Article

Study on the Chemical Composition, Enzyme Inhibition and Antioxidant Activity of *Ziziphora taurica* subsp. *cleonioides*

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Abstract: *Ziziphora* is a plant used in Turkish and Iran traditional medicine for its antibacterial activity, sedative and stomach soothing properties. Although the chemical profile of the essential oil of different *Ziziphora* species is well documented, data regarding plant extracts are incomplete. In this study extracts from *Ziziphora taurica* subsp. *cleonioides* were obtained using ethyl acetate, methanol and water and the chemical profile of the aerial part of the plant was elucidated. Among the compounds identified, rosmarinic acid was the most abundant (3375.67 ± 38.02 µg/mL), at the extract of methanol, followed by chlorogenic acid (3225.10 ± 16.44). Enzyme inhibition activity against α-amylase and tyrosinase was also estimated. The ethyl acetate extract showed the highest α-amylase activity (1.95 ± 0.04 mg/mL), while the best anti-tyrosinase activity was calculated for the methanolic extract (1.25 ± 0.01 mg/mL). In addition, total phenolic, flavonoid content and antioxidant activity were evaluated. According to our results, bioactivity of the plant is of great interest, nonetheless, at the same time, it is strongly depended on the solvent used during the extraction process. Our data suggest that the plant under study may be an important source to consider against metabolic, skin pigmentation and oxidative stress related disorders.

Keywords: antioxidant; enzyme inhibitory activity; flavonoid; LC–ESI–MS/MS; *Ziziphora taurica* subsp. *cleonioides*

1. Introduction

Plants produce a plethora of bioactive compounds, known as secondary metabolites. These compounds protect plants against herbivores and pathogens without interfering with their normal growth and development. Secondary metabolites from natural sources are being studied for their possible pharmacologic activity. Among them, flavonoids and phenolic acids are of particular interest, due to their multiple pharmacologic properties [1–3]. The most well studied activity of these biomolecules is their antioxidant capacity, which is discussed in numerous scientific studies [4,5]. Antioxidant properties of secondary metabolites are strongly related to the prevention and/or treatment of various disorders such as cancer, cardiovascular, neurodegenerative and age-related diseases [3,6].

In addition, emphasis is given to the possible therapeutic effect of natural products against metabolic disorders, such as diabetes, and skin hyperpigmentation disorders [7–11]. Current therapeutic treatment which uses synthetic formulations in order to encounter symptoms involving hyperglycemia and pigmentation abnormalities, is effective. However, many patients suffer from common or

more severe side effects. Given this fact, scientists are in search of alternative active molecules that could be used instead or in combination with the already available treatment, in order to minimize undesirable effects.

Various plants families have been studied for their potential pharmacologic activity. Among them *Ziziphora* species have been investigated for their possible use as therapeutic agents. *Ziziphora* are perennial herbs with fragrant leaves and are distributed in the Mediterranean area, Asia, North Africa and Turkey. *Ziziphora* belongs to the *Lamiaceae* family. Characteristic species of the Turkey flora are *Z. clinopodioiedes. Z. capitate. Z. persica. Z. tenuior. Z. taurica* subsp. *taurica* and *Z. taurica* subsp. *cleonioides* [12].

Regarding the chemical profile of the *Ziziphora* species, much interest has been given to the study of the essential oil of the plant. Pulegone is reported as the compound found in abundance in many *Z. clinopodioides* ecotypes [13]. In addition to pulegone also piperitone, 1,8-cineole, thymol, β-pinene and limonene have been reported [14,15]. Apart from the essential oil, aqueous and organic extracts of *Ziziphora* spp. have been studied in terms of their chemical composition. Bioactive compounds that have been identified in these extracts include flavonoids, phenolic compounds, caffeoyl derivates, sterols and fatty acids.

Apigenin, chrysin, luteolin, acacetin and diosmetin are among the phenolic compounds that have been detected in *Z. clinopodioiedes*, which is also the most studied species [16–18]. Various studies focused on possible biologic activity related to the chemical profile of the plant. These studies include reduction of vascular tension [19], antidiabetic, antihypertensive, antioxidant [20] and anticancer activity [18].

In particular, regarding *Z. taurica* subsp. *cleonioides*, the essential oil of the plant has been investigated for its antimicrobial and antioxidant activity [21,22].

According to these studies, *Ziziphora* species represent a valuable source of bioactive compounds and as a result may contribute to the ongoing therapeutic strategy regarding the use of natural products as therapeutic tools. However, regarding *Ziziphora taurica* subsp. *cleonioides* (Boiss) P. H. Davis extracts, little research is available. Therefore, this study aims to investigate its biological activity against key enzymes related to metabolic and skin pigmentation disorders, i.e., α-amylase and tyrosinase, to evaluate its antioxidant activity and to elucidate the chemical profile of the aerial part of the plant.

2. Materials and Methods

2.1. Chemicals and Reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH•), and 2,2-azino-bis (3-ethylbenzothiazloine-6-sulphonic acid) radical cation (ABTS+•), butylated hydroxyanisole (BHA), 3,4 dihydroxyphenylalanine (L-DOPA), tyrosinase, gallic acid, chlorogenic acid (purity > 95%), caffeic acid, syringic acid (purity > 95%), vanillin (purity > 95%), sinapic acid (purity > 95%), p-coumaric acid (purity > 95%), ferulic acid (purity > 95%), rosmarinic acid (purity > 95%), quercetin (purity > 95%), luteolin (purity > 95%), apigenin (purity > 95%), kojic acid, ethylenediaminetetraacetic acid (disodium salt) (EDTA), copper (II) chloride, sodium phosphate and, aluminum chloride, ammonium acetate, ammonium formate, ferric chloride, sulfuric acid, iodine potassium iodide solution, 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), formic acid (LC-MS), acarbose and α-amylase, were purchased from Sigma-Aldrich. Ferric chloride, Folin–Ciocalteu's reagent, and methanol were purchased from Merck. Vanillic acid (>95%), 4-hydroxybenzoic acid, 3-hydroxybenzoic acid, 3,4-dihydroxyphenylacetic acid, apigenin 7-glucoside (purity > 95%), luteolin 7-glucoside (purity > 95%), hesperidin (purity > 95 %), eriodyctiol (purity > 95%), and kaempferol (purity > 95%), were obtained from Fluka. Verbascoside (purity > 95 %), protocatechuic acid (purity > 95%), and hyperoside (purity > 95%), were purchased from HWI Analytik. Ammonium molybdate, ferrozine ion reagent, neocuproine hemihydrate, starch and ethyl acetate (HPLC grade), methanol (HPLC grade), and water (HPLC grade), were purchased from Acros Organics.

2.2. Plant Material

The aerial part of *Ziziphora taurica* subsp. *cleonioides* (Boiss) P.H. Davis (Lamiaceae), was collected from the Nemli region, Eskisehir (Turkey), (1010 m, 39° 44′ 06″ N 30° 13′ 02″ E) on June 2016. The identification was done by Dr Olcay Ceylan. The collected plant was then deposited at the department of Biology Mugla Sıtkı Koçman University (Mugla-Turkey). The voucher number was OC.3701. Plant material was cleaned, dried, covered in airtight bag and protected from light, for 7 days. The dry plant material was then shattered using a laboratory mill before the extraction.

2.3. Extraction Procedure

Five grams of the aerial part of the plant were macerated at room temperature for 24 h in ethyl acetate (100 mL). The maceration liquid was collected and the extract was concentrated to dryness under reduced pressure. The same procedure was repeated for the preparation of a methanolic extract. In addition, a water extract was prepared from 5 g of the dry plant material boiled for 15 min in deionized water (100 mL). The aqueous extract was then lyophilized and the received powdered material after the extraction procedure was kept at +4 °C until further analysis.

2.4. Total Phenolic and Total Flavonoid Content

Folin Ciocalteu was the method performed for the calculation of total phenolic content (TPC) [23]. Results were expressed as gallic acid equivalents (GAE) per gram of extract.

Total flavonoid content (TFC) was estimated using the Dowd method [24]. Results were expressed as quercetin equivalents

Detailed information regarding the experimental procedure for both the assays mentioned above is given in the Supplementary Materials.

2.5. Antioxidant Activity

The antioxidant activity was evaluated by using 6 assays: total antioxidant capacity of the phosphomolybdenum (TAC) [24], ferrous ion chelating activity [25], ferric reducing antioxidant power (FRAP) [26], cupric ion reducing capability (CUPRAC) [27], 1,1-diphenyl-2-picrylhydrazyl (DPPH•) radical and 2,2-azino-bis (3-ethylbenzothiazloine-6-sulphonic acid) radical cation (ABTS+•) [28,29]. The antioxidant activity was expressed as IC50 values (thus, the concentration of the sample capable of providing 50% antioxidant activity). Butylated hydroxyanisol (BHA) was used as positive control for all the assays performed except from the ferrous chelating assay, in which ethylenediaminetetraacetic acid (disodium salt) (EDTA) was the positive control used. The precise experimental procedure is described in the Supplementary Materials.

2.6. Enzyme Inhibition Activity

Enzyme inhibitory activity of the extracts were determined using α-amylase and tyrosinase, according to Yang et al. and Erdogan Orhan et al. [30,31]. Results of α-amylase and anti-tyrosinase activity were expressed as IC50 values. Acarbose and kojic acid were used as positive controls, respectively. Detailed experimental procedure is described in the Supplementary Materials.

2.7. Liquid Chromatography–Electrospray Tandem Mass Spectrometry (LC–ESI–MS/MS) Analysis

For the separation of the compounds presented at *Z. taurica* subsp. *cleonioides* extracts, an Agilent Technologies 1260 Infinity liquid chromatography system coupled to a 6420 Triple Quadrupole mass spectrometer was used [32]. Extracts were analyzed in both positive and negative ionization mode. Chromatographic separation was performed on a Poroshell 120 EC-C18 (100 mm × 4.6 mm I.D., 2.7 µm) column. Precise information regarding the mobile phase, the gradient program applied and the operation parameters, are given in the Supplementary Materials.

Standard solutions prepared the day of the experiment, were used to quantify the identified compounds.

Limits of detection (LoD) and limits of quantification (LoQ) were calculated according to the following formulas:

$$LoD = 3.3*(SD\ of\ the\ intercept)/S$$

$$LoQ = 10*(SD\ of\ the\ intercept)/S$$

SD refers to the standard deviation of the y intercept of the calibration curve and S is the slope of the calibration curve of the respective standard solution.

2.8. Statistical Analysis

Data were analyzed with one-way ANOVA using Tukey's test ($p < 0.05$) and Pearson's test. The assays for the biological activity were performed in triplicate and results are presented as mean values ± standard deviation (SD).

3. Results and Discussion

3.1. Extraction Yield

The ethyl acetate extract yielded the lowest amount (4.29% *w*/*w*), followed by the methanol extract (11.26% *w*/*w*). The yield of the aqueous extract presented the highest value (21.15% *w*/*w*) (Table 1).

Table 1. Extraction yield, total phenolic and flavonoid content of *Z. taurica* subsp. *cleonioides* extracts [x].

Extract	Yield (%)	Total Flavonoid Content (mg QEs/g Extract)	Total Phenolic Content (mg GAEs/g Extract)
Ethyl acetate	4.29	11.67 ± 0.03 [b]	37.45 ± 1.73 [a]
Methanol	11.26	14.76 ± 0.21 [a]	20.40 ± 1.36 [b]
Water	21.15	4.27 ± 0.42[c]	18.19 ± 0.17 [b]

[x] Within each column, means sharing the different superscripts show comparison between the extracts by Tukey's test at $p < 0.05$. GAEs and QEs refers to gallic acid and quercetin equivalents, respectively.

3.2. Total Phenolic and Flavonoid Content

In our study, the highest value of the total phenolic content was obtained by the ethyl acetate extract (37.47 ± 1.73 mg GAE/g extract). Significant differences between the amount of total phenolics in this extract and the other tested extracts were observed. The lowest value was calculated at the aqueous extract (18.19 ± 0.17 mg GAE/g extract) (Table 1).

Quantification of total flavonoid content showed significant differences between the aqueous and the organic extracts. The lowest value was calculated at the aqueous extract (4.27 ± 0.42 mg QE/g extract) while the methanol extract showed the highest flavonoid content (14.76 ± 0.21 mg QE/g extract) (Table 1).

Results of the percentage yield and the quantification of flavonoid and phenolic compounds, indicated that the solvent used, is a critical parameter to consider, for the estimation of TFC and TPC. Ethyl acetate, methanol and water are among the solvents used for the extraction of secondary metabolites and especially for polyphenolic compounds [33,34]. However, the different capacity of each individual compound presented in an extract to dissolve in a solvent as well as the polarity of the extraction solvent and the extraction technique, influence the results. Thus, differences between the total phenolic and flavonoid content are expected.

3.3. Antioxidant Activity

Different assays have been developed for the evaluation of the antioxidant activity of plant materials. Among them ORAC assay, that measures the capacity of a sample to neutralize peroxyl

radicals, FRAP and TAC methods which take place at low pH and depend on the reducing capacity of the compounds presented in a mixture, DPPH and ABTS assays that measure the overall capacity of a sample, ferrous chelating activity that depends on the chelating capacity of a component. When examining the antioxidant activity of a mixture, it is important not to perform only one antioxidant method in order to evaluate the overall activity. Antioxidant assays present differences in their mechanism of action and limitations as they react differently with the matrix. For example, FRAP assay cannot detect compounds that act by radical quenching [35], and DPPH method is more suitable when lipophilic antioxidant compounds are presented [36].

In our study, ethyl acetate extract showed the best antioxidant activity followed by the extract of methanol. The reducing activity measured with FRAP and CUPRAC assays was found to be better at the methanol and ethyl acetate extracts respectively. This result was expected, taking into account that reducing capacity of an extract is positively correlated to its phenolic content. Regarding the FRAP assay, no statistically significant difference between the activity of methanol (1.14 ± 0.06) and aqueous (1.54 ± 0.15) extracts was observed ($p > 0.05$) (Table 2).

Table 2. Antioxidant activity expressed as IC50 values (mg/mL) of *Z. taurica* subsp. *cleonioides* extracts [x].

Samples	Phosphomolybdenum	Ferric reducing antioxidant power (FRAP) Reducing	Cupric ion reducing capability (CUPRAC) Reducing	1,1-diphenyl-2-picrylhydrazyl (DPPH) Radical	2,2-azino-bis (3-ethylbenzothiazloine -6-sulphonic acid) radical cation (ABTS) Radical	Ferrous ion Chelating
Ethyl acetate	1.52 ± 0.02 ^{b}	2.99 ± 0.26 ^{c}	1.80 ± 0.03 ^{b}	10.60 ± 0.79 ^{c}	4.11 ± 0.09 ^{c}	6.47 ± 0.67 ^{c}
Methanol	1.82 ± 0.15 ^{bc}	1.14 ± 0.06 ^{b}	1.97 ± 0.02 ^{c}	4.75 ± 0.12 ^{b}	2.66 ± 0.02 ^{b}	1.66 ± 0.02 ^{b}
Water	2.71 ± 0.26 ^{c}	1.54 ± 0.15 ^{b}	2.49 ± 0.06 ^{d}	4.98 ± 0.15 ^{b}	2.61 ± 0.26 ^{b}	1.04 ± 0.01 ^{ab}
BHA	0.35 ± 0.01 ^{a}	0.25 ± 0.01 ^{a}	0.30 ± 0.01 ^{a}	0.32 ± 0.01 ^{a}	0.25 ± 0.01 ^{a}	-
EDTA	-	-	-	-	-	0.034 ± 0.001 ^{a}

[x] Within each column, means sharing the different superscripts show comparison between the samples by Tukey's test at $p < 0.05$. BHA and EDTA refers to butylated hydroxyanisol and ethylenediaminetetraacetic acid (disodium salt), respectively.

For the DPPH and ABTS assays, water and methanol extracts presented lower IC50 values than the ethyl acetate extract. However, our results, did not confirm the study of Prabhakar et al. [37], who observed that non-polar solvents are correlated with better antioxidant activity. Comparing the IC50 values between the two radical scavenging assays for each extract, a notable variation exists. Nonetheless, as it is already mentioned, different chemical properties and polarity of the compounds presented in an extract, can significantly affect its bioactivity [38]. In addition, no statistical difference between the methanolic and aqueous extracts was observed (Table 2).

Regarding the chelating activity of Fe_2+, ferrozine and ferrous ion form a stable colored solution. However, the presence of chelating compounds like phenolic compounds interrupts the formation of this stable complex. As a result, decrease in the color of the solution, which corresponds to decreased absorbance measurement occurs. In our study water extract had the best chelating activity, followed by the methanol and ethyl acetate extracts (Table 2).

Correlation between the total phenolic content and the antioxidant assays performed, ranged from r = 0.998 and r = 0.807, while the respective correlation regarding the total flavonoid content ranged from r = 0.825 and r = 0.157 (Table S1-Supplementary Material). According to these results, it can be concluded that the antioxidant activity of the extracts may be mostly attributed to the total phenolic content.

Total phenolic and flavonoid content of different *Ziziphora* species, as well as their potential antioxidant activity, is discussed in various studies [39,40]. Dastjerdi and Mazoji [39] evaluated the bioactivity of *Ziziphora clinopodioides* from Iran, regarding the antioxidant activity and the total phenolic and flavonoid content. They concluded that the aqueous methanol extract of the plant had the highest phenolic content and the best antioxidant activity. Furthermore, Tian et al. [41] performed a similar

study, using different extraction solvents, in which it was evaluated that the ethyl acetate extract of *Ziziphora clinopodioides* Lam., showed the best results regarding the total phenolic content and the antioxidant activity.

3.4. Enzyme Inhibition Activity

The α-amylase inhibitory activity of the extracts, given as IC50, varied from 1.95 ± 0.04 mg/mL to 36.99 ± 0.13 mg/mL.

The lowest IC50 value was obtained by the ethyl acetate extract (1.95 ± 0.04 mg/mL), which is close to that calculated for the standard solution acarbose (1.21 ± 0.07 mg/mL). Water extract presented the highest IC50 value (Table 3). The α-amylase inhibitory activity was correlated with the total phenolic content (r = 0.931), phosphomolybdenum test (r = 0.982), and the CUPRAC reducing power (r = 0.967) assays (Table S1-Supplementary Material).

Table 3. Enzyme inhibition activity (IC50: mg/mL) of *Z. taurica* subsp. *cleonioides* extracts [x].

Samples	α-Amylase Inhibition	Tyrosinase Inhibition
Ethyl acetate	1.95 ± 0.04 [b]	1.40 ± 0.06 [b]
Methanol	3.97 ± 0.08 [c]	1.25 ± 0.01 [b]
Water	36.99 ± 0.13 [d]	2.71 ± 0.42 [c]
Acarbose	1.21 ± 0.07 [a]	-
Kojic acid	-	0.37 ± 0.02 [a]

[x] Within each column, means sharing the different superscripts show comparison between the samples by Tukey's test at $p < 0.05$.

Alpha-amylase is a key enzyme implicated in different metabolic disorders such as diabetes and obesity. It is responsible for the hydrolysis of 1,4-α-glucoside bonds of the oligo and polysaccharides, so as the final product, glucose, can be utilized by the organism to generate energy [40]. However, α-amylase hyperreactivity, can lead to excessive production of glucose, which finally results to high blood sugar levels. In this way, different therapeutic approaches aim at inhibiting the α-amylase activity, by using synthetic drug formulations. Nonetheless, undesirable side effects are common. For this reason, medicinal plants are being studied for their potential α-amylase inhibitory activity. Different plant extracts have been tested, while emphasis is given on the α-amylase inhibitory activity of the flavonoids which is strongly related to their structure [42].

The tyrosinase inhibitory activity of the extracts given as IC50, varied from 1.25 ± 0.01 mg/mL to 2.71 ± 0.42 mg/mL. All the extracts inhibited tyrosinase. In particular, the lowest IC50 value was estimated for the methanolic extract of, the plant, while the highest IC50 value was calculated for the aqueous extract (Table 3). In addition, tyrosinase inhibitory activity was correlated with luteolin and TFC (r = 0.985 and 0.995 respectively).

Tyrosinase is an enzyme implicated in melanogenesis. It catalyzes the hydroxylation of L-tyrosine to 3,4 dihydroxyphenylalanine (DOPA) and the oxidation of DOPA to dopaquinone. Melanin is responsible for skin pigmentation and also a skin defense compound. However, overproduction can lead to a variety of diseases such as dermal melanocytosis, melasma and post-inflammatory hyperpigmentation. Downregulation of tyrosinase activity is a therapeutic approach regarding hyperpigmentation disorders. Among the most common depigmentation agents used is hydroquinone, which is however related to various adverse effects [43]. For this reason, various plant extracts have been tested for their capacity to inhibit tyrosinase [44]. Results of these studies are promising, as they indicate that molecules produced by natural sources are great inhibitors of tyrosinase.

Data regarding the enzyme inhibition activity of *Z. taurica* subsp. *cleonioides* extracts against α-amylase and tyrosinase are not previously available. However, in a recent study, *Ziziphora taurica* subsp. *taurica*, was examined for its α-amylase and tyrosinase inhibitory activity [45]. Our data are

in accordance with Tomaczyk et al. [45], and potentiate the fact that *Ziziphora* species are potential alternative candidates against glucose metabolism and hyperpigmentation disorders.

3.5. LC–ESI–MS/MS Analysis

Tentative identification of the compounds presented was performed in the multiple reaction monitoring (MRM) mode, by comparing their retention time and precursor ions with authentic standard solutions. Quantification of the detected compounds was achieved by comparing the peak areas of the detected compounds with those of standard solutions. Correlation coefficients of all the generated calibration curves were higher than R^2 = 0.99 (Table S2-Supplementary Material).

Caffeic acid derivates, phenolic compounds and hydrobenzoic acids were identified and quantified (Figure 1). Esters of caffeic acid, namely rosmarinic acid and chlorogenic acid, were presented in all the tested extracts. In particular, rosmarinic acid was the predominant compound identified at the methanolic extract (3375.67 ± 38.02 µg/g), followed by chlorogenic acid (3225.10 ± 16.44 µg/g). In addition, hesperidin (711.57 ± 44.79 µg/g), luteolin-7-glucoside (597.46 ± 12.85 µg/g), luteolin (327.88 ± 0.55 µg/g), apigenin glucoside (260.64 ± 3.61 µg/g), caffeic acid (175.14 ± 9.14 µg/g) and protocatechuic acid (110.16 ± 2.92 µg/g), were identified in abundance, respect to the other compounds, at the extract of methanol. In general, the use of methanol as an extraction solvent, appeared to be the most efficient, in terms of quantification, for the majority of the compounds presented.

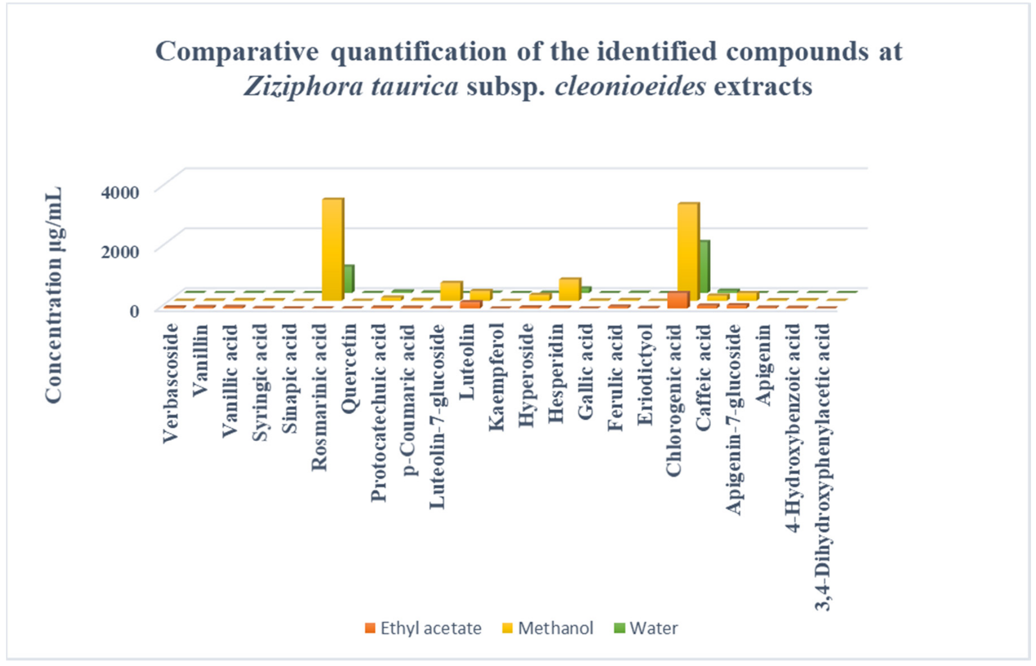

Figure 1. Comparative quantification of the identified compounds at *Ziziphora taurica* subsp. *cleonioides* extracts.

Phytochemical analysis of various *Ziziphora* species has been previously performed. Reports mention the presence of flavonoids, phenolic acids, caffeic and rosmarinic acid. According to these studies, *Ziziphora* can be considered an important source of bioactive compounds [17,20,46]. In our study, compounds 6, 8, 10, 11, 12, 19, 20, and 21 confirmed previous results. However, compounds 1-5, 7, 9, 13–18, 22, and 23, according to our knowledge, are reported herein for the first time.

As it is already mentioned, *Ziziphora* species have been used in folk medicine. Infusions of the plant were used to treat infections, heart and lung diseases, as sedative and antispasmodic agents [47].

In addition, as has being described by various researchers, rosmarinic and chlorogenic acid act as strong antioxidants and hypoglycemic agents [48–52]. Thus, the antioxidant and enzyme inhibition activity of the plant, can be explained due to the properties of the above-mentioned compounds,

in combination to the presence of the other identified compounds. Given the rich chemical profile of *Ziziphora taurica* subsp. *cleonioides* in polyphenols and taking into consideration that these molecules are suggested to improve human wellbeing, it can be concluded that the plant under study can be classified as a promising candidate to use in therapeutics.

4. Conclusions

In this study we examined the enzyme inhibition activity and antioxidant capacity of *Ziziphora taurica* subsp. *cleonioides* extracts, using ethyl acetate, methanol and water as extraction solvents. Our results indicated that the plant under study may be effective against glycemia control and skin hyperpigmentation disorders. A-amylase and tyrosinase were successfully inhibited by the ethyl acetate and methanolic extracts, which simultaneously showed a promising antioxidant activity. Chemical analysis revealed that *Ziziphora taurica* subsp. *cleonioides* is a plentiful source of bioactive compounds, known for their multiple therapeutic activities. Our findings are challenging and may motivate future *in vivo* experiments in order to validate the beneficial role of the plant, either used as dietary supplement or as medicinal agent.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/9/24/5515/s1, Table S1: Correlation between phenolic compounds and assays; Table S2: Concentration (μg/g extract) and analytical characteristics of *Z. taurica* subsp. *cleonioides* extracts

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