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Total phenolic content, cyclooxygenases, α-glucosidase, acetylcholinesterase, tyrosinase inhibitory and DPPH radical scavenging effects of *Cornus sanguinea* leaves and fruits

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ABSTRACT: The aim of the present study was to investigate total phenolic content and biological effects of methanol extracts from *Cornus sanguinea* L. leaves (LME) and fruits (FME). Total phenolic contents, COX-1/COX-2, α-glucosidase, AChE, tyrosinase inhibitory and DPPH radical scavenging effects of both extracts were investigated by using spectrophotometric methods. The total phenolic contents of LME and FME were determined as 191.14 ± 4.84 and 31.51 ± 2.68 mg GAE/g dry weight, respectively. LME inhibited COX-1 enzyme 70.71 ± 1.88% and 79.38 ± 0.92% at 50 and 100 µg/mL. LME had higher COX-1 and COX-2 inhibitory effects than that of FME. LME inhibited α-glucosidase stronger than positive control, acarbose. On the other hand, both extracts showed lower AChE inhibition actions compared to positive control, galantamine. Moreover, LME had higher tyrosinase inhibitory effect than FME. Both extracts scavenged DPPH radical in a concentration-dependent manner. Also, LME had stronger scavenging effect than that of FME. To our knowledge, current work is the first report on tyrosinase, AChE, as well as COX-1 inhibitory properties of *C. sanguinea.* These results suggested that LME of *C. sanguinea* have a promising potential for the treatment of several disorders but further studies are needed to support the this assumption.

KEYWORDS: Acetylcholinesterase; antioxidant; cyclooxygenase; *Cornus sanguinea*; α-glucosidase; tyrosinase.

1. INTRODUCTION

The species of *Cornus* have significant biological effects due to the presence of ascorbic acid, phenolic compounds, flavonoids, anthocyanins [1]. Their leaves and fruits have antioxidant, antimicrobial, anti-inflammatory, cytoprotective, antidiabetic effects [2]. The genus *Cornus* is represented by two species in Turkey. *Cornus sanguinea* L. (Cornaceae), is a small tree with purplish-black drupe fruits [3]. An infusion prepared from *C. sanguinea* barks is used as an astringent and it is applied topically to sore eyes in Serbia [4]. *C. sanguninea* fruits are consumed as food and for medicinal purposes in Turkey. They are eaten as jam and also are used the treatment of diarrhea [5,6]. Fruits and leaves of the plant are rich in phenolic compounds [2,7]. Presence of some quercetin derivatives, including quercetin-3-*O*-glucuronide, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-rhamnoside and quercetin-3-*O*-rutinoside in fruits determined by LC-MS/MS [8]. An ethanol extract of leaves, flowers and stems (2:1:1) of *C. sanguinea* inhibited methicillin resistant *Staphylococcus aureus* and *Candida albicans* with MIC values of 8 and 12.6 mg/ml [9].

Inflammation is a basic defense mechanism against injury or infection and characterized by many symptoms such as redness, heat, pain, and swelling [10]. The main metabolic process in the inflammatory is the arachidonic acid (AA) pathway. In this pathway, the cyclooxygenase (COX) enzymes form prostaglandins which are responsible for the pain and associated with process of inflammation [11]. Its processes are involved in severe degenerative disorders including rheumatoid arthritis, cardiovascular disease, asthma, diabetes, epilepsy, Alzheimer's disease, neurotoxicity etc. [12].

Diabetes mellitus (DM), is one of the degenerative diseases, cause to severe complications such as diabetic nephropathy, neuropathy, and cardiovascular diseases etc. α-Glucosidase enzyme is a key role for the hydrolysis and absorption of carbohydrates [13]. Its inhibitors are used for the treatment of DM since

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they regulate postprandial blood glucose and insulin levels [14]. In recent years, researchers have been looking for new, natural, and with less side effects α-glucosidase inhibitors against DM due to the gastrointestinal side effects of drugs used in the clinic.

Alzheimer disease (AD) is one of the disorders that cause decreased cognitive functions and memory loss [15]. Acetylcholinesterase (AChE) catalyzes the hydrolysis of the neurotransmitter acetylcholine to choline and acetic acid [16]. Improving cholinergic function by inhibition of AChE is an important method for the symptomatic treatment of AD. Cholinesterase inhibitors increase cholinergic transmission by inhibiting the AChE [17].

Tyrosinase is widely found in plant, animals, fungi and bacteria and catalyzes by oxidizing monophenols to diphenols and diphenols to quinones and finally quinones generate melanine which prevent ultraviolet damage from sunlight on the skin [18]. However, the excessive generation of melanin triggers several disorders such as melanoma, age spots, neurodegenerative disesases etc [19]. In addition, tyrosinase causes degradation of nutritional values due to browning problems for fruits and vegetables [19]. So, tyrosinase inhibitors have been extremely interesting in the cosmetic and pharmaceutical as well as food industry in recent years.

The aim of present study was to investigate total phenolic content and biological effects of methanol extracts from *C. sanguinea* leaves (LME) and fruits (FME).

2. RESULTS

2.1. Estimation of total phenolic content

The total phenolic contents of LME and FME were determined as 191.14 ± 4.84 mg GAE/g dry weight, 31.51 ± 2.68 mg GAE/g dry weight, respectively.

2.2. COX-1 and COX-2 inhibitory effects of LME and FME

The *in vitro* COX-1 and COX-2 inhibitory effects of LME and FME were presented in Figures 1 and 2. The obtained results were expressed as percent of inhibition (%) and IC_{50} values. As shown in Figure 1, LME inhibited COX-1 enzyme with 45.31 ± 1.49%, 62.80 ± 2.53%, 70.71 ± 1.88% and 79.38 ± 0.92% at 12.5, 25, 50 and 100 µg/ml. It showed significant COX-1 inhibitory effect when compared to diclofenac at 25 and 100 µg/ml ($p < 0.01$). In addition, the IC_{50} value of LME (13.60 ± 2.04 µg/ml) was lower than diclofenac (17.55 ± 0.91 µg/ml). On the other hand, FME had low inhibitory properties against COX-1. In COX-2 inhibition assay, At 100 µg/ml, LME displayed significant inhibition as compared to celecoxib ($p < 0.001$). The IC_{50} value of LME was determined as 11.39 ± 2.39 µg/ml on COX-2.

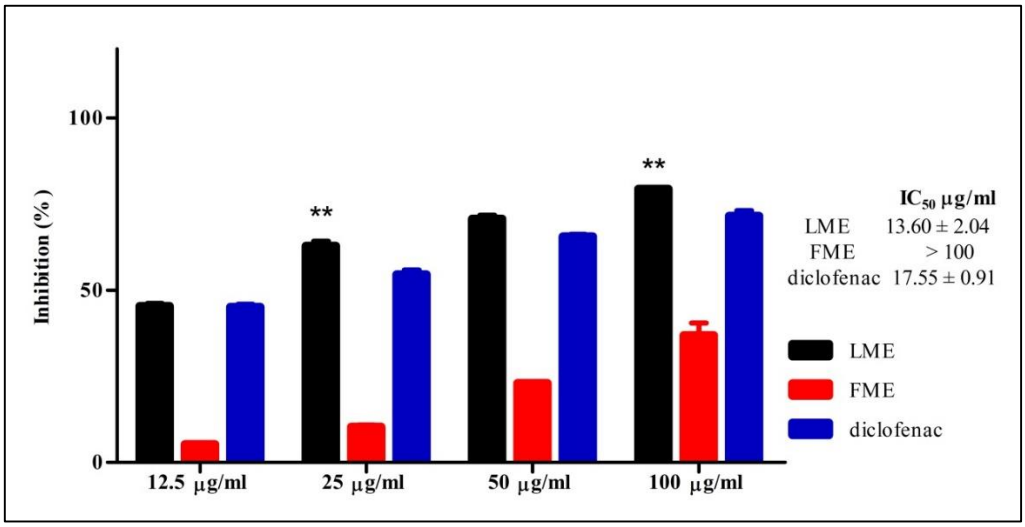

Figure 1. Inhibitory effects (%) of LME and FME on COX-1. **p < 0.01 comparing positive control at same concentrations.

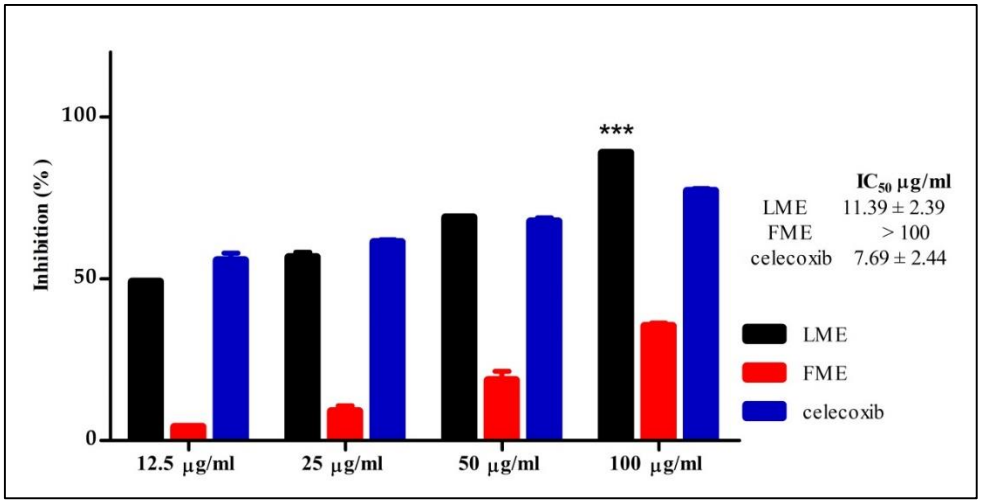

Figure 2. Inhibitory effects (%) of LME and FME on COX-2. ***p < 0.001 comparing positive control at same concentrations.

2.3. α-Glucosidase inhibitory effects of LME and FME

The *in vitro* α-glucosidase inhibitory properties of the extracts were given in Table 1. The obtained results were expressed as percent inhibition (%) and IC_{50} values. LME inhibited α-glucosidase in a concentration-dependent manner as shown in Table 1. At 25, 50 and 100 µg/ml, LME had significant inhibition actions when compared to acarbose ($p < 0.001$). The IC_{50} value of LME was determined as 19.14 ± 0.21 µg/ml. On the other hand, FME did not show any inhibitory action at studied concentrations.

Table 1. Inhibitory effects (%) of LME and FME on α-glucosidase.

	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	IC_{50} µg/ml
LME	23.57 ± 4.24	74.81 ± 5.03***	86.09 ± 0.45***	88.76 ± 0.06***	19.14 ± 0.21
FME	nd	nd	nd	nd	nd
Acarbose	20.64 ± 2.53	39.78 ± 0.53	47.85 ± 1.41	62.85 ± 1.41	51.24 ± 1.27

***p < 0.001 comparing positive control at same concentrations.
nd: not determined.

2.4. AChE inhibitory effects of LME and FME

The *in vitro* AChE inhibitory potentials of the extracts were given in Table 2 and the obtained results were expressed as percent inhibition (%) and IC_{50} values. As given in Table 2, LME showed inhibitory effects on AChE with 16.84 ± 2.55%, 25.54 ± 1.47%, 36.71 ± 0.55% and 50.89 ± 1.49% at 12.5, 25, 50 and 100 µg/ml. While FME did not display inhibition on AChE at 12.5 and 25 µg/ml, it showed inhibitory effects with 11.59 ± 1.51% and 24.58 ± 1.49% at 50 and 100 µg/ml. The IC_{50} values of LME and FME were 93.64 ± 2.98 and > 100 µg/ml, respectively. The results showed that both extracts showed lower inhibitory effects when compared to galantamine which used as a positive control.

Table 2. Inhibitory effects (%) of LME and FME against AChE.

	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	IC_{50} µg/ml
LME	16.84 ± 2.55	25.54 ± 1.47	36.71 ± 0.55	50.89 ± 1.49	93.64 ± 2.98
FME	nd	nd	11.59 ± 1.51	24.58 ± 1.49	> 100
Galantamine	76.52 ± 2.12	85.44 ± 1.59	90.96 ± 0.34	92.46 ± 1.99	< 12.50

nd: not determined

2.5. Tyrosinase inhibitory effects of LME and FME

The *in vitro* tyrosinase inhibitory effects of the both extracts were presented in Figure 3 and the obtained results were expressed as percent of inhibition (%) and IC_{50} values. LME had higher inhibition action than that of FME but kojic acid showed stronger inhibitory effect than both extracts at studied concentrations. At 12.5, 25, 50 and 100 µg/ml, both extracts inhibited tyrosinase with 12.97 ± 0.61%, 36.42 ± 0.80%, 60.00 ± 2.51% and 66.49 ± 0.38% for LME and 9.23 ± 1.72%, 23.95 ± 0.42%, 49.50 ± 2.81% and 82.49 ± 1.32% for FME, respectively. The IC_{50} values of LME and FME were 44.45 ± 3.31 and 65.06 ± 2.41 µg/ml, respectively.

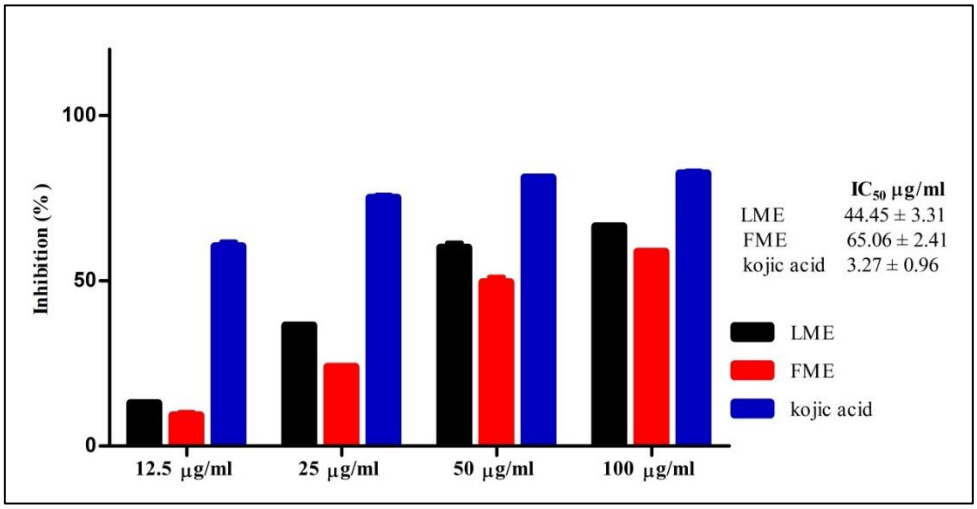

Figure 3. Inhibitory effects (%) of LME and FME on tyrosinase.

2.6. DPPH radical scavenging effects of LME and FME

The DPPH radical scavenging effects of the both extracts were shown in Table 3. The obtained results were expressed as percent of inhibition (%) and IC_{50} values. Both extracts scavenged DPPH radical in a concentration-dependent manner as presented in Table 3. At 50 and 100 µg/ml, LME had remarkable radical scavenging actions with 75.76 ± 2.56% and 92.34 ± 0.87%, respectively. The IC_{50} values of LME was 27.27 ±1.30 µg/ml. In addition, LME had stronger scavenging effects that of FME at studied concentrations.

Table 3. DPPH radical scavenging effects (%) of LME and FME.

	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	IC_{50} µg/ml
LME	23.52 ± 0.22	44.14 ± 3.10	75.76 ± 2.56	92.34 ± 0.87	27.27 ± 1.30
FME	3.79 ± 2.57	8.05 ± 4.00	16.10 ± 3.71	29.51 ± 3.32	> 100
Gallic acid	89.78 ± 0.64	91.76 ± 0.38	92.19 ± 0.35	92.92 ± 0.74	< 12.50

3. DISCUSSION

Plants and their extracts have been used for treatment of many disorders. Stankovic and Topuzovic reported that the species of the genus *Cornus* are used in veterinary medicine, pharmacy, and traditional medicine since they have rich in phenolic compounds and show various biological activities such as antioxidant, anti-inflammatory, cytoprotective, and antidiabetic etc. [2]. To the best of our knowledge, tyrosinase, AChE, as weel as COX-1 inhibitory properties of the plant investigated for the first time by current work. In this study, the total phenolic contents of LME and FME were found to be 191.14 ± 4.84 mg GAE/g dry weight, 31.51 ± 2.68 mg GAE/g dry weight, respectively. LME had higher total phenolic content than that of FME about six-fold. Stankovic and Topuzovic reported that the total phenolic and flavonoid contents in the methanol extracts of *C. sanguinea* leaves and fruits were determined as 205.74 ± 0.49 mg GA/g extract and 34.19 ± 0.25 mg GA/g extract, 23.24 ± 0.16 mg rutin/g extract, 14.40 ± 0.09 mg rutin/g extract, respectively [2]. Their results showed that *C. sanguniea* leaves contain more phenolics and flavonoids

compared to the fruits. Therefore, it is thought that LME showed higher activity than FME for *in vitro* biological activity studies that we used. In this study, we investigated *in vitro* COX-1 and COX-2 inhibitory effects of LME and FME by using spectrophotometric method. The obtained results demonstrated that LME had higher COX-1 and COX-2 inhibitory actions than FME and showed significant COX-1 inhibitory effect when compared to diclofenac at 25 and 100 µg/ml ($p < 0.01$). For COX-2, LME showed significant inhibition as compared to celecoxib at 100 µg/ml ($p < 0.001$). Popovic et al. reported that methanol extract of *C. sanguinea* fresh fruit were analyzed using LC-MS/MS and determined contents of neochlorogenic acid, quercetin-3-*O*-glucuronide, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-rhamnoside, and quercetin-3-*O*-rutinoside [20]. Kim et al. reported that neochlorogenic acid, a natural polyphenolic compound found in dried fruits, suppresses COX-2 protein expression on murine microglial BV2 cells [21]. Mandour et al. reported that quercetin-3-*O*-glucuronide inhibited COX-1 and COX-2 enyzmes with 37.60% and 60.00% at 25 µg/ml and quercetin-3-*O*-galactoside showed COX-1 and COX-2 with 39.20% and 60.70% at same concentration [22]. In another study, Comolada et al. reported that quercetin-3-*O*-rhamnoside showed benefical effects of *in vivo* model of intestinal inflammation [23]. Ning et al. reported that quercetin-3-*O*-glucuronide showed inhibitory effect against α-glucosidase enzyme [24]. As shown in Table 1, LME inhibited α-glucosidase in a concentration-dependent manner and it had significant inhibitory effect compared to acarbose at 25, 50 and 100 µg/ml ($p < 0.001$). On the other hand, FME displayed no α-glucosidase inhibitory effects at studied concentrations. Suprisingly, α-glucosidase activity increased in the presence of FME at 12.5, 25, 50 and 100 µg/ml. On the contrary of our work, Truba et al. reported that aqueous-ethanolic extract of *C. sanguinea* fruit exhibited inhibitory effect against α-glucosidase enzyme and the IC_{50} value of it was determined as 70.07 ± 16.62 µg/ml [25]. In this study, the results of *in vitro* AChE inhibitory potentials of the extracts showed that LME showed higher inhibitory effects that of FME but it had lower inhibitory action than galantamine. To the best of our knowledge, there was not any report on the cholinesterase inhibitory effect of *C. sanguinea* but there are few studies about the same activitiy of genus *Cornus* so far. Bhakta et al. isolated seven compounds from the fruits of *Cornus officinalis*, cornuside, 1,2,3-tri-*O*-galloyl-*β*-D-glucose, 1,2,3,6-tetra-*O*-galloyl-*β*-D-glucose, tellimagradin I, tellimagrandin II, and isoterchebin and investigated AChE inhibitory effects of them using Ellman's method [26]. The IC_{50} values of cornuside, 1,2,3-tri-*O*-galloyl-*β*-D-glucose, 1,2,3,6-tetra-*O*-galloyl-*β*-D-glucose, tellimagradin I, tellimagrandin II, and isoterchebin were calculated as >100 µM, >100 µM, 85.64 ± 0.03 µM, 87.52 ± 0.32 µM, 11.86 ± 0.56 µM, 47.55 ± 0.54 µM, respectively. In addition, tellimagradin I, tellimagrandin II, and isoterchebin inhibited AChE via mixed, competitive, and mixed inhibitory type [26]. The tyrosinase inhibitory effects of the both extracts were presented in Figure 3 and LME had higher inhibitory action that of FME but kojic acid showed stronger inhibitory effects than both extracts. These obtained results showed compatibility with other inhibitory activities. An et al. reported the tyrosinase effects of methanol extract of *Cornus officinalis* fruits on melan-a cells. Their results showed that methanol extract upregulated tyrosinase activitiy on this cell line [27]. DPPH radical scavenging assay is the common technique to evaluate antioxidant effect due to being a cost-effective, fast and sensitive method [28]. The obtained results displayed that both extracts scavenged DPPH radical in a concentration-dependent manner. LME had remarkable radical scavenging action at 50 and 100 µg/ml and it had stronger scavenging effect than FME. Stankovic and Topuzovic reported that IC_{50} values of DPPH radical scavenging effects of methanol extracts of *C. sanguinea* leaves and fruits collected from Serbia were 19.84 ± 0.11 and 358.59 ± 1.14 µg/ml. Their results showed that leaves have higher antioxidant activity when compared to fruits [2]. Yousfbeyk et al. reported that IC_{50} value of methanol (1%HCl) extract from *C. sanguinea* in Iran was found to be 90.43 µg/ml [7]. The results showed that enzyme inhibitory and antioxidant effects depend on the polarity of solvent, plant part, extraction method etc. However, our results were consistent with data given in literature.

4. CONCLUSION

In this study, we investigated total phenolic content, COX-1/COX-2, α-glucosidase, AChE, tyrosinase inhibitory and DPPH radical scavenging effects of LME and FME obtained *C. sanguinea* from Turkey. The total phenolic contents of LME and FME were found to be 191.14 ± 4.84 and 31.51 ± 2.68 mg GAE/g dry weight, respectively. LME had higher total phenolic content than that of FME about six-fold. LME inhibited COX-1 enzyme with 70.71 ± 1.88% and 79.38 ± 0.92% at 50 and 100 µg/ml. It showed significant COX-1 inhibitory effect when compared to diclofenac at 25 and 100 µg/ml. LME had higher COX-2 inhibitory effects than that of FME. LME inhibited α-glucosidase in a concentration-dependent manner and it had significant inhibition actions as compared to acarbose. Both extracts showed lower AChE inhibition effects as

compared to galantamine. In tyrosinase inhibition assay, LME had higher inhibition action than that of FME but kojic acid showed stronger inhibitory effects than both extracts. Both extracts scavenged DPPH radical in a concentration-dependent manner. In addition, LME had stronger scavenging effects that of FME at studied concentrations. These results suggested that LME of *C. sanguinea* have a promising potential for the treatment of several degenerative disorders such as including rheumatoid arthritis, diabetes, Alzheimer's disease, and Parkinson's disease etc but *in vivo* researchs are needed to support the therapeutic actions.

5. MATERIALS AND METHODS

5.1. Plant material

C. sanguinea was collected from Çamiçi plateau, Niksar, Tokat (40°, 38', 52'' N, 36°, 50', 55'' E) on 28 Agust 2019. The plant material was collected and identified by Assoc. Prof. Dr. Didem Şöhretoğlu. A voucher specimen was deposited in Herbaryum of Hacettepe University, Faculty of Pharmacy (HUEF 19072).

5.2. Extraction

The shade-dried leaves (3 g) were macerated with MeOH (50 ml) at room temperature for overnight and then extracted at 40 °C for 8 h. The same process repeated 3 times, and the extracts were combined. The concentrated MeOH extract was suspended in H_2O and lyophilized (0.38 g). Fruits are kept -20 °C then the same extraction procedure was applied to 3 g fruits. After this procedure, 0.65 g extract was obtained.

5.3. Estimation of total phenolic content

The total phenolic content of LME and FME was determined by using the Folin-Ciocalteu reagent according to method by Kahkönen [29]. The extracts and diluted Folin-Ciocalteu reagent (Sigma-Aldrich, 47641), were placed in each well of a 96 well plate. After then sodium carbonate was added. The mixtures were incubated for 30 min in the dark. Afterwards, the absorbance was measured at 760 nm. The total phenolic contents of LME and FME were expressed as mg gallic acid equivalents (GAE) per g of dry weight of extract.

5.4. Biological studies

5.4.1. COX-1 and COX-2 inhibition assay

The COX-1 and COX-2 inhibitory effects of LME and FME were carried out by using spectrophotometric methods [30]. Diclofenac and celecoxib were used positive controls for COX-1 and COX-2, methanol (%1) as blank. COX-1 from sheep (Sigma-Aldrich, C0733) and COX-2 from human (Sigma-Aldrich, C0858) were treated the extracts (12.5-100 µg/ml) in 96-well plate and incubated for 5 min. After incubation, endpoint assay mix (Tris.Cl pH 8.1 (1 mM), hemin (100 µM), N,N,N′,N′-tetramethyl-p-phenylenediamine (17 mM), arachidonic acid (10 mM)) (Sigma-Aldrich) was added and reaction mixture was incubated for 15 min. The absorbance was measured at 611 nm using microplate reader. The COX-1 and COX-2 inhibition (%) and IC_{50} values (µg/ml) of samples was determined as follows [Eq. 1]:

$$\text{Inhibition (\%)}=[((A_{control}) - A_{extract}))/A_{control}]\times 100 \quad \text{[Eq. 1]}$$

5.4.2. α-Glucosidase inhibition assay

The α-glucosidase inhibitory effect was investigated according to our previous study with minor modifications [31]. Acarbose was used as a positive control, whereas methanol (1%) was used as blank. α-Glucosidase from *Saccharomyces cerevisiae* (Sigma-Aldrich, G5003) was treated with the extracts (12.5-100 µg/ml) in 96-well plate and incubated for 10 min. After incubation, *p*-nitrophenyl-α-glucopyranoside (5 mM) (Sigma-Aldrich, N1377) was added to reaction mixture and incubated for 15 min at room temperature. The absorbance was measured at 405 nm using microplate reader. The α-glucosidase inhibition (%) and IC_{50} values (µg/ml) of samples was determined by using equation mentioned above.

5.4.3. AChE inhibition assay

The inhibitory properties of the extracts on AChE were determined according to our previous study with minor modifications [32]. Galantamine was used as a positive control, whereas methanol (1%) was used

as blank. AChE from Electrophorus electricus (Sigma-Aldrich, C3389) were treated with the extracts (12.5-100 µg/ml) in 96-well plate and incubated for 10 min. After incubation, Tris.Cl buffer pH 8 (50 mM), 5,5-dithio-bis(2-nitrobenzoic)acid (3 mM) (Sigma-Aldrich, D8130) were added to mixtures and incubated for 15 min at room temperature. After incubation, acetylthiocholine iodide (Sigma-Aldrich, A5751) was added as a substrate to start enzymatic reaction. The absorbance was measured at 412 nm using microplate reader. The AChE inhibition (%) and IC_{50} values (µg/ml) of samples was determined by using equation mentioned above.

5.4.4. Tyrosinase inhibition assay

The inhibitory properties of the extracts on tyrosinase were investigated according to our previous study with minor modifications [19]. Kojic acid was used as a positive control and methanol (1%) was used as blank. Tyrosinase from mushroom (Sigma-Aldrich, T3824) in phosphate buffer pH 6.8 (100 mM) were treated with the extracts (12.5-100 µg/ml) in 96-well plate and incubated for 10 min. Afterwards, 3,4-dihydroxy-L-phenylalanine (3 mM) (Sigma-Aldrich, D9628) was added to mixtures and incubated for 15 min at room temperature. The absorbance was measured at 475 nm using microplate reader. The tyrosinase inhibition (%) and IC_{50} values (µg/ml) of samples was determined by using equation mentioned above.

5.4.5. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical scavenging effects of the extracts were carried out according to our previous study with slight modification [33]. Gallic acid was used as a positive control and methanol (1%) was used as blank. DPPH solution (0.2 mM) (Sigma-Aldrich, D9132) was added the extracts (12.5-100 µg/ml) in 96-well plate and incubated for 30 min. The absorbance was measured at 517 nm using microplate reader. The DPPH radical scavenging effects (%) and IC_{50} values (µg/ml) of samples were determined by using equation mentioned above.

5.5. Statistical analysis

The data were analyzed using GraphPad Prism 5.0 and data were expressed as the mean ± SD. Statistical analysis was investigated with two-way analysis of variance (ANOVA) followed by Bonferroni tests.

Author contributions: Concept – B.B., D.Ş.; Design – B.B., D.Ş.,; Supervision – D.Ş.; Resources – B.B., D.Ş.; Materials – B.B.; Data Collection and/or Processing – B.B., D.Ş.; Analysis and/or Interpretation – B.B., D.Ş.,; Literature Search – B.B., D.Ş.,; Writing – B.B.; D.Ş.; Critical Reviews – B.B., D.Ş.

Conflict of interest statement: The authors declared no conflict of interest.

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