

Antioxidant and anti-inflammatory activities of *Phlomis pungens* and *Coridothymus capitatus*

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ABSTRACT: The purpose of this study was to comparatively reveal for the first time the antioxidant, anti-inflammatory effects of *Phlomis pungens* and *Coridothymus capitatus* methanol extracts obtained by using maceration extraction method.

According to the results of anti-inflammatory activity, *P. pungens* methanolic extract demonstrated a more prominent and intensive anti-inflammatory effect with 24.7% of inhibitive capacity in the altered edema size after the first hour of carrageenan injection compared to *C. capitatus* methanolic extract. *P. pungens* methanolic extract inhibitory effect increased during three hours and reached maximum by 41.9%. According to the results obtained from antioxidant activity experiments, methanol extract of *C. capitatus* exhibited stronger free radical scavenging (DPPH), cupric reducing (CUPRAC), ferric reducing/antioxidant power (FRAP) and ABTS radical cation scavenging activity than *P. pungens* extract. In addition, *C. capitatus* extract had higher ABTS radical cation scavenging and ferric reducing/antioxidant power activity than ascorbic acid, BHT and BHA, respectively. Therefore, this extract can be used in both medicine and food industry as a natural antioxidant source.

KEYWORDS: *C. capitatus*; *P. pungens*; antioxidant; anti-inflammatory

1. INTRODUCTION

Plants are used worldwide in traditional medicines and these are all potential reservoirs for new drugs. Traditional medicinal plants have become very important in novel drug discovery because of the bioactive components [1]. The plants contain phenolic compounds such as phenolic acids, flavonoids and tannins and therefore plants are potential sources of natural antioxidants [2].

The genus *Phlomis*, belonging to the family Lamiaceae, is distributed throughout Euro-Asia and North Africa continents. *Phlomis* L. genus is represented by 46 species in Turkish flora [3]. *Phlomis* species have different uses from one country to another. The flowering parts of the plant are often used to treat gastrointestinal problems and to protect the liver, kidney, and cardiovascular system. *Phlomis* species are used to treat various diseases such as diabetes, gastric ulcer, hemorrhoids, inflammation and wounds. In addition, some of the *Phlomis* species are used as tonic and stimulant in Anatolia [2].

Previous phytochemical studies have shown that *Phlomis* species contain iridoids, flavonoids, phenylpropanoids, phenylethanoids, lignans, neolignans, diterpenoids, alkaloids and essential oils. *Phlomis pungens* Willd. is a member of the

genus *Phlomis*. The traditional use of *Phlomis pungens* in folk medicine has not been reported, but it has been found that some species of *Phlomis* such as *P. lychnitis* or *P. rotata* and *P. purpurea* have antihemorrhoid and cardiotoxic activity [1, 2].

Coridothymus capitatus (L.) Rchb.f. [Syn: *Thymus capitatus* Hoff.et Link, *Satureja capitata* L., *Thymbra capitata* (L.) Cav.] is a member of the genus *Coridothymus* belongs to family Lamiaceae [3]. Aerial parts of *Coridothymus capitatus* are used against cold, influenza, throat infection and in cosmetics, flavoring and pharmaceutical industry [4].

To the best of our belief, there have been no studies on the antioxidant and anti-inflammatory activities of methanol extract from *Phlomis pungens* and *Coridothymus capitatus* aerial parts. Therefore, the aim of this study is to determine antioxidant and anti-inflammatory activities of *Phlomis pungens* and *Coridothymus capitatus* aerial parts.

2. RESULTS AND DISCUSSION

2.1. Extract yield percentage

The extract yield percentage of methanol extracts from plants was found to be in the order of: *P. pungens* (2.80%)>

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C. capitatus (2.60%). According to this study, *P. pungens* was found to have higher recovery than *C. capitatus*.

2.2. In vitro antioxidant activity of plant extracts

Antioxidant activities and total phenolic contents of methanol extracts obtained from the plants were given in Table 1.

In the DPPH radical scavenging activity assay, it was found that the *C. capitatus* extract (IC_{50} 0.011±0.002 mg/mL) has stronger free radical scavenging activity than *P. pungens* extract (IC_{50} 0.064±0.005 mg/mL). When the activity results of all the extracts were compared with the standards, it was found that these extracts had lower activity than standard compounds [BHT (IC_{50} 0.0081±0.0007 mg/mL) and ascorbic acid (IC_{50} 0.0023±0.0001 mg/mL)].

In this study, It was found that the methanol extract obtained from *C. capitatus* (263.08±0.006 mM trolox/mg extract) exhibited stronger ABTS radical cation scavenging activity than standard compounds [ascorbic acid (260.70±0.06 mM trolox/mg extract), BHT (153.07±0.05 mM trolox/mg extract)] and *P. pungens* extract (23.36±0.005 mM trolox/mg extract).

According to the results obtained from the FRAP assay, *C. capitatus* methanol extract (56.34±0.08 mM Fe^{2+} /mg extract) has stronger ferric reducing antioxidant power activity than BHA (16.91±0.02 mM Fe^{2+} /mg extract) and *P. pungens* extract (8.60±0.008 mM Fe^{2+} /mg extract).

In this study, according to the results obtained from the CUPRAC assay, *C. capitatus* extract (2.72±0.07 mM trolox/mg extract) showed stronger cupric reducing antioxidant capacity than *P. pungens* extract (0.39±0.03 mM trolox/mg extract).

When the amounts of the phenolic compounds in the extracts were compared, it was found that the *C. capitatus* extract (120±0.02 mgGAE/g extract) contained more phenolic contents than the *P. pungens* extract (30±0.004 mgGAE/g extract).

According to the four antioxidant activity tests, *C. capitatus* methanol extract exhibited stronger antioxidant activity than *P. pungens* extract. It was also found that this extract had higher total phenolic contents than other extract. Therefore, a linear relationship was found between antioxidants and phenolic compounds in this study.

According to the literature review, there are some studies on the antioxidant activity of different extracts (excluding methanol extract obtained from maceration method) from plants. The antioxidant activity of ethanol and water extracts from *Phlomis pungens* Willd. var. *hispida* leaves and flowers have been reported before. In that study, the antioxidant capacity of extracts was assayed using different solvents for extraction. This study have reported that water extract (100 µg/mL) from *P. pungens* flowers exhibited stronger ABTS⁺ (90.6 %), superoxide anion radical scavenging (98.3%), and metal chelating activity (23.43%) than other extract [2].

In contrast to the above study, in present study, the antioxidant activity of methanol extract from *P. pungens* aerial parts was examined using DPPH, FRAP and CUPRAC methods. When the results obtained from the ABTS assay were compared with results of ours, it was found that methanol extract from *P. pungens* aerial parts had lower activity than water extract from *P. pungens* var. *hispida* flowers.

According to another study, antioxidant activities of ethyl acetate, methanol (both extracts obtained from Soxhlet method) and water extracts from *P. pungens* var. *pungens* aerial parts were screened by β – carotene bleaching, phosphomolybdenum, DPPH, ABTS, superoxide anion, CUPRAC, FRAP and metal chelating assays. In addition, in this study, total phenolic and flavonoid contents of these extracts were determined. The water and methanol extracts of plant exhibited the highest total phenolic (57.68 mgGAE/g extract; 41.10 mgGAE/g extract), flavonoid contents (58.22 mgRE/g extract; 56.08 mgRE/g extract) and DPPH (IC_{50} 1.17 mg/mL; IC_{50} 2.28 mg/mL), ABTS (143.27 mg trolox/g extract; 142.39 mg trolox/g extract), superoxide anion (57.96 mg trolox/g extract; 42.37 mg trolox/g extract), CUPRAC (126.74 mg trolox/g extract; 115.07 mg trolox/g extract), FRAP (102.36 mg trolox/g extract; 75.20 mg trolox/g extract) and metal chelating (27.64 mg EDTAE/g extract; 21.08 mg EDTAE/g extract) activity, respectively [5].

Contrary to the above study, in this study, maceration method was used to obtain methanol extract from plant. In our study found that, the amount of total phenolic contents contained in the methanol extract obtained from maceration method exhibited lower than in methanol extract from Soxhlet method. In addition, when the results obtained were compared with results of ours, it was found that methanol extract from *P. pungens* aerial parts exhibited stronger DPPH free radical scavenging activity than water and methanol extracts from *P. pungens* var. *pungens* aerial parts.

There are 3 studies on antioxidant activity of different extracts obtained from *C. capitatus* [4, 6, 7]. According to the work of Mokhtar and co-worker, ethyl acetate extract (IC_{50} 1.50 µg/mL) from *C. capitatus* showed stronger DPPH radical scavenging activity than diethyl ether extract (IC_{50} 1.92 µg/mL) [6]. When the results obtained from the DPPH assay were compared with results of ours, it was found that *C. capitatus* methanol extract showed lower free radical scavenging activity than ethyl acetate and diethyl ether extracts from this plant.

According to another study, ethanol extract (200 µg/mL) of plant showed moderate DPPH radical scavenging (54 %) and β -carotene bleaching (15 %) activity. It was also found that the plant contained a moderate amount of total phenolic compounds (103.5 µg pyrocatechol equivalent/mg extract) [4]. When the result of the above study was compared with ours, in our study, the methanol extract from the plant showed higher DPPH radical scavenging activity than the ethanol extract.

According to the study of Harmandar and co-workers, the total antioxidant activity measured by the β -carotene bleaching test was the highest in *C. capitatus* water extract (95.7 %). In addition, the amount of phenolic compounds in water extract was found higher than the value expected [55.70 ± 1.59 pyrocatechol equivalent ($\mu\text{g mg}^{-1}$)]. It was also found that the ethanol extract (90.7 %) showed the strongest free radical scavenging activity in this study [7]. Unlike the above study, in our study, the methanol extract from plant exhibited stronger DPPH radical scavenging activity than ethanol extract.

Additionally, essential oil compositions of *P. pungens* var. *pungens* and *C. capitatus* have been reported before [8-10]. Germacrene D, n-hexadecanoic acid, hexahydrofarnesyl acetone, β -caryophyllene and linalool was found as the major compound in the oils of *P. pungens* var. *pungens* [8]. According to the work of Lagouri and co-workers, the main compounds in *C. capitatus* essential oil were found as carvacrol, *p*-cymene, γ -terpinene, *trans*-thujanol, β -caryophyllene and *cis*-pinane [9, 10].

Table 1.2.3. Correlation analysis

The results showed that there is a correlation between total phenolic contents and antioxidant activity of the methanol [DPPH ($Y=0.4794x+30.90$ $R^2=0.8735$ ($p<0.05$)), ABTS ($Y=0.8908x-7,568$ $R^2=0.9832$ ($p<0.05$); FRAP ($Y=0.022x+0.077$ $R^2=0.9988$, ($p<0.05$)] extract of *C. capitatus*.

In addition, there is a strong correlation between total phenolic contents and antioxidant activity of the methanol [DPPH ($Y=0.6531x-0.5491$ $R^2=0.9986$ ($p<0.05$)), ABTS ($Y=0.3146x+0.8663$ $R^2=0.9999$ ($p<0.05$); FRAP ($Y=0.0138x+0.0129$ $R^2=0.9968$, ($p<0.05$)] extract of *P. pungens*.

Correlation coefficients and regression analyses showed that phenolic compounds were responsible for the antioxidant activity in plants extracts. Our results showed that there is a linear relationship between antioxidant activity and phenolic compounds.

Table 1. The antioxidant activities and total phenolic contents of *C. capitatus* and *P. pungens* extracts

Extracts/ Standards	DPPH (IC_{50} ; mg/mL)	ABTS (mMtrolox/ mg extract)	FRAP assay (mMFe ²⁺ / mg extract)	CUPRAC (mMtrolox/ mg extract)	Total phenolic contents (mg gallic acid equivalents/g extract)
<i>P. pungens</i>	0.064 \pm 0.005 ^a	23.36 \pm 0.005 ^a	8.60 \pm 0.008 ^a	0.39 \pm 0.03 ^a	30 \pm 0.004 ^a
<i>C. capitatus</i>	0.011 \pm 0.002 ^b	263.08 \pm 0.006 ^b	56.34 \pm 0.08 ^b	2.72 \pm 0.07 ^b	120 \pm 0.02 ^b
BHT	0.0081 \pm 0.0007 ^c	153.07 \pm 0.05 ^c			
Ascorbic acid	0.0023 \pm 0.0001 ^d	260.70 \pm 0.06 ^d		5.70 \pm 0.02 ^c	
BHA			16.91 \pm 0.02 ^c		

Values are mean of triplicate determination (n = 3) \pm standard deviation

Statistically significant at $p<0.05$

2.4. In vivo anti-inflammatory activity of plant extracts

The injection of carrageenan illustrated a rapid increase in the volume of paw, reaching its maximum 4h post-injection [11]. Indomethacin (5 mg/kg) indicated maximum anti-inflammatory effect 4 hours after carrageenan injection by 69.43 % (Table 2, $p<0.001$). *Phlomis pungens* methanolic extract demonstrated a more prominent and intensive anti-inflammatory effect with 24.7% of inhibitive capacity in the altered edema size after the first hour of carrageenan injection compared to *Coridothymus capitatus* methanolic extract. *P. pungens* methanolic extract inhibitory effect increased during three hours and reached maximum by 41.9% (Table 2, $p<0.001$).

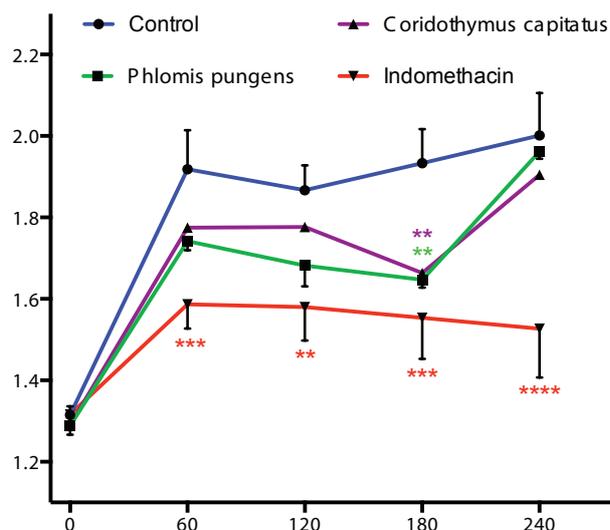
Carrageenan-induced edema inflammation is assumed to be biphasic, of which the first phase is mediated by the release of histamine and serotonin followed by kinin release and then prostaglandin in the later phase. Acute single-dose of *P. pungens* and *C. capitatus* markedly decreased rat paw edema

volume, especially at 3 hour after carrageenan injection. Some phenolic compounds have been reported to pose inhibition on histamine release in mast cells, which is responsible for early phase of inflammation induced by carrageenan. Hence, plants's acute anti-inflammatory effects may stem from its phenolic composition [12]. As a result phenolic compounds might be the effective components, which trigger the anti-inflammatory action, but further investigations are needed to discover precise mechanisms.

To the best of our belief, there have been no studies of anti-inflammatory activities of methanol extracts from *P. pungens* and *C. capitatus* aerial parts. In this study, anti-inflammatory activity of plants was examined for the first time and according to the obtained results, *P. pungens* extract showed higher anti-inflammatory activity than *C. capitatus* extract.

Table 2. Results of % inhibition of paw edema in *P. pungens* and *C. capitatus*.

Treatment	60 min	120 min	180 min	240 min
<i>P. pungens</i>	24.71 %	28.62 %	41.91 %	1.89 %
<i>C. capitatus</i>	19.57 %	11.78 %	39.64 %	8.95 %
Indomethacin	55.22 %	52.36 %	61.81 %	69.43 %

**Figure 1.** Anti-inflammatory effect of *P. pungens* and *C. capitatus* on carrageenan-induced paw edema

Values are expressed as Mean \pm SEM (Standard error mean); Values are calculated using two-way ANOVA followed by Tukey posttests; **** P <0.0001, *** P <0.001, ** P <0.01 and * P <0.05 when compared to control group; n=6.

3. CONCLUSION

In this study, antioxidant and anti-inflammatory activities of *P. pungens* and *C. capitatus* methanol extracts obtained from maceration method were investigated for the first time. According to the results obtained from this study, *C. capitatus* extract had higher free radical scavenging and cupric reducing antioxidant activity than *P. pungens* extract. Also, this extract exhibited stronger ABTS radical cation scavenging and ferric reducing/antioxidant power activity than standards (ascorbic acid, BHT, BHA) and *P. pungens* extract. Therefore, methanol extract of *C. capitatus* can be used as natural antioxidants in the future for food and pharmaceutical industry. In addition, *P. pungens* methanolic extract demonstrated a more prominent and intensive anti-inflammatory effect than *C. capitatus* extract.

4. MATERIALS AND METHODS

4.1. Chemicals

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonate) and butylated hydroxytoluene was sourced from Fluka. 2,4,6-Tripyridyl-s-triazine, 2,2-diphenyl-1-picryl-hydrazyl (DPPH[•]), Folin Ciocalteu's phenol reagent, gallic acid, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4''-disulfonic acid sodium salt, ascorbic acid were obtained from Sigma Chemical Co. All other reagents were of analytical grade.

4.2. Collection and identification of plant material

Phlomis pungens and *Coridothymus capitatus* aerial parts were collected during the flowering stage from Ankara-Beynam and Çanakkale-Belenköyü, Turkey at 2014, respectively. The taxonomic identity of the plant was confirmed by Assist. Prof. Dr. Gizem Bulut. The voucher specimens were deposited in the herbarium of the Faculty of Pharmacy, Marmara University; herbarium numbers: MARE 14852, MARE 16723 respectively.

4.3. Preparation of plants extract

P. pungens and *C. capitatus* aerial parts was dried in shade at 25 °C and ground in a mechanic grinder to fine powder and weighed accurately. Then, 100 g plant powder was extracted with methanol for 96 h at 25 °C.

4.4. Extract yield percentage

The extraction yield is a measure of the solvent's efficiency to extract specific components from the original material [13].

The percentage yield was obtained using this formula $A_2 - A_1 / A_0 \times 100$.

Where A_2 is the weight of the extract and the container, A_1 the weight of the container alone and A_0 the weight of the initial dried sample.

4.5. Determination of total phenolic contents in extract

Extracts prepared at different concentrations (1-5 mg/mL) were taken in 0.1 mL tubes and 4.5 mL of water was added to them. Then the Folin-Ciocalteu reagent (diluted 1/3 with

distilled water) and 0.3 mL of 2% sodium carbonate solution were added to the mixture. The mixture was allowed to stand at room temperature for 2 hours, and then the absorbance was measured at 760 nm against the reference. The total phenolic compounds contained in the extracts were given as mg gallic acid equivalents /g extract [14].

4.6. Determination of DPPH radical scavenging activity

The free radical scavenging activity of the extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical [15]. DPPH solution (0.1 mM, 3.9 mL) was added to the of extracts (0.1 mL) prepared at different concentrations (1-5 mg/mL). Then the mixture was allowed to stand at room temperature for 30 minutes. The absorbance of the mixture was measured against the reference at 517 nm. The experiment was repeated three times and the averages of the values and the standard deviation were calculated using the Graphpad Prism 5 program. The concentration of extracts and standard substance, which causes a 50 % reduction in initial DPPH concentration, is defined as IC_{50} . The results obtained in the experiment were given as IC_{50} = mg/mL.

4.7. Determination of ABTS radical cation scavenging activity

7 mM ABTS ammonium salt was dissolved in water and reacted with 2.45 mM potassium peroxodisulphate. The $ABTS^{\cdot+}$ stock solution was allowed to stand at room temperature for 12-16 hours to achieve a dark blue color. The $ABTS^{\cdot+}$ stock solution was prepared by diluting with distilled water to have an absorbance of 0.70 (\pm 0.02) at 734 nm. 40 μ L of extracts prepared at different concentrations (1-5 mg/mL), 3960 μ L of $ABTS^{\cdot+}$ working solution were combined. The absorbance of the mixture was measured against the reference at 734 nm for 6 minutes. The data obtained in this study were expressed as mM trolox/mg extract [16].

4.8. Ferric reducing antioxidant power activity (FRAP)

The FRAP reagent was prepared by mixing 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of the TPTZ solution (10 mM TPTZ in 40 mM HCl) and 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$. Then the FRAP reagent was kept at 37°C for 30 minutes in incubator device (Nuve). 3.8 mL of the FRAP reagent was mixed with 0.2 mL of extract and after 4 minutes the absorbance of the mixture was measured against the reference at 593 nm. FRAP values of the extracts were expressed as mM Fe^{2+} /mg extract [17].

4.9. Cupric reducing antioxidant capacity (CUPRAC)

Cupric reducing antioxidant capacity assay was carried out according to the method of Apak et al. [18]. The scheme

for normal measurement is summarized as follows: 1 mL of 10 mM Cu (II)+1 mL of 7.5 Mm neocuprine+1 mL of 1 M ammonium acetate buffer (pH 7)+1 mL of extracts solution+0.1 mL of H_2O ; total volume = 4.1 mL, measure A_{450} against a reagent blank after 1 h of reagent addition. In this study, CUPRAC values of the extracts were expressed as mM trolox/mg extract.

4.10. In vivo-anti-inflammatory activity of plant extracts

Carrageenan-induced edema is commonly used as an experimental animal model for acute inflammation and is believed to be biphasic, of which the first phase is mediated by the release of histamine and 5-HT followed by kinin release and then prostaglandin in the later phase [19]. Carrageenan paw edema was induced in Sprague-Dawley rats by sub-plantar injection of 0.1 ml of 1 % (w/v) carrageenan in saline in the right paw of the rats. The different groups were treated orally with *Phlomis pungens* (100 mg/kg, p.o.) and *Coridothymus capitatus* (100 mg/kg, p.o.), indomethacin (5 mg/kg, p.o.) and saline (10 ml/kg, p.o.) 60 min. before the administration of carrageenan [20, 21]. The volume of the edema development and its duration was determined for 4 hours using plethysmometer 37140 (UgoBasile, Italy). The inhibitory activity was calculated according to the formula: % Inhibition = [(Vt-Vo) control-(Vt-Vo)]/[(Vt-Vo) control] x 100. Where Vt is the paw volume at time t, Vo is the paw volume before carrageenan injection, (Vt-Vo) is edema in paw after time t. The study was approved by the Marmara University, Animal Experiments Local Ethics Committee (MÜHDEK-58.2017.mar).

4.11. Statistical analysis

All the experiments were done in triplicates. All data from the study were shown as mean \pm SD and analysed by the Graphpad Prism 5. Statistical differences between the experimental groups were analysed using one-way and two-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison test. Mean values were considered statistically significant when $p < 0.05$.

Authorship statement

Author contributions: Concept – T.T., M.E.Ç., L.B., L.K.; Design – T.T., L.B.; Supervision – T.T.; Resource – A.N.H.Y., G.B., L.K., T.T., M.E.Ç.; Materials – T.T., A.N.H.Y., M.E.Ç., G.B.; Data Collection and/or Processing – L.K., G.B., L.B., T.T., M.E.Ç., A.N.H.Y.; Analysis and/or Interpretation – A.N.H.Y., M.E.Ç., T.T., L.B., G.B.; Literature Search – M.E.Ç., T.T., A.N.H.Y.; Writing – T.T., M.E.Ç., A.N.H.Y.; Critical Reviews – T.T., M.E.Ç., G.B., A.N.H.Y., L.K., L.B.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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