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ORIGINAL ARTICLE / ÖZGÜN MAKALE

EXAMINATION OF THE AERIAL PARTS OF *CLINOPODIUM PAMPHYLICUM* SUBSP. DAVISII (CONTANDR. & QUÉZEL) GOVAERTS IN TERMS OF ANTIOXIDANT AND ENZYME INHIBITION POTENTIALS, TOGETHER WITH PHENOLIC PROFILE

CLINOPODIUM PAMPHYLICUM SUBSP. DAVISII (CONTANDR. & QUÉZEL) GOVAERTS TOPRAK ÜSTÜ KISIMLARININ ANTİOKSİDAN VE ENZİM İNHİBİSYON POTANSİYELİ VE FENOLİK PROFİLİNİN İNCELENMESİ

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ABSTRACT

Objective: *The family Lamiaceae, consists of medicinal and aromatic plants, is represented by 46 genera and 571 species in Turkey. Traditional uses for Calamintha Miller species include utilizing as a spice, stimulant, antispasmodic, diaphoretic, expectorant, as well as treatment for stomach and throat aches. C. pamphylica subsp. davisii (Contandr. & Quézel) P.H. Davis is accepted as synonym of Clinopodium pamphylicum subsp. davisii (Contandr. & Quézel) Govaerts. C. pamphylicum subsp. davisii is an endemic species to Turkey. Until now, no biological and phytochemical research has been reported on this plant except for its essential oil research. Examining the antioxidant, and enzyme inhibition effects of C. pamphylicum subsp. davisii, as well as determining its phytochemical composition were the targets of the current study.*

Material and Method: *To evaluate in vitro antioxidant potential (DPPH, ABTS, iron chelating, total phenol and flavonoid amounts) and enzyme inhibitory activities, such as acetylcholinesterase, butyrylcholinesterase, α-glucosidase, α-amylase and tyrosinase for C. pamphylicum subsp. davisii were measured with spectrophotometric methods. The quantities of different phenolic acids and flavonoids were measured using HPLC to analyze the phenolic composition of the plant extracts.*

Result and Discussion: *Our results indicated that except for iron chelating methanol extract exhibited higher antioxidant properties over water extract using DPPH, and ABTS methods. In addition, methanol extract displayed more inhibition on the tested enzymes except for acetylcholinesterase, and α-glucosidase than water extract. As for HPLC findings, although*

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chlorogenic acid was signified as the main phenolic compound for the methanol extract, caffeic acid was specified as the major phenolic for the water extract. These results provide a scientific basis for C. pamphylica subsp. davisii, which possessed the traditional uses in Turkish folk medicine.
Keywords: *Antioxidant activity, clinopodium pamphylicum, enzyme inhibition activity, phenolic compounds*

ÖZ

Amaç: *Tıbbi ve aromatik bitkilerden oluşan Lamiaceae familyası Türkiye'de 46 cins ve 571 tür ile temsil edilmektedir. Calamintha Miller türlerinin geleneksel kullanımları arasında baharat olarak, uyarıcı, spazm giderici, terletici, balgam söktürücü etkileri ve bunun yanısıra mide ve boğaz ağrılarının tedavisi yer alır. C. pamphylica subsp. davisii (Contandr. & Quézel) P.H. Davis Clinopodium pamphylicum subsp. davisii (Contandr. & Quézel) Govaerts bitkisinin sinonim ismi kabul edilmiştir. C. pamphylicum subsp. davisii Türkiye'ye endemik bir türdür. Şimdiye kadar, bu bitki üzerinde uçucu yağ araştırmaları dışında herhangi bir biyolojik ve fitokimyasal araştırma bildirilmemiştir. C. pamphylicum subsp. davisii'nin antioksidan ve enzim inhibisyonu etkilerinin incelenmesi ile birlikte fitokimyasal bileşenlerinin değerlendirilmesi bu çalışmanın amaçlarıdır.*
Gereç ve Yöntem: *C. pamphylicum subsp. davisii'nin in vitro antioksidan potansiyelini (DPPH, ABTS, demir şelasyon, Toplam fenolik madde ve flavonoit miktarı) ve asetilkolinesteraz, butirilkolinesteraz, α-glukozidaz, α-amilaz ve tirozinaz gibi enzim inhibitör aktiviteleri spektrofotometrik yöntemler kullanılarak ölçülmüştür. Bitki ekstrelerinin fenolik bileşimini HPLC kullanarak analiz etmek için birçok fenolik asit ve flavonoitin miktarları hesaplanmıştır.*
Sonuç ve Tartışma: *Sonuçlarımız demir şelasyon dışındaki DPPH ve ABTS yöntemleri ile metanol ekstresinin sulu ekstreden daha yüksek antioksidan özellik sergilediğini göstermiştir. Ayrıca, metanol ekstresi, test edilen asetilkolinesteraz ve α-glukozidaz dışındaki enzimler üzerinde sulu ekstreden daha fazla inhibisyon sergilemiştir. HPLC sonuçlarına gelince, klorojenik asit methanol ekstresi için ana fenolik bileşik olarak belirlenirken, kafeik asit su ekstresinde başlıca fenolik olarak belirlenmiştir. Bu sonuçlar, Türkiye'de halk arasında geleneksel kullanımları olan C. pamphylica subsp. davisii için bilimsel bir temel oluşturmaktadır.*
Anahtar Kelimeler: *Antioksidan aktivite, clinopodium pamphylicum, enzim inhibisyon aktivite, fenolik bileşikler*

INTRODUCTION

Diseases including cancer, cardiovascular issues, diabetes, cataracts, and neurological conditions are all influenced by reactive oxygen species, which are created by several cellular metabolic processes and environmental pollutants [1]. Plants and foods rich in phenolic compounds when they are taken into the body can prevent the development of these disorders by destroying free radicals that accumulate due to their antioxidant activity.

Diabetes mellitus is an important metabolic chronic disease that negatively affects the lives of many people in the world. Diabetes can be controlled by preventing α-amylase, which is in duty of the digestion of carbs, from doing its function. Diabetes is also managed by blocking α-glucosidase from doing its work, which involves absorbing glucose in the digestive system and causing postprandial hyperglycemia to decrease. Targeting digestive enzymes including α-amylase and α-glucosidase has proven to be successful in lowering blood sugar levels [2]. In addition, free radicals found to play a crucial part in the development of secondary diabetic disorders, because of the propensity to harm lipids, proteins, and DNA [3]. Agents of natural origin have become more attractive because they have minimal side effects and are better tolerated than other oral hypoglycemic agents currently used in antidiabetic therapy. To control diabetes, using plants with strong enzyme inhibitor and antioxidant properties can be a good treatment strategy.

Alzheimer's disease (AD) is a neurological disease that is frequently seen in the elderly and negatively affects their lives. According to studies, a significant decrease in the neurotransmitter called acetylcholine in the cerebral cortex is an important factor for this disease. Therefore, inhibition of acetylcholinesterase (AChE), which is responsible for the degradation of acetylcholine, has become one of the target treatment strategies in the treatment of AD [4]. The tyrosinase enzyme is involved in the melanin synthesis pathway in mammals, and by inhibiting this enzyme in skin problems caused by

overproduction of melanin, the synthesis of melanin is reduced, thus preventing hyperpigmentation [5]. In addition, tyrosinase inhibitors, including kojic acid and hydroquinone gain importance in cosmetology due to their skin lightening properties. Synthetic anti-pigmenting agents with tyrosinase inhibitory effect usually cause inflammation of the skin, so natural compounds with less side effects are sought as an alternative to synthetics. In recent years, the search for compounds with anti-pigmentation effect has increased for the use of plants to cosmetic purposes against skin diseases [6]. In addition, tyrosinase is closely associated with neurodegenerative processes in Parkinson's disease. Therefore, the search for tyrosinase enzyme inhibitors has become important, mostly in agricultural, cosmetic, and pharmaceutical sectors [7].

In Turkey, the Lamiaceae family, which includes bioactive and aromatic plants, is represented by 46 genera and 571 species [8]. Among the Lamiaceae genus, the habitat of Calamintha Miller includes North Africa, Eurasian, European, and East Mediterranean. Turkey contains 12 taxa and 9 species belonging to the genus, 5 of which are endemic to the country with a 45 percent endemism frequency [9]. The Turkish names for the Calamintha species include Güzel Nane, Dağ Nanesi, Miskotu, Tıbbi Miskotu, and Yabani Oğulotu. They are traditionally utilized as spice, stimulant, spasmolitic, diaphoretic, expectorant, as well as to improve nervous, and gastrointestinal system, and treat renal diseases, stomach aches, and throat pain. Calamintha pamphylica Boiss. & Heldr. is an endemic species to Turkey, which has three subspecies, subsp. pamphylica, subsp. Davisii (Contandr. & Quézel) P.H. Davis, and subsp. alanyense S. Alan & Ocak. Until now, no biological and phytochemical research has been reported on thes plants except for its essential oil research, and morphological comparison on these subspecies [10-14]. C. pamphylica subsp. davisii, hairy perennial herbaceous plant, is known as "kemer fesleğeni" (synonym, *Clinopodium pamphylicum* subsp. davisii (Contandr. & Quézel) Govaerts) and grown in limestone rocks, often forests of *Pinus brutia*, and *Cupressus sempervirens* [14,15]. The objectives of our investigation were to investigate the phenolic profiles of *C. pamphylicum* subsp. davisii extracts by HPLC, and to assess the antioxidant properties and enzyme inhibition potentials of the extracts.

MATERIAL AND METHOD

Plant Material

C. pamphylicum subsp. davisii, as a whole plant was collected from Yarikpinar canyon, located in Antalya province of Turkey in April 2018. The plant material was identified by a botanist specialist (Hayri Duman, Professor at Department of Biology), works in Gazi University. Voucher specimen was kept with herbarium number of 26912 in the KNYA, Selçuk University Faculty of Science Herbarium, Turkey.

Extract Preparation

After the aerial part of plant material was dried in the shade, it was pulverized into fine powder by laboratory type miller. 10g of the powder were extracted in 100 ml methanol for 24h with shaking occasionally and filtered with Whatman filter paper No. 1. A rotary evaporator (Buchi, Switzirland) was used to concentrate the filtrates under vacuum at 40°C. The procedure was repeated for three times. The residue of plant materials was subjected to maceration with distilled water. After filtering, these extracts were refrigerated and lyophilized to yield water extract (Table 2). The extracts were kept in the refrigerator until utilization for *in vitro* assays.

Quantitative Analysis of Phenolic Compounds by HPLC

The extracts were subjected to chromatographic analysis with High-Performance Liquid Chromatography (HPLC), provided with diode array detector (DAD, G1315B). HPLC (Agilent Technologies, Wilmington, DE, USA), equipped with quaternary pump (G1311A), automatic injector system (G1329A), and thermostatted column (G1316A). A wavelength of 280 nm, which is typically employed for the simultaneous measurement of various phenolic compounds, was set on the DAD detector. During assessment, 1 ml of methanol was used to dissolve 25 mg of dry crude extract, and 10 µl of sample solution was injected. Separations on ACE 5 C18 (250 x 4.6mm; 5µm) column were carried

out at 30 °C with 0.8 ml/min flow rate. Gradient elution was utilized for the analysis, with the mobile phase A: B: C (80:12:8) water with 0.1% acetic acid (A), methanol with 0.1% acetic acid (B), and acetonitrile with 0.1% acetic acid (C). Elution program was proceeded with 80:12:8 (A: B: C) at 8 min, and the polarity was gradually increased by 25:60:15 at 40-45 min, then back to the mobile phase (80:12:8) to the recondition of column for 5 min. Before processing HPLC injections, extract samples and solvents for mobile phases were filtered via a 0.22 μm filtration system (Millipore Corp., Billerica, MA). Each sample was examined triplicate.

Total phenol (TPC) and flavonoid contents (TFC)

The TPC and TFC for the methanol and water extracts of *C. pamphylicum* subsp. davisii were determined using spectrophotometric methods, such as Folin-Ciocalteu and aluminum chloride. TPC were expressed with mg gallic acid equivalents per g dry extract weight (mg GAE/g DW). The TFC were calculated mg equivalence of quercetin over g dry extract weight (mg QE/g DW).

Antioxidant Activity

Antioxidant capabilities of plant-derived substances or extracts must be measured using methods that consider the mechanism of antioxidant activity. Therefore, in this investigation, the iron chelating and free radical scavenging tests were used to assess the antioxidant potential of the extracts. The DPPH and ABTS reduction spectrophotometric tests were used to estimate capability of the extracts for scavenging free radicals [16, 17]. The relationship between the extracts and the development of the ferrozine-Fe2+ complex controlled the iron chelating properties of the extracts [18].

Enzyme inhibition potentials

Anticholinesterase effects were assessed by altering the spectrometric techniques designed in advance [19]. The tyrosinase inhibition effect was also carried out using the reported earlier technique, with L-dopa serving as the substrate, and kojic acid serving as a standard agent [20]. Moreover, as initially disclosed, α-glucosidase inhibition effect was measured [21]. Additionally, the α-amylase inhibition experiment was subjected by adapting the procedure used by Caraway-Somogi iodine/potassium iodide method [22].

Statistical Data

Every bioactivity test was conducted in tri plicate during the experimental procedures. Three parallel assessments' mean were utilized to summarize the results. The Tukey's test, and one-way ANOVA were used to show the correlation with Graphpad Prism 8.0 program.

RESULT AND DISCUSSION

HPLC Analysis of Phenolics

As shown in Table 1, the phenolic compounds of the methanol, and water extracts were quantified using HPLC. Chlorogenic acid, catechin, and vanillic acid were revealed to be the main constituents in the methanol extract of *C. pamphylicum* subsp. *davisii* (Figure 1), whereas caffeic acid, 4-hydroxy benzoic acid, and sinapic acid were shown to be the more predominate phenolic compounds in the water extract (Figure 2). As mentioned in the study, the phytochemical profiles of essential oils from *Calamintha* taxa were reviewed with the main compounds being piperitone oxide, pulegone, menthone, menthol, and menthyl acetate [11]. It was recorded that *C. pamphylicum* subsp. *davisii* essential oil mainly contained pulegone, menthol, menthone, and menthyl acetate in other study [12]. In another study, menthone (9.7-21.7%), menthyl acetate (9.8-20.9%), pulegone (5.6-19.7%), and menthol (7.4-15.4%) were determined as main components of the essential oil samples of *C. pamphylicum* subsp. *davisii* collected from different site [10]. According to the literature, there is no study on phytochemical investigation on the extracts of *C. pamphylicum* subsp. *davisii*. However, in a study, caffeoylquinic acid was determined as major compound for *C. nepeta* 80% methanol extract in the range 2.76 to 14.69 mg/g by HPLC [23]. In other study, phytotoxic phenolics of the ethyl acetate fraction from the aerial parts of *C. nepeta* were detected as gallic, and ferulic acids by HPLC using characterization and quantification

techniques [24]. Using LC-MS/MS method, acacetin and caffeic acid derivatives were identified as major components of hydroalcoholic extracts of *C. nepeta* in another investigation [25].

Table 1. The phenolic contents of *C. pamphylicum* subsp. *davisii* extracts (µg/mg, n=3)

Analyte	Retention time (min)	Methanol extract	Water extract
Gallic acid	4.69	-	-
3,4-dihydroxy benzoic acid	6.98	0.479	0.028
Catechin	7.97	1.217	-
Chlorogenic acid	8.79	1.517	0.03
4-hydroxy benzoic acid	10.65	-	0.547
1,2-dihydroxy benzene	11.09	0.111	-
Epicatechin	11.40	-	0.028
Vanilic acid	11.80	1.025	0.042
Caffeic acid	12.18	-	0.668
Vanilin	17.63	0.028	0.161
***p*-Coumaric acid**	18.27	0.026	-
Sinapic acid	19.17	0.125	0.270
***Trans*-Ferulic acid**	20.07	0.093	-
Elagic acid	21.17	0.230	-
Rutin	22.40	0.124	0.061
Salicylic acid	32.88	0.299	0.188
Quercetin	36.26	0.636	0.153
Kaempferol	39.97	0.132	0.033

TPC and TFC

The highest TPC of *C. pamphylicum* subsp. *davisii* was measured in the methanol extract (104. 16 mg GAE/g), followed by the water extract (24.05 mg GAE/g). The highest TFC was recorded for the methanol extract (81.37 mg QE/g), followed by the water extract (69.62 mg QE/g) (Table 2). Previous research has found that the amount of phenolic and flavonoid compounds in plant extracts is affected by

Table 2. Extract yield, total phenol and flavonoid amounts, and antioxidant activities of *C. pamphylicum* subsp. *davisii* methanol and water extracts[a]

Extract/ Reference	Extract yield (%, g/g)	Total phenolic (*m*g GAEs/g)[b]	Total flavonoids (*m*g QEs/g)[c]	Antioxidant activity (IC_{50} µg/ml)		
				DPPH	ABTS	Iron chelating
Methanol	10.88	104. 16 ± 1.16	81.37 ± 5.89	506.0 ± 2.02	6.29 ± 0.42	3818 ± 1.33
Water	12.88	24.05 ± 5.31	69.62 ± 4.79	14685 ± 0.59	122.0 ± 0.91	619. 8 ± 1.54
Quercetin		-	-	9.62 ± 0.09	-	
BHT		-	-	-	0.50 ± 0.23	
EDTA		-	-	-	-	437.3 ± 2.31

a: The data was presented as the averages ± standard deviations of three parallel calculations. b: GAEs. Gallic acid equivalents (y = 0.003x + 0.0578 gallic acid (mg) (r^2 = 0.999)); c: QEs. quercetin equivalents (y = 0.0068x + 0.0928 quercetin (mg) (r^2 = 0.9982)).

the polarity of solvents used in the extraction procedures [26]. In accordance with our results, recent investigation indicated that *C. vulgaris* methanol extract contained 39.41 mg GAE/g for phenolic, and

12.03 mg QE/g for flavonoid contents [27]. As for another work, total phenol ingredients of the methanol, and ethyl acetate extracts obtained from *C. nepeta* were identified as 143.52, and 351.47 mg chlorogenic acid equivalents/g dry plant material, respectively [24].

Antioxidant Activity

The free radical scavenging abilities of the extracts of *C. pamphylicum* subsp. *davisii* were examined by using DPPH and ABTS techniques. The results were displayed in Table 2. The methanol extract exhibited the most superior levels of DPPH and ABTS radical scavenging abilities with IC_{50} values of 506.0 ± 2.02 and 6.29 ± 0.42 µg/ml, respectively. This may be due to the strong radical scavenging activity of some phenolic compounds presents in the methanol extract. However, higher ferrous ion chelating activity was found in water extract (IC_{50}: 619. 8 ± 1.54 µg/ml) than the methanol extract (IC_{50}: 3818 ± 1.33 µg/ml). In a study, *C. grandiflora* methanol extract (IC_{50}: 24 µg/ml) was shown to have more superior DPPH radical scavenging ability than that of *C. nepeta* subsp. *glandulosa* (IC_{50}: 67 µg/ml) [28]. In another study, the antioxidant activities of *C. glandulosa* plant extracts were assessed that the extracts were able to reduce DPPH radicals (IC_{50}: 0.51-4.40 mg/ml), and ABTS radicals (IC_{50}: 0.36-34.29 mg/ml) [29]. Our findings were found as lower than these results. The ferrous ion chelating potential of the essential oil from *C. incana* was observed to have low activity (1.94 mg EDTA equivalents per gram of oil) [30].

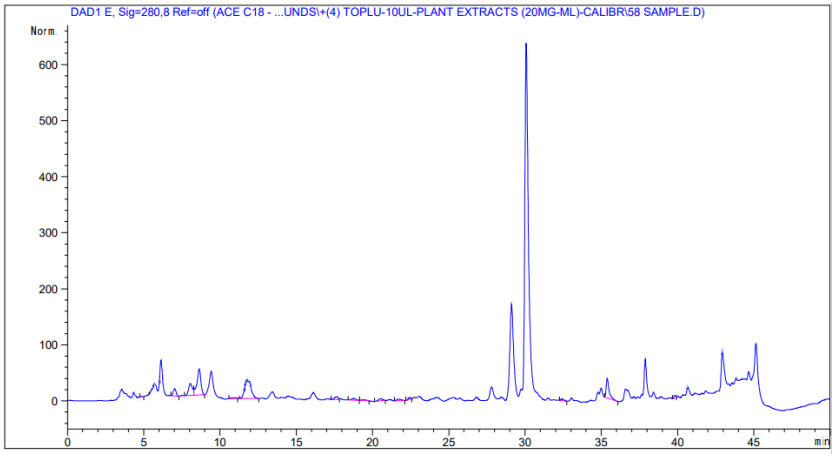

Figure 1. PHLC chromatogram of *C. pamphylicum* methanol extract

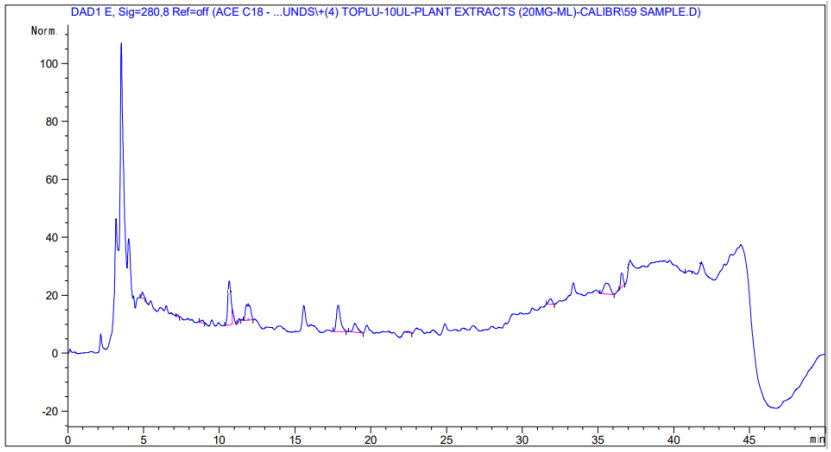

Figure 2. PHLC chromatogram of *C. pamphylicum* water extract

Enzyme Inhibition Activity

In our work, enzyme inhibition effects of methanol and water extracts of *C. pamphylicum* subsp. *davisii* on AChE, BChE, tyrosinase, α-glucosidase, and α-amylase were presented an overview (Table 3). Both the extracts showed low anticholinesterase effects with IC_{50} 459.3-33462 μg/ml as compared with positive control galanthamine (IC_{50} 28.16, and 27.34 μg/ml for AChE, and BChE inhibition, respectively). The highest anti-BChE activity was exhibited in the methanol extract. In a research, AChE inhibition effects of the ethanol extracts of *C. grandiflora* and *C. officinalis* were shown to be moderate (around 62.5%), and the ethanol extract of *C. sylvatica* were exhibited to be low (around 25%) at 1 mg/ml concentration [31]. A comparison of these results with ours reveals that *C. pamphylicum* subsp. *davisii* water extract showed lower acetylcholinesterase inhibitory activity with 40.12% at the highest concentration (1000 μg/ml) than the other *Calamintha* species above mentioned. In another investigation, C. *nepeta* essential oil, and its water extract were found to possess AChE inhibition effects with IC_{50} values 205.6, and 983.9 μg/ml, respectively [32]. According to our findings, *C. pamphylicum* subsp. *davisii* was not active against AChE inhibitory than *C. nepeta*. In another study, *C. nepeta* methanol extract inhibited AChE with 48.78% at very high concentration (10 mg/ml) [23]. As for our findings, the highest BChE inhibitory activity was exhibited at the highest concentration (1000 μg/ml) on the methanol extract of *C. pamphylicum* subsp. *davisii* with 65.59%. In similar research, *C. incana* essential oil was found to have no effect on the inhibition of AChE, and BChE [32]. Conversely, C. *nepeta* essential oil and its water extract possessed BChE inhibition effects with IC_{50} values 88.3, and 1669.9 μg/ml, respectively [32].

As for anti-tyrosinase effects, of *C. pamphylicum* subsp. *davisii* methanol extract exhibited inhibition on tyrosinase (IC_{50}= 9431 ± 0.27 μg/ml), while the water extract was observed to be ineffective. In comparison with that of kojic acid (IC_{50}=107.3 ± 0.66 μg/ml), the methanol extract showed very weak inhibition effect. Otherwise, the methanol, and water extracts showed tyrosinase inhibition effects at 1000 μg/ml with 36.65, and 32.70%, respectively. In a previous work, it was discovered that the essential oil of *C. incana* has a tyrosinase inhibition effect for 2.10 mg kojic acid equivalents for each gram of oil [30]. In other work, *C. nepeta* methanol extract exhibited anti-tyrosinase activity 38.45% inhibition at very high concentration (10 mg/ml) [23]. As compared this study, we found that similar inhibition data on *C. pamphylicum* at lower concentration (0.1 mg/ml).

C. pamphylicum subsp. *davisii* extracts were compared with positive control acarbose which inhibited *α*-amylase and *α*-glucosidase with IC_{50} values 259 and 825.7 μg/ml, respectively. Although the methanol extract for *α*-amylase inhibition effect exerted greater (IC_{50}=5756 μg/ml) than water extract, the water extract for *α*-glucosidase inhibition effect was found to have (IC_{50}=549.3 μg/ml). As for the literature, the essential oil of *C. incana* showed acarbose equivalents/g essential oil on *α*-amylase and *α*-glucosidase inhibitory effects with 0.34 and 0.10 mmol, respectively [30]. Furthermore, *C. nepeta* methanol extract exhibited inhibitory activity 16.45 and 66.62% against *α*-amylase and *α*-glucosidase at 10 mg/ml [23]. In our results, the water extract of *C. pamphylicum* subsp. *davisii* exhibited inhibition effects of *α*-amylase and *α*-glucosidase with 35.12, and 76.64% respectively. As compared with study on *C. nepeta*, we observed that *C. pamphylicum* subsp. *davisii* possessed higher inhibition effects on *α*-amylase and *α*-glucosidase.

Table 3. Enzyme inhibition effects of *C. pamphylicum* subsp. *davisii* methanol and water extracts* (IC_{50} μg/ml)

Samples	AChE	BChE	Tyrosinase	α-glucosidase	α-amylase
Methanol extract	7469 ± 0.65[a]	459.3 ± 1.05[a]	9431 ± 0.27[a]	N.A.	5756 ± 0.68[a]
Water extract	1640 ± 0.78[a]	33462 ± 0.47[a]	N.A.	549.3 ± 1.98[a]	15104 ± 0.27[a]
Galanthamine	28.16 ± 2.01[b]	27.34 ± 1.86[b]	-	-	-
Kojic acid	-	-	107.3 ± 0.66 [b]	-	-
Acarbose	-	-	-	825.7±1.03[b]	259.4 ±2.02[b]

*: IC_{50} values were presented as the averages plus standard deviations of three parallel calculations; a: Values were determined using the negative control method; b: Reference agent; N.A.: not active

Assessment of the potential benefits of *C. pamphylicum* subsp. *davisii* extracts on inhibition of oxidative stress and enzyme activities, as well as phytochemical contents were presented. This study indicated that methanol extract was primarily observed to be quite effective than water extract with regard to antioxidant, and enzyme inhibition activities. Our phytochemical findings also indicated that chlorogenic acid, catechin, and vanillic acid would be responsible for these bioactivities. These experiments supported that *C. pamphylicum* subsp. *davisii* may be good new source for developing natural agents against several critical diseases related with oxidative stress, and enzymatic mechanisms. Extensive research also needs to be conducted on *C. pamphylicum* subsp. *davisii* to investigate mechanism of biological effects, and further medicinal properties.

AUTHOR CONTRIBUTIONS

Concept: F.A., N.E.; Design: F.A., N.E.; Control: F.A., N.E.; Sources: F.A., N.E.; Materials: N.E.; Data Collection and/or Processing: N.E.; Analysis and/or Interpretation: N.E.; Literature Review: F.A., N.E.; Manuscript Writing: N.E.; Critical Review: F.A., N.E.; Other: F.A., N.E.

CONFLICT OF INTEREST

There is no actual, possible, or apparent conflict of interest for this manuscript.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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