavenging and reducing power capacities (Guendouze et al., 2015; Chaouche et al., 2018; Aryal et al., 2019; Dewi et al., 2020; Phuyal et al., 2020**).** Therefore**,** phenolics possess one or

more aromatic rings, and one or more hydroxyl groups that are disposed to donate a hydrogen atom or an electron to a free radical (Chaouche et al., 2018; Aryal et al., 2019). The antioxidant activity of flavonoids isolated by Nazemiyeh et al. (2008) is a consequence of the presence of the phenolic moieties in the structures (Nazemiyeh et al., 2008).it was demonstrated the particular substitution pattern of free groups on the flavonoid skeleton influenced the potential of antioxidant activity. Thus, the presence of hydroxyl and-carbonyl group in the favonoid skeleton resulted in high FRAP potential and the presence of 2,3-double bond in conjugation with the 4-oxo function in the C-ring resulted potent radical scavenging ability (Selamoglu, 2017a; Afsar et al., 2018).

Conclusions

The results obtained from this study suggested that leaves and flowers extracts exhibited good antioxidant properties and might be used advantageously as an antioxidant agents for the protection against oxidative stress related to various cell damages, demonstrating by two different methods, namely DPPH and reducing power. Results obtained in this study demonstrated that the potency of electron /or hydrogen donors of these extracts of *E. arborea* separated parts grown in Algeria varies with the type of solvent, the vegetal part and their content on antioxidant compounds such as phenolic, flavonoids and condensed tannins. Moreover, the results also indicated that ethyl acetate sub-fraction of flowers extract exhibited the highest DPPH scavenging when compared with other sub-fractions of all vegetal parts used and also the crude extract of leaves exhibited the highest reducing properties. The results also showed correlation between phenolic, flavonoids and condensed tannins content and the antioxidant capacity of the various sub-fractions. Thereby, these results are preliminary and further testing of the activity of high sub-fractions and isolating procedures of the responsible antioxidants molecules of *E. arborea* would be interesting.

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Conflicts of Interest

Authors declare no conflict of interest.

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