

## Antioxidant and anti-inflammatory activity of capitula, leaf and stem extracts of *Tanacetum cilicicum* (Boiss.) Grierson

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**Abstract:** In this study, various extracts obtained from different parts of *Tanacetum cilicicum* were investigated for *in vitro* antioxidant and anti-inflammatory activity. Antioxidant activity was tested with three methods; namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity, 2,2-azino-bis(3-ethylbenzothiazolin-6-sulphonic acid) (ABTS) radical cation scavenging capacity, and ferric-reducing antioxidant power (FRAP) assays. Total phenolic and flavonoid contents of extracts were determined by Folin-Ciocalteu and aluminum chloride methods, respectively. Also, anti-inflammatory activity of these extracts was evaluated by 5-lipoxygenase inhibition assay. Ethyl acetate extract of capitula of *T. cilicicum* (TCCEA) showed the highest antioxidant activity with IC<sub>50</sub> values of 22.44 and 30.86 µg/mL against DPPH and ABTS radicals, respectively. At the same time, the highest ferric reducing power was found in the TCCEA (42.2 mg TE/g extract). The highest total phenolic contents have been detected in TCCEA and ethyl acetate extract of leaves of *T. cilicicum* (TCLEA) with values of 174.1 and 175.6 mg GAE/g extract, respectively. Similarly, the highest total flavonoid contents have been detected in TCCEA and TCLEA with values of 26.94 and 30.48 mg QE/g extract, respectively. TCCEA exhibited strong anti-inflammatory activity with IC<sub>50</sub> value of 9.44 µg/mL when compared to standard indomethacin (22.39 µg/mL). These results demonstrate that TCCEA has a significant antioxidant and anti-inflammatory activity. Also, the results show that TCCEA is a good candidate for further bioactivity-guided fractionation in the search for new active anti-inflammatory and antioxidant compounds.

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## 1. INTRODUCTION

Inflammation is a physiopathological response of living tissues and occurs against injuries causing accumulation of local plasmatic fluid and blood cells [1]. Inflammation is a complex process involving many different factors such as prostaglandins, leukotrienes and platelet activating factor (PAF) [2]. Although it is a defense mechanism of the body, complex events and mediators involving the inflammatory reaction can induce, or even aggravate, many

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diseases. Thus, anti-inflammatory agents are known to be effective agents in the treatment of these pathologies [1].

Chronic inflammatory diseases, one of the most important health problems in the world, are increasingly developing worldwide. Today, both steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) are used to heal inflammation. Although steroids have an important role in the treatment of inflammatory diseases, they can be used only for short periods due to their toxicity. Prolonged use of NSAIDs can cause serious side effects, especially gastrointestinal bleeding [3]. It is known that some natural products are used as a good anti-inflammatory agent without the risk of side effects [4]. Also, It is generally recognized that free radicals play an important role in the development of tissue damage and pathological events [5]. There are free radicals in the pathology of many diseases including cancer, atherosclerosis, malaria, rheumatoid arthritis and neurodegenerative diseases [6]. Endogenous and exogenous antioxidants act as free radical scavengers by preventing and repairing the damage caused by free radicals and can therefore increase immune defense and reduce the risk of cancer and degenerative disease [7]. Antioxidant compounds derived from plants can reduce the formation of free radicals and cure the diseases caused by oxidative stress. Medicinal plants are known to have an important antioxidant and anti-inflammatory activity. Especially, phenolic and flavonoid compounds derived from medicinal plants contribute to the antioxidant activity of plants and function as anti-inflammatory agents [8].

The genus *Tanacetum* L., the third largest genus of the Asteraceae family, grows in the temperate regions of Europe and West Asia and comprises about 200 species. In Turkey, the genus *Tanacetum* is represented by 47 species or 61 taxa including subspecies and varieties and 27 of those are endemic [9,10]. *Tanacetum cilicicum* is 60-70 cm tall. Cauline leaves is greyish, 5-15 cm tall and pinnatisect [11]. *Tanacetum* species have been used as antipyretic and to treat headache, tinnitus, dizziness in traditional medicine for centuries in Turkey [12]. For example, *T. parthenium* has been used for years for the therapy of fever, migraine, menstrual disases, stomachache, toothache due to its anti-inflammatory activity [13]. Also, *Tanacetum* species are used as tonic, appetizers, anthelmintics, diuretics, carminatives, stimulants, emmenagogues in Turkey [14]. The members of this genus are rich in phenolic compounds which are responsible for their biological activities such as anti-inflammatory, antimicrobial, antifeedant, cytotoxic, insecticidal etc. [15,16]. Also, it is reported that different *Tanacetum* species have anticancer activity [17]. It has been demonstrated in previous study that different parts of *T. cilicicum* contain *p*-coumaric acid, ferulic acid, gallic acid, gentisic acid, chlorogenic acid, quercetin, naringenin and catechin [18]. *Tanacetum* species also contain essential oil ( -pinene, sabinene, limonene, eucalyptol, camphor, linalool, -terpineol, borneol) and sesquiterpene lactones as secondary metabolites [19-21].

In this study, various extracts obtained different parts of *Tanacetum cilicicum* were examined for *in vitro* antioxidant and anti-inflammatory activity. According to our detailed literature search, there is a limited number of the study on *T. cilicicum*. There are only two study in literature regarding antioxidant properties of *T. cilicicum* [9,18] but antioxidant activities of different extracts of *T. cilicicum* obtained by using different solvents such as hexane, chloroform, ethyl acetate, ethanol were examined for the first time in the present study. Also, there is no study regarding anti-inflammatory properties of *T. cilicicum*. Therefore, this is the first study that *T. cilicicum* extracts were evaluated according to *in vitro* anti-inflammatory method.

## 2. MATERIAL and METHODS

### 2.1. Plant material and chemicals

Plant samples were collected at their flowering period from the Pülümür district of Tunceli in Turkey and identified by Dr. Ahmet Do an, a botanist of the Faculty of Pharmacy, University of Marmara. Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Marmara University (MARE No: 17768).

Chemicals and solvents of 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2 -Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, Folin–Ciocalteu reagent sodium nitrite, aluminum chloride hexahydrate, sodium hydroxide, iron (II) chloride, 2,4,6-tris (2-pyridyl)-s-triazine, type V soybean lipoxygenase, sodium acetate, boric acid, ferric chloride, gallic acid, quercetin, ascorbic acid, indomethacine, linoleic acid, ferrozine, methanol, ethanol, hexane, chloroform, ethyl acetate, asetic acid, hydrochloric acid were obtained from Merck (Darmstadt, Germany), Sigma–Aldrich (St. Louis, MO, USA) and were of analytical grade.

### 2.2. Extraction

About 15 g of each dried and ground the capitula (TCCE), leaves (TCLE), and stem (TCSE) of *Tanacetum cilicicum* were extracted with 8×200 mL EtOH, using an ultrasonic bath. After filtration and evaporation, the ethanol extracts were dissolved in 30 mL 60% aqueous ethanol, and subjected to solvent-solvent partition between *n*-hexane (5×50 mL), chloroform (3×50 mL), and ethyl acetate extract (2×50 mL). The *n*-hexane, chloroform, ethyl acetate extract and aqueous ethanol extracts of *T. cilicicum* capitula obtained by this method were coded as TCCH, TCCC, TCCEA and TCCAE, respectively. The *n*-hexane, chloroform, ethyl acetate extract and aqueous ethanol extracts of *T. cilicicum* leaves obtained by this method were coded as TCLH, TCLC, TCLEA and TCLAE, respectively. The *n*-hexane, chloroform, ethyl acetate extract and aqueous ethanol extracts of *T. cilicicum* stem obtained by this method were coded as TCSH, TCSC, TCSEA and TCSAE, respectively. Extraction yields have been summarized in Table 1. All extracts were stored under refrigeration for further analysis.

### 2.3. Antioxidant activity

#### 2.3.1. DPPH radical scavenging activity

DPPH radical scavenging capacity of each extract was determined by the method of Zou et al. [22]. Briefly, 10 µL of extracts in DMSO at different concentrations (250-0.048 µg/mL) were added to 190 µL methanol solution of DPPH (0.1 mM) in a well of 96-well plate. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 min. Absorbance readings were taken at 517 nm. The percent radical scavenging activity of extracts and standard against DPPH were calculated according to the following:

$$\text{DPPH radical-scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where  $A_0$  is the absorbance of the control (containing all reagents except the test compounds), and  $A_1$  is the absorbance of the extracts/standard. Extract concentration providing 50% inhibition ( $IC_{50}$ ) was calculated from the graph plotting inhibition percentage against extracts concentration. Tests were carried out in triplicate. Ascorbic acid was used as positive control.

#### 2.3.2. ABTS radical-scavenging activity

ABTS radical cation scavenging activity assay was carried out according to the method described by Zou et al. [22]. ABTS radical cations were prepared by mixing equal volume of ABTS (7 mM in  $H_2O$ ) and potassium persulfate (4.9 mM in  $H_2O$ ), allowing them to react for

12-16 h at room temperature in the dark. Then, ABTS radical solution was diluted with 96% ethanol to an absorbance of about 0.7 at 734 nm. 10  $\mu$ L of extracts in DMSO at different concentrations (250-0.048  $\mu$ g/mL) were added to 190  $\mu$ L of ABTS radical solution in a well of 96-well plate. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 min. Absorbance readings were taken at 734 nm. The percent radical scavenging activity of extracts and standard against ABTS were calculated according to the following:

$$\text{ABTS radical-scavenging activity (\%)} = [(A_0 - A_1)/A_0] \times 100$$

where  $A_0$  is the absorbance of the control (containing all reagents except the test compounds), and  $A_1$  is the absorbance of the extracts/standard. Extract concentration providing 50% inhibition ( $IC_{50}$ ) was calculated from the graph plotting inhibition percentage against extracts concentration. Tests were carried out in triplicate. Trolox was used as positive control.

#### 2.4. Determination of total phenolic contents (TPC)

Total phenolic contents of *Tanacetum cilicicum* extracts were measured using Folin–Ciocalteu reagent [23]. The assay was adapted to the 96 well microplate format. 10  $\mu$ L of extracts in various concentrations (151.52-18.94  $\mu$ g/mL) were mixed with 20  $\mu$ L Folin–Ciocalteu reagent (Sigma), 200  $\mu$ L of  $H_2O$ , and 100  $\mu$ L of 15%  $Na_2CO_3$ , and the absorbance was measured at 765 nm after 2 h incubation at room temperature. Gallic acid was used as a standard and the total phenolics were expressed as mg GAE/g extract.

#### 2.5. Determination of total flavonoid contents (TFC)

Total flavonoid content was determined following a method by Zhang et al. [24]. The assay was adapted to the 96 well microplate format. 25  $\mu$ L extract of in various concentrations were mixed 125  $\mu$ L of ultra pure water and 7.5  $\mu$ L of 5 %  $NaNO_2$ . After 6 min, 15  $\mu$ L of 10%  $AlCl_3 \cdot 6H_2O$  was added. After 5 min, 50  $\mu$ L  $NaOH$  (1 M) was added and this solution completed with 250  $\mu$ L of ultra pure water. The absorbance was measured against the reagent blank at 510 nm. The standard curve for total flavonoids was made using quercetin standard solution (0.488 to 250  $\mu$ g/mL) under the same procedure as earlier described. The total flavonoids were expressed as milligrams of quercetin equivalents per g of dried fraction.

#### 2.6. Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power assay was carried out according to the method described by Zou et al. [22]. This method was developed to measure the ferric reduction ability at a low pH. When the ferric 2,4,6-tripyridyl-s-triazine complex ( $Fe^{3+}$ -TPTZ) is reduced to the ferrous form ( $Fe^{2+}$ -TPTZ), an intense blue color is developed. Briefly, the FRAP reagent was prepared by mixing 10 volumes of 250 mM acetate buffer (pH 3.6), with 1 volume of 10 mM TPTZ in 40 mM HCl and with 1 volume of 20 mM  $FeCl_3 \cdot 6H_2O$ . A total of 10  $\mu$ L of properly diluted samples and 30  $\mu$ L of distilled water was added to 260  $\mu$ L of freshly prepared FRAP reagent in a well of a 96-well plate. The mixture was incubated at 37 °C throughout the reaction. After 8 min, the absorbance was read at 593 nm against reagent blank. Tests were carried out in triplicate. The FRAP value of extracts was expressed as mg trolox/g extract.

#### 2.7. In vitro anti-inflammatory activity

The anti-inflammatory activity was evaluated with slight modifications according to the method described by Phosrithong et al. [25]. The method was adapted to the 96 well transparent microplate with some modification. 10  $\mu$ L at different concentrations of *Tanacetum cilicicum* extracts (5000-9.77  $\mu$ g/mL) or standard indomethacine (250-0.49  $\mu$ g/mL) were added to 20  $\mu$ L ethanol, 20  $\mu$ L pure water, 25  $\mu$ L of sodium borate buffer solution (0.1 M, pH 9) followed by addition of 25  $\mu$ L of type V soybean lipoxygenase solution in buffer (pH 9, 20.000 U/mL).

After the mixture was incubated at 25 °C for 5 min, 100 µL of 0.6 mM linoleic acid solution was added, mixed well and the change in absorbance at 234 nm was recorded for 6 min. Indomethacin was used as a reference standard. The percent inhibition was calculated from the following equation:

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

A dose-response curve was plotted to determine the IC<sub>50</sub> values. IC<sub>50</sub> is defined as the concentration sufficient to obtain 50% of a maximum anti-inflammatory activity. All tests and analyses were performed in triplicates.

## 2.8. Statistical analysis

The data were given as means ± standard deviations and analysed by one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparison tests using GraphPad Prism 5. Differences between means at p < 0.05 levels were considered significant.

## 3. RESULTS

As shown in Table 1, all plant extracts were found to possess concentration-dependent inhibitory activity against DPPH radical except for hexane extracts. A low IC<sub>50</sub> value shows the high antioxidant activity. Therefore, TCCEA with IC<sub>50</sub> value of 22.44 µg/mL was superior than other extracts of *T. cilicicum* for DPPH assay. DPPH radical scavenging activity of *T. cilicicum* extracts decreased in the following order: TCCEA TCLEA TCCE TCCC TCLE TCSEA TCLAE TCCAE TCLC TCSE TCSC TCCH TCLH TCSH (Table 1). In ABTS assay, TCCEA with IC<sub>50</sub> value of 30.86 µg/mL was better than other extracts of *T. cilicicum* for ABTS assay. ABTS radical scavenging activity of *T. cilicicum* extracts decreased in the following order: TCCEA TCLEA TCCE TCCC TCSEA TCLAE TCLC TCLE TCSC TCCAE TCSE TCLH TCLAE TCSH TCCH (Table 1). FRAP activity of the plant extracts were determined according to the equation ( $y = 0.0021x + 0.0118$ ,  $R^2 = 0.9922$ ) and expressed in terms of trolox equivalent (mg trolox/g extract). It was found that TCCEA (42.02 mg trolox/g extract) possessed the highest antioxidant activity in FRAP assay. Ferric reducing antioxidant power for extracts were reduced in the following order: TCCEA TCCE TCCC TCSEA TCLE TCSC TCLEA TCLC TCLAE TCSE TCCAE TCSAE TCLH TCSH TCCH.

Total phenolic contents were calculated according to the equation ( $y = 0.0056x + 0.0466$ ,  $R^2 = 0.9978$ ) obtained from calibration curve as gallic acid equivalent (mg/g extract), while total flavonoid contents were calculated according to the equation ( $y = 0.004x + 0.0514$ ,  $R^2 = 0.9967$ ) obtained from calibration curve as quercetin equivalent (mg/g extract). As shown in Table 2, the amount of total phenolics in extracts varied from 6.106 to 175.6 mg of gallic acid equivalent per gram dried extract and the total flavonoid contents varied from 6.136 to 30.48 mg quercetin equivalent per gram dried extract. The highest total phenolic and flavonoid levels have been detected in TCLEA and TCCEA which showed highest activity in DPPH, ABTS tests.

TCCEA inhibited 5-lipoxygenase activity by 99.21% at a concentration of 250 µg/mL. TCCEA exhibited very strong anti-lipoxygenase activity with IC<sub>50</sub> values of 9.44 µg/mL when compared to standard (IC<sub>50</sub> for indomethacin: 22.39 µg/mL) (Table 1). Also, TCSEA extract at a concentration of 250 µg/mL showed lowest anti-lipoxygenase activity with inhibition rate of 34.41% (Figure 1).

**Table 1.** Antioxidant and anti-inflammatory activities of various extracts obtained from different parts of *Tanacetum cilicicum*

| Extracts*/Standards | Yield (%) | DPPH activity<br>IC <sub>50</sub> (µg/mL) | ABTS activity               |                               | FRAP activity<br>(mg trolox/g extract)** | Anti-inflammatory activity<br>IC <sub>50</sub> (µg/mL) |
|---------------------|-----------|---|-----------------------------|-------------------------------|--|--|
|                     |           |   | (mg trolox/g extract)**     | IC <sub>50</sub> (µg/mL)      |  |  |
| TCCE***             | 18.35     | 117.5±0.42 <sup>c</sup>                   | 326.1 ± 1.89 <sup>c</sup>   | 42.32±0.13 <sup>d</sup>       | 41.89±0.90 <sup>a</sup>                  | 156.0 ± 1.20 <sup>c,d</sup>                            |
| TCCH                | 25.87     | 981.00 ± 1.49 <sup>h</sup>                | 47.47 ± 0.00 <sup>j</sup>   | 238.4 ± 0.0 <sup>k</sup>      | 7.18 ± 0.05 <sup>i</sup>                 | 167.5 ± 0.21 <sup>c,d,e</sup>                          |
| TCCC                | 24.40     | 126.5 ± 0.28 <sup>c</sup>                 | 306.1 ± 7.55 <sup>d</sup>   | 45.62 ± 0.59 <sup>d,e</sup>   | 40.94 ± 0.76 <sup>a,b</sup>              | 127.0 ± 2.05 <sup>b,c</sup>                            |
| TCCEA               | 12.07     | 22.44 ± 0.057 <sup>a</sup>                | 423.5 ± 0.00 <sup>a</sup>   | 30.86 ± 0.0 <sup>b</sup>      | 42.02 ± 1.31 <sup>a</sup>                | 9.44 ± 3.60 <sup>a</sup>                               |
| TCCA                | 31.33     | 137.8 ± 0.35 <sup>c,d</sup>               | 214.1 ± 1.89 <sup>g</sup>   | 61.66 ± 0.17 <sup>h</sup>     | 16.82 ± 0.69 <sup>g</sup>                | 240.9 ± 24.25 <sup>f,g</sup>                           |
| TCSE***             | 6.69      | 183.0 ± 1.27 <sup>e</sup>                 | 212.8 ± 7.54 <sup>g</sup>   | 63.97 ± 0.84 <sup>h</sup>     | 30.39 ± 0.32 <sup>f</sup>                | 255.7 ± 14.99 <sup>g</sup>                             |
| TCSH                | 6.46      | 2052.00 ± 19.80 <sup>i</sup>              | 54.14 ± 9.43 <sup>i,j</sup> | 210.4 ± 4.46 <sup>j</sup>     | 7.28 ± 0.15 <sup>i</sup>                 | 202.9 ± 12.02 <sup>d,e,f,g</sup>                       |
| TCSC                | 10.53     | 191.0 ± 0.64 <sup>e</sup>                 | 272.8 ± 1.88 <sup>f</sup>   | 51.73 ± 0.20 <sup>g</sup>     | 38.80 ± 0.10 <sup>b,c,d</sup>            | 112.4 ± 6.08 <sup>b,c</sup>                            |
| TCSEA               | 4.62      | 132.2 ± 0.35 <sup>c,d</sup>               | 291.5 ± 1.89 <sup>d,e</sup> | 46.75 ± 0.18 <sup>d,e,f</sup> | 40.32 ± 0.12 <sup>a,b</sup>              | 395.1 ± 2.76 <sup>i</sup>                              |
| TCSSE               | 69.65     | 557.5 ± 4.60 <sup>g</sup>                 | 78.14 ± 1.89 <sup>h</sup>   | 179.7 ± 0.78 <sup>i</sup>     | 11.11 ± 0.72 <sup>h</sup>                | 360.3 ± 50.63 <sup>h</sup>                             |
| TCLE***             | 22.55     | 130.3 ± 0.21 <sup>c</sup>                 | 267.5 ± 1.89 <sup>f</sup>   | 51.48 ± 0.13 <sup>f,g</sup>   | 39.77 ± 0.17 <sup>a,b,c</sup>            | 187.4 ± 2.83 <sup>d,e,f</sup>                          |
| TCLH                | 17.62     | 1455.00 ± 9.19 <sup>i</sup>               | 64.80 ± 1.88 <sup>h,i</sup> | 175.3 ± 0.71 <sup>i</sup>     | 10.12 ± 0.17 <sup>h</sup>                | 149.8 ± 3.75 <sup>c,d</sup>                            |
| TCLC                | 17.94     | 153.9 ± 0.28 <sup>d</sup>                 | 279.5 ± 0.00 <sup>e,f</sup> | 50.45 ± 0.46 <sup>f,g</sup>   | 36.57 ± 0.1 <sup>d,e</sup>               | 148.1 ± 1.06 <sup>c,d</sup>                            |
| TCLEA               | 7.41      | 61.49 ± 0.16 <sup>b</sup>                 | 387.5 ± 1.89 <sup>b</sup>   | 35.43 ± 0.33 <sup>c</sup>     | 37.55 ± 1.62 <sup>c,d</sup>              | 85.18 ± 3.50 <sup>b</sup>                              |
| TCLSE               | 57.93     | 136.8 ± 0.14 <sup>c,d</sup>               | 278.1 ± 5.66 <sup>e,f</sup> | 49.69 ± 0.59 <sup>e,f,g</sup> | 34.44 ± 0.03 <sup>e</sup>                | 220.3 ± 4.31 <sup>e,f,g</sup>                          |
| AA****              |           | 17.6 ± 0.37 <sup>a</sup>                  |                             | 14.5 ± 0.32 <sup>a</sup>      |  |  |
| Trolox****          |           | 14.54 ± 0.18 <sup>a</sup>                 |                             | 13.00 ± 0.21 <sup>a</sup>     |  |  |
| BHA****             |           | 57.15 ± 0.09 <sup>b</sup>                 |                             | 17.06 ± 0.58 <sup>a</sup>     |  |  |
| BHT****             |           | 213.6 ± 15.20 <sup>f</sup>                |                             | 26.82 ± 1.12 <sup>b</sup>     |  |  |
| INDO                |           |   |                             |                               |  | 22.39 ± 0.2546 <sup>a</sup>                            |

\* Abbreviations: TCCE, TCCH, TCCC, TCCEA, TCCA show the ethanol extracts and its *n*-hexane, chloroform, ethyl acetate, and aqueous ethanol fractions of the capitula of *Tanacetum cilicicum*, respectively. TCSE, TCSH, TCSC, TCSEA, TCSAE show the ethanol extracts and its *n*-hexane, chloroform, ethyl acetate, and aqueous ethanol fractions of the stems of *Tanacetum cilicicum* respectively. TCLE, TCLH, TCLC, TCLEA, TCLAE show the ethanol extracts and its *n*-hexane, chloroform, ethyl acetate, and aqueous ethanol fractions of the leaves of *Tanacetum cilicicum*, respectively.

\*\* Results were expressed as trolox equivalent for ABTS and FRAP.

\*\*\* The yield of these extracts was calculated from the powdered dry plant. The yield of the remaining extracts was calculated from dry ethanol extracts.

\*\*\*\* AA: Ascorbic acid; BHA: Butylhydroxyanisole; BHT: Butylhydroxytoluene; INDO: Indomethacine

\*\*\*\*\* Each value in the table is represented as mean ± standard deviation (n=3). Different letter superscripts in the same column indicate significant differences (P<0.05).

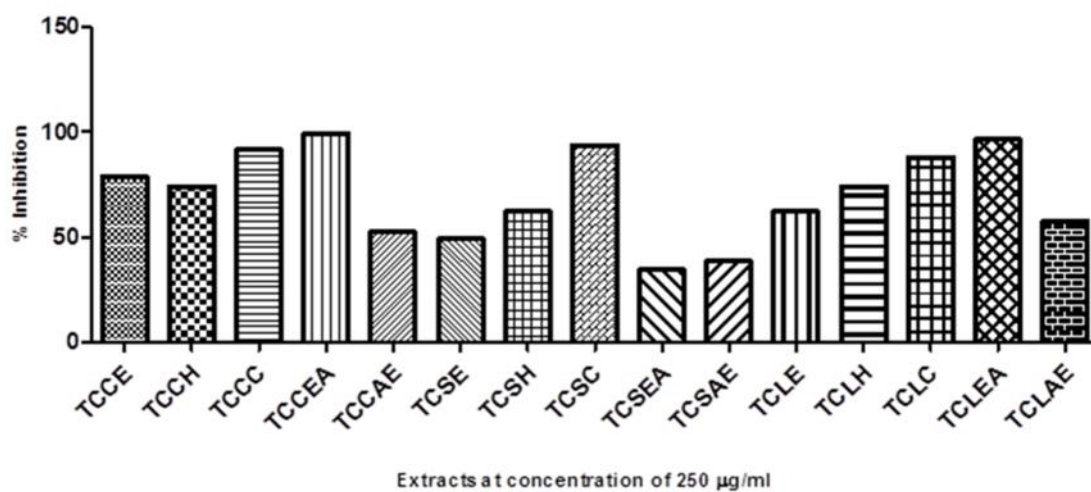
**Table 2.** Total phenol and total flavonoid contents of various extracts obtained from different parts of *T. cilicicum*

| Extracts*/<br>Standards | TPC<br>(mg GAE/g<br>extract)** | TFC<br>(mg QE/g<br>extract)**   |
|-------------------------|--------------------------------|---------------------------------|
| TCCE                    | 87.01 ± 0.64 <sup>b</sup>      | 14.25 ± 0.64 <sup>c,d</sup>     |
| TCCH                    | 10.28 ± 0.20 <sup>ij</sup>     | 7.82 ± 0.15 <sup>d,e,f</sup>    |
| TCCC                    | 72.37 ± 4.57 <sup>c,d</sup>    | 13.68 ± 1.55 <sup>c,d,e</sup>   |
| TCCEA                   | 174.1 ± 2.72 <sup>a</sup>      | 26.94 ± 0.56 <sup>a,b</sup>     |
| TCCAE                   | 42.45 ± 0.48 <sup>g</sup>      | 8.57 ± 1.94 <sup>d,e,f</sup>    |
| TCSE                    | 28.44 ± 0.16 <sup>h</sup>      | 9.55 ± 0.21 <sup>d,e,f</sup>    |
| TCSH                    | 6.11 ± 0.13 <sup>j</sup>       | 5.84 ± 3.58 <sup>f</sup>        |
| TCSC                    | 68.85 ± 0.48 <sup>d,e</sup>    | 12.40 ± 0.57 <sup>c,d,e,f</sup> |
| TCSEA                   | 77.36 ± 0.70 <sup>c</sup>      | 19.25 ± 2.17 <sup>b,c</sup>     |
| TCSAE                   | 12.08 ± 0.0 <sup>ij</sup>      | 6.14 ± 1.31 <sup>e,f</sup>      |
| TCLE                    | 57.58 ± 1.62 <sup>f</sup>      | 11.43 ± 0.39 <sup>c,d,e,f</sup> |
| TCLH                    | 13.63 ± 0.22 <sup>i</sup>      | 9.03 ± 2.94 <sup>d,e,f</sup>    |
| TCLC                    | 63.28 ± 0.35 <sup>e,f</sup>    | 15.29 ± 2.72 <sup>c,d</sup>     |
| TCLEA                   | 175.6 ± 0.0 <sup>a</sup>       | 30.48 ± 0.48 <sup>a</sup>       |
| TCLAE                   | 40.59 ± 0.19 <sup>g</sup>      | 11.16 ± 3.80 <sup>d,e,f</sup>   |

\* Abbreviations: TCCE, TCCH, TCCC, TCCEA, TCCAE show the ethanol extracts and its *n*-hexane, chloroform, ethyl acetate, and aqueous ethanol fractions of the capitula of *Tanacetum cilicicum*, respectively. TCSE, TCSH, TCSC, TCSEA, TCSAE show the ethanol extracts and its *n*-hexane, chloroform, ethyl acetate, and aqueous ethanol fractions of the stems of *Tanacetum cilicicum* respectively. TCLE, TCLH, TCLC, TCLEA, TCLAE show the ethanol extracts and its *n*-hexane, chloroform, ethyl acetate, and aqueous ethanol fractions of the leaves of *Tanacetum cilicicum*, respectively.

\*\* Results were expressed as gallic acid equivalent (GAE) for TPC, as quercetin equivalent for TFC.

\*\*\* Each value in the table is represented as mean ± standard deviation (n=3). Different letter superscripts in the same column indicate significant differences (P<0.05).

**Figure 1.** Anti-inflammatory activity of various extracts obtained from different parts of *T. cilicicum*

#### 4. DISCUSSION

In the present study, the antioxidant and anti-inflammatory activities of ethanol extracts of the capitula, leaves, and stem of *Tanacetum cilicicum* and their *n*-hexane, chloroform, ethyl acetate and aqueous ethanol fractions were evaluated. TCCEA against DPPH radical, TCCEA, TCLEA against ABTS radical, and TCCEA, TCCE, TCCC, TCSEA against FRAP exhibited strong antioxidant activity.

The antioxidant activity of the extracts was investigated by three methods; DPPH radical-scavenging activity, ABTS radical-scavenging activity, and Ferric reducing antioxidant power (FRAP) assay. DPPH is a purple colored radical that transforms into a yellow non-radical DPPH in the presence of a strong antioxidant molecule. This color change occurs when the DPPH radical receives a hydrogen from the antioxidant molecule [26]. The ABTS radical cation decolorization assay is a method for screening antioxidant activities of molecules and is applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxysinamates, carotenoids and plasma antioxidants. Pre-formed radical monocation of 2,2 -Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS<sup>•+</sup>) is produced by oxidation of ABTS with potassium persulfate and decreases in the presence of hydrogen-donating antioxidants [27]. The FRAP test measures the reduction capacity of the antioxidant agents by reducing Fe<sup>3+</sup> to Fe<sup>2+</sup>. In this reaction, the bright red salt of potassium ferricyanide solution is reduced to the potassium ferrocyanide complex by taking an electron from the antioxidant molecule. The color of reaction mixture changes from green-yellow to Perl's Prussian blue color [26].

In one study, antioxidant activity of methanol extracts of flower, leaf and stem of *T. cilicicum* were investigated by various antioxidant experiments. It was reported that leaf, flower and stem of this species showed DPPH scavenging activity with IC<sub>50</sub> values of 25.95 µg/mL, 30.26 µg/mL and 117.48 µg/mL, respectively [18]. In another study, it was found that methanol extract prepared with aerial parts of *T. cilicicum* had an IC<sub>50</sub> value of 249.17 µg/mL against DPPH radical [9]. When these studies compare with activities of ethanol extracts (because they have approximately the same polarity) prepared from capitula (117.5 µg/mL), stem (183.0 µg/mL), and leaves (130.3 µg/mL) of *T. cilicicum* in our current study, we found that they had lower activity than the results of the first study but higher than the results of the second study. Also, ethyl acetate extract prepared from capitula of *T. cilicicum* (TCCEA) in our present study showed a strong activity with IC<sub>50</sub> value of 22.44 µg/mL against DPPH radical. TCCEA was found to have higher activity than BHT (213.6 µg/mL) and BHA (57.15 µg/mL) and close to ascorbic acid (17.6 µg/mL) and trolox (14.54 µg/mL) compared to the standards.

Arituluk et al. (2016) revealed that trolox equivalent antioxidant capacity (TEAC) of methanol extract of *T. cilicicum* aerial parts in ABTS test was 66.98 mg trolox equivalent per g extract (TE)[9]. When this study compare with activities of ethanol extracts (because they have approximately the same polarity) prepared from capitula (326.1 mg TE/g), stem (212.8 mg TE/g), and leaves (267.5 mg TE/g) of *T. cilicicum* in our current study, we found that they had the higher trolox equivalent antioxidant capacity. Also, ABTS activity of TCCEA in our present study, it was found to be quite high compared to the activities of all extracts (423.5 mg TE/g or IC<sub>50</sub>: 30.86 µg/mL). Also, ethyl acetate extract prepared from capitula of *T. cilicicum* (TCCEA) in our present study showed a significant activity with IC<sub>50</sub> value of 30.86 µg/mL against ABTS radical. TCCEA was found to have lower activity than BHA (17.06 µg/mL), ascorbic acid (14.5 µg/mL) and trolox (13.00 µg/mL) and close to BHT (26.82 µg/mL) compared to the standards.

Arituluk et al. (2016) showed that methanol extract of aerial parts of *T. cilicicum* has ferric reducing antioxidant power of 198.19 mg QE/g extract [9]. In contrast to this study, ethanol extracts were obtained from the plant in our current study and the results were expressed as trolox equivalent, not as quercetin. When this study compare with activities of ethanol



extracts (because they have approximately the same polarity) prepared from capitula (41.89 mg TE/g), stem (30.39 mg TE/g), and leaves (39.77 mg TE/g) of *T. cilicicum* in our current study, we found that they had the lower ferric reducing antioxidant power. Also, TCCEA possessed the highest FRAP activity with 42.02 mg trolox/g extract while TCCH had the lowest FRAP activity with 7.175 mg trolox/g extract.

Gecibesler et al. (2016) reported that total phenolic amount of methanol extracts obtained from different parts of *T. cilicicum* were found ranging from  $99.53 \pm 2.39$  to  $268.02 \pm 0.97$  mg GAE/g extract. They found that phenolic contents for the methanol extract of flower of *T. cilicicum* were higher than leaf and stem of *T. cilicicum* [16]. In other study, Arituluk et al. (2016) indicated that amount of total phenol of methanol extract of *T. cilicicum* aerial parts was 33.14 mg gallic acid equivalent per g extract (GAE) [9]. When compared to our present study, total phenolic amounts for ethanol extracts of capitula (87.01 mg/g), leaves (57.58 mg/g) and stem (28.44 mg/g) of *T. cilicicum* were found to be lower than the results of the first study but higher than the results of the second study except for TCSE. Also, total phenol contents of TCLEA and TCCEA were found to be considerably high with values of 175.6 mg GAE/g extract and 174.1 mg GAE/g extract, respectively. The lowest phenolic amount was found in hexane extract of flower of *T. cilicicum* (6.106 mg GAE/g).

Gecibesler et al. (2016) reported that total flavonoid amounts of methanol extracts obtained from different parts of *T. cilicicum* varied from  $26.76 \pm 3.53$  to  $86.41 \pm 1.62$  mg QE/g of the extract. It was found that the flavonoid content of the flower parts ( $26.76 \pm 3.53$  mg QE/g extract) was the lowest followed by stem ( $29.59 \pm 2.58$  mg QE/g extract). However, the leaves had the highest total flavonoid content. ( $86.41 \pm 1.62$  mg QE/g extract). [16]. In other study, Arituluk et al. (2016) indicated that amount of total flavonoid of methanol extract of *T. cilicicum* aerial parts was 18.73 mg quercetin equivalent per g extract (QE) [9]. When compared to our present study, total flavonoid contents for ethanol extracts of capitula (14.25 mg/g), leaves (11.43 mg/g) and stem (9.55 mg/g) of *T. cilicicum*, were found to be lower than the results of the previous studies. However, total flavonoid content of TCLEA and TCCEA were found to be high with values of 30.48 and 26.94 mg QE/g extract when compared to the study of Arituluk et al. (2016), respectively. The lowest flavonoid content was observed in TCSH with 5.837 mg QE/g extract.

In addition, the total phenol and flavonoid contents of TCCEA and TCLEA, which had a high antioxidant activity compared to other extracts, were found to be high. Therefore, these compounds can be considered responsible for the antioxidant activity of the extract. Although the antioxidant activity of TCCEA was higher than TCLEA, the total amount of phenolic and flavonoid compounds was lower than TCLEA (although there was no statistically significant difference). This indicates that the activity of TCCEA may be due to not only phenolic compounds found in the extract but also non-phenolic compounds such as terpenoid compounds. It supports this view in a previous study conducted by Ali et al. In this study, the sesquiterpene and triterpene compounds were isolated from the ethyl acetate extract of a different *Tanacetum* species, *Tanacetum abrotonifolium* [28].

Polar solvents are more effective in obtaining phenolic compounds from plants compared to less polar solvents [26]. Less polar solvents such as hexane and  $\text{CHCl}_3$ , carry non-polar and non-phenolic compounds such as terpenoids and methoxylated flavonoids [26,29-30]. For example, in our current study, polar extracts as EtOAc and MeOH extracts had higher total phenolic and flavonoid content than hexane and  $\text{CHCl}_3$  extracts. The results of present study are similar to those of See et al. (2017).

No study has been found about anti-inflammatory activity of the plant in literature. However, some parts of *Tanacetum* species traditionally have been used by the people for anti-inflammatory purposes [31] and there are some studies regarding anti-inflammatory properties

of other *Tanacetum* species [13]. In our current study, it was found that especially the TCCEA had significant anti-inflammatory activity with IC<sub>50</sub> value of 9.44 µg/mL. In addition, it has been found that it has a higher anti-lipoxygenase activity than indomethacin (22.39 µg/mL) which is used as standard. These results confirm ethnobotanical use of the *Tanacetum* species. Also, flavonoids and sesquiterpene lactones are known as important compounds with anti-inflammatory activity [32]. Therefore, the reason why TCCEA exhibits good anti-inflammatory activity may be due to the presence of high phenolic compounds.

## 5. CONCLUSION

These results show that ethyl acetate extract from capitula of *Tanacetum cilicicum* has a strong anti-inflammatory and antioxidant potency. In addition to the *in vivo* experiments that will be carried out on TCCEA, it is clear that more scientific studies are needed to elicit compounds that are responsible for antioxidant and anti-inflammatory effect.

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