Anticholinergic, anti-diabetic and antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts: polyphenol contents analysis by LC-MS/MS

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ABSTRACT

Ethanolic (EEC) and aqueous (WEC) extracts of cinnamon (*Cinnamomum verum*) were evaluated for their antioxidant profiles by eight distinguished bioanalytical antioxidant methods. Their inhibitory effects were tested against some enzymes including acetylcholinesterase, butyrylcholinesterase, α-glycosidase and α-amylase, which linked to different diseases. Additionally, the antioxidant properties were determined and polyphenolic compositions of the both extracts were evaluated by LC-MS/MS analysis. According to the LC-MS/MS experiments, thirteen compounds were found in WEC and EEC. Also, p-hydroxybenzoic acid (321.1 mg/kg extract), p-coumaric acid (291.4 mg/kg extract), and pyrogallol (142.4 mg/kg extract) were found to be the most abundant ingredients in the WEC. On the other hand, pyrogallol (264.3 mg/kg extract), ferulic acid (224.7 mg/kg extract) and p-coumaric acid (170.2 mg/kg extract) were found as the most plentiful chemicals in the EEC. For the estimation of the antioxidant capacities of the both extracts (WEC and EEC), DPPH· and ABTS⁺ scavenging activities, as well as Fe³⁺-Fe²⁺ and Cu²⁺-Cu⁺ reducing assays were studied. The IC₅₀ values of the WEC and EEC indicated that they were potent effective DPPH· (21.25 and 15.71 μg/mL) and ABTS⁺ (6.52 and 5.79 μg/mL) scavengers, as well as AChE (221.33 and 110.26 μg/mL), BChE (461.69 and 94.93 μg/mL), α-glycosidase (206.86 and 220.00 μg/mL) and α-amylase (189.86 and 200.86 μg/mL) inhibitors. As a conclusion, both EEC and WEC had rich phenolic contents and demonstrated effective anticholinergic, antidiabetic and antioxidant effects.

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Introduction

Cinnamon (*Cinnamomum verum*) is predominantly employed in cuisine as a condiment and flavoring material. It has been widely used in traditional folk medicine and cuisine for a long time. It belongs to the Lauraceae family and possesses significant biological activities including antimicrobial, antifungal, antiviral, antiallergic, antitumor, antihyperlipidemic, antidiabetic, antipyretic, antiluetic, antihypertensive, gastroprotective, and immunomodulatory and anesthetic effects.[1,2] Also, it is used for several conditions...
such as flatulence, diarrhea, amenorrhea, toothache, ever, leucorrhea, common cold and headache. \[2\] The bark yields an essential oil containing cinnamaldehyde and eugenol. Several biological activities such as peripheral vasodilatory, antitumor, antifungal, cytotoxic and antimutagenic activities have been attributed to cinnamaldehyde.\[3\] Plants are potential sources for the improvement of novel products in foods, cosmetics, and pharmaceuticals.\[4\–\6\] Recently, biologically active compounds of plants have proven to be desirable post natural agents. Medicinal plants have a crucial role in basic healthcare and the cosmetic industry in many developing and developed countries.\[7\–\9\] They contain significant amounts of bioactive compounds and have organoleptic, as well as biochemical properties.\[10\,\,11\] Turkey possesses rich plant biodiversity for use in foods, cosmetics, and the pharmaceutical industry.\[11\,\,12\] Additionally, secondary metabolites of plants have had extensive usage in traditional medicine due to their well-established potential pharmaceutical and biological properties. These metabolites have preventive roles in plants and demonstrate different biological and pharmaceutical properties with health benefit effects.\[13\–\15\] Biologically active secondary metabolites including phenolics have been isolated and used for the treatment of some health disorders. Recently, there have been growing demands and many studies for the validation, production and utilization of herbal secondary metabolites in medicines to treat many diseases.\[12\,\,16\]

Reactive oxygen species (ROS) are described as short-lived molecules, ions and radicals. Their half-lives differ from nanoseconds to hours.\[17\,\,18\] They occur in many chemical reactions and during some biological processes such as the respiration and electron transport chain.\[19\–\21\] Increased ROS levels can provoke oxidative stress and lead to hazardous cellular and molecular damages. As a result of this, various types of diseases such as neurological disorders, lung diseases, cardiovascular diseases, cancer and inflammation can occur in living systems.\[22\–\24\] Recently, oxidative stress and ROS have been accepted as important environmental risks for different types of chronic disorders such as cancer, immunodeficiency syndrome, age-related pathologies, cardiovascular diseases, arteriosclerosis, diabetes, and obesity.\[25\,\,26\] ROS occur in living organisms during normal cellular metabolism and can be harmful to decisive biomolecules including proteins, nucleic acids lipids and carbohydrates.\[27\–\30\] The antioxidant defense system includes antioxidant components and antioxidant enzymes.\[31\,\,32\] In terms of pharmaceutical products, they can easily scavenge ROS and reduce the lipid autoxidation of foods and pharmaceutical products during production and storage processes.\[33\,\,34\] In terms of food products, antioxidants are described as molecules that prevent oxidation in low quantities or concentrations. Additionally, they easily delay or inhibit the oxidation of substrates.\[22\,\,35\] Therefore, attention has recently turned to research for effective antioxidants including phenolic compounds from natural and accessible resources.\[36\,\,37\] Plants include many biological active phytochemicals such as phenols and polyphenols that possess structural features that have antioxidant activities.\[38\–\40\] Thus, there are growing demands for safer natural antioxidants from plant origins for pharmaceutical and food applications.\[41\] Phenolics are active secondary metabolites that scavenge ROS and eliminate oxidative damage.\[42\] Their biological effects including the antioxidant ability of the phenols from medicinal plants make them crucial products for their protective effects against some degenerative disorders including diabetes, cancer, arteriosclerosis, hypercholesterolemia and cardiovascular diseases.\[43\–\46\] Also, there has been a general desire to replace synthetic food additives with natural antioxidants. Therefore, intensive researches are being carried out on the extraction, characterization and utilization of natural antioxidants from plant and food sources.\[47\] The natural antioxidants are primarily plant phenolics that may occur in all parts of the plants such as barks, fruits, nuts, vegetables, seeds, leaves and roots.\[48\] Plant phenolics are multifunctional effects and act as reducing agents, metal chelators and singlet oxygen quenchers. Phenolic compounds exist in spices, which show antioxidant properties, have been studied for substitution of synthetic antioxidants, due to possible side effects of synthetic antioxidants, which may in some circumstances act deleterious to living organisms.\[49\] Antioxidants can interfere with oxidation by reacting with free radicals, chelating metals and also by acting as ROS scavengers.\[50\]

Alzheimer’s disease (AD) generally affects the memory and behavior of elderly people worldwide. This neurological disease clinically includes the growing degeneration of brain tissue, which is effected by an acetylcholine (ACh) deficiency.\[51\,\,52\] The acetylcholinesterase (AChE), as a component in the nervous
systems, converts acetylcholine (ACh) to choline and (Ch) acetate.\cite{52,53} It was reported that the reduced levels of Ach in the hippocampus and cortex had great biochemical changes in patients with AD.\cite{54} Natural substances such as AChEIs (AChE inhibitors) were commonly used in clinical trials, especially for AD treatment. Phenolics have also been identified as AChEIs and provide pioneering molecules for AD treatment.\cite{55,56}

Diabetes mellitus (DM) is a metabolic disease that is hallmarked by abnormal levels of glucose in the blood, as well as some disorders including neuropathy, retinopathy, cardiovascular diseases, atherosclerosis, and neural damage.\cite{57} It was reported that oxidative stress and ROS are major mediators of DM. In the case of hyperglycemia, the excessive glucose loading triggers ROS generation in mitochondria. In this case, the mitochondrial functions are impaired.\cite{58} Digestive enzymes hydrolyze polysaccharides into monosaccharide units. Thus, digestive enzyme inhibition had a crucial therapeutic route for the treatment of DM.\cite{59,60} α-Amylase and α-glycosidase enzymes were released from small intestine cells. Both digestive enzymes hydrolyze oligosaccharide and polysaccharide molecules to monosaccharides such as glucose.\cite{61} In humans, digestive enzyme inhibitors are very important for controlling diabetes and hyperglycemia.\cite{60} Digestive enzyme inhibitors can decrease the absorption of carbohydrates and suppress postprandial glucose levels, hyperglycemia and Type-2 diabetes mellitus (T2DM). Therefore, these inhibitors compete with the oligosaccharides to bind to the active site of digestive enzymes. Thus, they efficiently reduce the postprandial monosaccharide units such as glucose in T2DM.\cite{62,63}

The main goal of this study is to determine the antioxidant activity of ethanolic (EEC) and aqueous (WEC) extracts of cinnamon (Cinnamomum verum) using by distinct bioanalytical methods including the cupric (Cu$^{2+}$) and ferric (Fe$^{3+}$) ions reducing abilities, as well as ABTS$^{\cdot+}$, DPPH$^{-}$, and DMPD$^{\cdot+}$ scavenging activities. Also, LC-MS/MS analysis was used for determination of the phenolic profiles of tested for both extracts. Furthermore, another significant aim of this study is to demonstrate the inhibitory abilities of the WEC and EEC against the acetylcholinesterase, butyrylcholinesterase, α-amylase and α-glycosidase enzymes, which are linked to global and common health diseases.

**Materials and methods**

**Chemicals and plant materials**

Neocuproine, ABTS, BHA, DPPH-, BHT, α-tocopherol, and trolox were purchased from Sigma-Aldrich (Germany). The other compounds were used for analytical grade and were obtained from either Merck or Sigma-Aldrich. Dried cinnamon (Cinnamomum verum) was obtained from a local market at Erzurum, Turkey.

**Preparation of the water and ethanol extracts**

The lyophilized aqueous extraction of cinnamon (Cinnamomum verum) (WEC) was performed according the previous method.\cite{64} For this purpose, 30 g dried cinnamon barks was powdered, mixed with boiling water (0.5 L) and stirred for 30 min. The water extract was double-filtered over cheesecloth and Whatman paper (No.1). The residue was frozen in a freezer at −84°C (Sanyo, Japan). Finally, the frozen extract was lyophilized in a lyophilizator (50°C, 5 mm-Hg). Then, the dried WEC was transferred to a bottle and stored until use (−20°C).

Ethanol extraction of cinnamon (Cinnamomum verum) (EEC) was realized according to previous studies.\cite{65} For this purpose, 30 g plant cinnamon barks was powdered and mixed with ethanol (500 mL). Then, the extracted sample was filtered through paper (Whatman No.1) and evaporated at 40°C.\cite{66} The residue of the plant was re-extracted under similar extraction conditions until the ethanol became colorless. Finally, the extract was evaporated in an evaporator. Then, the dried EEC was transferred to a bottle and then stored until use (−20°C).
**Total phenolic and flavonoid contents**

The quantification of the total phenolics in both extracts of WEC and EEC was performed according to the Folin-Ciocalteau method.\(^{[67]}\) The appropriate 0.5 mL of diluted sample was transferred to Folin-Ciocalteau reagent (1.0 mL). Then, the solution was vigorously mixed and neutralized with carbonate (0.5 mL, 1%). After 2 h of incubation in the dark at r.t., the absorbance was recorded at 725 nm. The phenolic contents were determined as milligram of gallic acid equivalents (GAE) per gram of WEC and EEC.

The total flavonoids in the both extracts were calculated according to aluminum chloride (AlCl\(_3\)) method.\(^{[68]}\) Briefly, both extraction solutions (0.5 mL) were mixed with ethanol (1.5 mL, 95%), the same volume of AlCl\(_3\) (10%, 1.5 mL), potassium acetate solution (1.0 M, 0.5 mL) and distilled deionized water (2.3 mL). After incubation (25°C, 30 min), the absorbance was measured at 415 nm. Total flavonoids were determined as milligram equivalents of quercetin (QE) per gram of WEC and EEC.

**LC-MS/MS analysis**

During the experiments, the samples were kept at 15°C in the autosampler. Additionally, the chromatographic conditions, instrumental procedure optimization, linearity, repeatability, recovery, precision, limits of quantification (LOQ) and determination (LOD), identification of uncertainty sources, and identification of standard uncertainties were performed according to our previous study (Table 1).\(^{[69]}\)

**Reducing ability assays**

The Fe\(^{3+}\) reducing capacities of WEC and EEC was determined according to Oyaizu’s method\(^{[70]}\) with minor revisions, as described previously.\(^{[71,72]}\) Briefly, different concentrations of the WEC and EEC in distilled water (10–50 µg/mL) were transferred into the same volume of phosphate buffers (1.25 mL, pH = 6.6; 0.2 M) and K\(_2\)Fe(CN)\(_6\) solution (1.25 mL, 1%). The mixtures were incubated at 50°C for 20 min and acidified with TCA (1.25 mL, 10%). Finally, an aliquot of FeCl\(_3\) solution (0.1%, 0.5 mL) was transferred to the mixtures, and the absorbances of the WEC and EEC were measured at 700 nm.

The Cu\(^{2+}\) reducing effects of the WEC and EEC were measured based on the previous methodologies.\(^{[73,74]}\) To this end, the same volumes of 250 µL of CuCl\(_2\) solution (10 mM, 0.25 mL), neocuproine solution (7.5 mM) and CH\(_3\)COONH\(_4\) buffer (0.25 mL, 1.0 M) were added to the WEC and EEC solutions (10–50 µg/mL) in glass test tubes. The total mixture volumes were adjusted to 2 mL with deionized and distilled water. Then, the glass tubes were closed and retained at r.t. Finally, their absorbances were spectrophotometrically recorded at 450 nm.\(^{[75]}\)

**Radical scavenging activities**

The DPPH\(^{•}\) scavenging effects of the WEC and EEC were determined according to Blois’s method\(^{[76]}\) as described in a previous study.\(^{[77]}\) The N-centered DPPH\(^{•}\) is used for the estimation of the radical scavenging capacity of a pure substance or plant extract. For this aim, an aliquot of DPPH\(^{•}\) (0.5 mL, 0.1 mM) was added to sample solution (1.5 mL) in ethanol (10–50 µg/mL) and incubated for 30 min in the dark. Finally, the absorbance of the sample was spectrophotometrically recorded at 517 nm.\(^{[78]}\)

The ABTS\(^{•+}\) scavenging method is based on a previously described method.\(^{[32]}\) The 2.0 mM ABTS solution with 2.3 mM K\(_2\)S\(_2\)O\(_8\) oxidizing agent resulted in ABTS cation radicals (ABTS\(^{•+}\)). The initial absorbance (0.700 ± 0.025) was adjusted with buffer (0.1 mM; pH = 7.4) at 734 nm. Finally, 3.0 mL of the WEC and EEC at various concentrations (10–50 µg/mL) was mixed with 1.0 mL of ABTS\(^{•+}\), and the remaining absorbance was spectrophotometrically recorded at 734 nm.

The radical scavenging capacities (RSC) of the WEC and EEC were found as millimolar in the reaction mixture. All radicals (DPPH\(^{•}\) and ABTS\(^{•+}\)) scavenging effects (RSC) were calculated as follows: RSC (%) = (1 – As/Ac) x 100, where Ac and As are the absorbances of the control and
Table 1. Validation and uncertainty parameters for antioxidant phenolic acids, LC-MS/MS parameters of selected compounds and the amounts of antioxidants in the WEC and EEC in mg/kg (WEC: Lyophilized water extract of cinnamon (Cinnamomum verum), EEC: Evaporated ethanolic extract of cinnamon (Cinnamomum verum)).

| No | Compound                  | Linear regression equation | $r^2$ | Linear range (ppm) | LOD/LOQ (ppb) | Recovery (%) | Parent Ion | Daughter Ion | Collision Energy (V) | $U_{95}$ (%) | Phenolics (mg/kg) | EEC | WEC |
|----|---------------------------|----------------------------|-------|--------------------|---------------|--------------|------------|-------------|----------------------|-------------|------------------|-----|-----|
| 1  | Caffeic acid              | $y = 4.1981x + 0.0831$     | 0.995 | 0–0.5              | 0.6/2.3       | 90.0         | 179        | 135         | 10                   | 7.76        | 91.2             | 9.8 | 9.8 |
| 2  | Ferulic acid              | $y = 2.483x - 0.0347$      | 0.996 | 0–1                | 0.2/0.8       | 94.1         | 193        | 133         | 15                   | 3.97        | 224.7            | 88.8 | 88.8 |
| 3  | Syringic acid             | $y = 1.599x - 0.0131$      | 0.997 | 0–1                | 0.3/1.5       | 94.7         | 197        | 181.6       | 10                   | 3.12        | nd               | nd  | nd  |
| 4  | Ellagic acid              | $y = 0.2358x + 0.0003$     | 0.992 | 0–1                | 0.2/1         | 99.2         | 301        | 150         | 10                   | 2.53        | nd               | nd  | nd  |
| 5  | Quercetin                 | $y = 0.245x - 0.0001$      | 0.992 | 0–1                | 1.2/4.2       | 100.1        | 301        | 178.6       | 10                   | 1.64        | nd               | nd  | nd  |
| 6  | α-Tocopherol              | $y = 0.0743x - 0.0079$     | 0.986 | 0–2.5              | 10/50         | 104.0        | 429        | 162.6       | 20                   | 3.43        | nd               | nd  | nd  |
| 7  | Catechol                  | $y = 0.0246x + 0.0154$     | 0.991 | 1–25               | 7.5/25        | 98.2         | 109        | 64.8        | 35                   | 2.40        | nd               | nd  | nd  |
| 8  | Pyrogallol                | $y = 0.411x - 0.0107$      | 0.993 | 0–1                | 1.4/5         | 101.5        | 125        | 80          | 16                   | 2.06        | 264.3            | 142.4 | 142.4 |
| 9  | p-Hydroxybenzoic acid     | $y = 5.664x - 0.0436$      | 0.998 | 0–1                | 0.5/2         | 100.7        | 138        | 92.5        | 16                   | 1.84        | 29.4             | 321.1 | 321.1 |
| 10 | Vanillin                  | $y = 5.516x-0.0732$        | 0.997 | 0–1.0              | 0.6/2         | 99.0         | 150.7      | 135.4       | 12                   | 1.93        | 89.4             | 101.2 | 101.2 |
| 11 | p-Coumaric acid           | $y = 10.976x - 0.1661$     | 0.996 | 0–1                | 0.2/1         | 93.6         | 163        | 118.5       | 12                   | 3.98        | 170.2            | 291.4 | 291.4 |
| 12 | Gallic acid               | $y = 2.236x - 0.046$       | 0.996 | 0–1                | 0.4/1.4       | 101.3        | 18.6       | 124         | 13                   | 1.87        | 39.6             | 29.8  | 29.8 |
| 13 | Ascorbic acid             | $y = 0.0171x-0.00114$      | 0.995 | 0.1–10             | 15/50         | 108.0        | 175        | 114.6       | 12                   | 2.28        | 31.3             | nd  | nd  |

nd: Not determined. These values are below the limits of the quantification (<LOD).
samples, respectively. The half maximal scavenging of the chelating concentration (IC\textsubscript{50}) was estimated by plotting the percentages against the WEC and EEC sample concentrations (μg/mL).\textsuperscript{[79]}

**Enzyme inhibition studies**

AChE inhibition effects of the WEC and EEC were done according to Ellman’s method\textsuperscript{[80]} as described previously.\textsuperscript{[81]} Briefly, different amount of WEC and EEC solutions (10–30 μg/mL) in 100 μL of buffer solution (Tris/HCl, 1.0 M, pH = 8.0) were added to 50 μL (5.32 × 10\textsuperscript{-3} EU) of the enzyme solution. The mixtures were incubated for 15 min at 20°C. A 50 μL aliquot (0.5 mM) of DTNB (5,5\textsuperscript{'}-dithio-bis(2-nitro-benzoic)acid) and acetylthiocholine iodide (AChI) were transferred to the mixtures. The reaction was started and absorbances of the incubated mixtures were spectrophotometrically recorded at 412 nm.\textsuperscript{[82]}

**BChE activity and inhibition studies**

BChE inhibition activities of the WEC and EEC were done according to Ellman’s method\textsuperscript{[80]} as described previously.\textsuperscript{[83]} Briefly, different amount of WEC and EEC solutions (10–30 μg/mL) in 100 μL of buffer solution (Tris/HCl, 1.0 M, pH = 8.0) were added to 50 μL (5.32 × 10\textsuperscript{-3} EU) of the enzyme solutions. The mixtures were incubated for 15 min at 20°C. A 50 μL aliquot of DTNB (5,5\textsuperscript{'}-dithio-bis(2-nitro-benzoic)acid) (0.5 mM) and butrylcholine iodide (BChI) were transferred to the mixtures, and the reaction was started. The absorbances of the incubated mixtures were spectrophotometrically measured at 412 nm.\textsuperscript{[84]}

**α-Glycosidase inhibition studies**

The inhibitory effects of WEC and EEC on α-glycosidase enzyme were performed according to the previous method.\textsuperscript{[85]} For this aim, different concentrations of WEC and EEC were added to phosphate buffer (75 μL, pH = 7.4). Then, 20 μL of α-glycosidase solution in indicated buffer was added and incubated for 10 min. An aliquot (50 μL) of p-nitrophenyl-D-glycopyranoside (p-NPG) in the same buffer was added to the final mixture, which was reincubated at physiological temperature (37°C), and the absorbance value was spectrophotometrically calculated at 405 nm.

**α-Amylase activity**

The inhibition effects of WEC and EEC on α-amylase enzyme were measured according to Xiao et al.\textsuperscript{[86]} Briefly, 1 g of starch was dissolved in 40 mL of alkaline solution (0.4 M) and then heated at 80°C for a half an hour. After cooling, the pH was set to 6.9 and the total volume was completed to 100 mL using deionized water. Then, different concentrations of WEC and EEC and the same volume (35 μL) of starch and phosphate buffer (pH: 6.9) were mixed. Next, 20 μL of α-amylase solution was transferred to the final mixture and incubated at 35°C for one hour. Finally, the reaction was stopped by the addition of 50 μL of HCl (0.1 M), and the absorbance value was recorded at 580 nm.

**Statistical analysis**

The results of experiments are the average of triplicate analyses. The experimental data were calculated as the mean ± standard deviation and analyzed. Variance ANOVA including one-way analysis was realized. Significant differences between means were recorded by Duncan’s multiple range tests. \(P < .05\) was regarded as significant, and \(p < .01\) was very significant.
Results and discussion

Phenolic composition

Phenolic compounds as plant metabolites contribute to the biological activities of plant extracts.\(^{[87]}\) It is known that the amount of extracted phenolics depends on some parameters including the extraction temperature, time, pH, and solvent polarity.\(^{[88]}\) Additionally, the aerial parts of the plants used for the extraction have a great importance because of different patterns of secondary metabolites.\(^{[89]}\) For this purpose, water, ethanol, and water and acetone are considered the effective solvents for the extraction of the polyphenol contents from plants.\(^{[90]}\) It was reported that the potential health benefits of cinnamon derived primarily from bioactive ingredients such as polyphenols and flavonoids as antioxidants.\(^{[91]}\) Pharmaceutical analyses of its essential oil revealed that cinnamaldehyde, cinnamic acid, cinnamate, procyanidins, eugenol, and catechins are the major elements of cinnamon.\(^{[92,93]}\) The WEC and EEC had high phenolic contents of 153.5 and 205.5 GAE/g extract, respectively. A high phenolic content in any extract indicates its high antioxidant capability. On the other hand, flavonoids are the most abundant chemical group in medicinal plants as secondary metabolites. The total flavonoids in the WEC and EEC were found spectrophotometrically as 16.67 and 11.25 QE/g extract, respectively. The results showed that there is a good correlation between the total flavonoids in the WEC and EEC and the antioxidant activity.

In this study, LC-MS/MS analysis was performed for the determination of the phenolic profiles of the WEC and EEC for the quantification and identification of twenty-five phenolics, as summarized in Table 1 and Figure 1. According to the LC-MS/MS experiments, the WEC is rich with regards to the phenolic content. Also, \(p\)-hydroxybenzoic acid (321.1 mg/kg extract), \(p\)-coumaric acid (291.4 mg/kg extract), pyrogallol (142.4 mg/kg extract), vanillin (101.2 mg/kg extract) and ferulic acid (88.8 mg/kg extract) were found to be the most abundant ingredients in the WEC, respectively. On the other hand, pyrogallol (264.3 mg/kg extract), ferulic acid (224.7 mg/kg extract), \(p\)-coumaric acid (170.2 mg/kg extract), caffeic acid

![Figure 1. LC-MS/MS chromatograms of WEC (A) and EEC (B) from cinnamon (Cinnamomum verum). [WEC: Lyophilized water extract of cinnamon (Cinnamomum verum), EEC: Evaporated methanolic extract of cinnamon (Cinnamomum verum)].](image-url)
(91.2 mg/kg extract) and vanillin (89.4 mg/kg extract) were found as the most plentiful chemicals in the EEC, respectively (Table 1). The indicated main phenolics could be responsible for the antioxidant effects of the WEC and EEC. Plant phenols or polyphenols are the major antioxidant compounds that possess different biological activities. They have multifunctional properties and can act as ROS and singlet oxygen scavengers, reducing agents, and hydrogen atom donors. Additionally, phenolics especially flavonoids are capable of metal chelating and reducing capacities.

**Antioxidant results**

The reducing potentials of the WEC and EEC were determined by two distinct reducing systems including CUPRAC, and Fe\[(CN-)_6\]3 reducing abilities. A plant extract can be a reductant and inactivate oxidant agents and ROS. It was reported that cinnamon (*Cinnamomum verum*) contains a number of antioxidant compounds, which can effectively scavenge ROS.\[91\] This method can easily measure the reduction of Fe\[(CN-)_6\]3.\[95\] The addition of Fe\(^{3+}\) to the WEC and EEC leads to the formation of the Fe\([Fe(CN^-)_6]_3\) complex, which had a maximum absorbance at 700 nm.\[96\] In this context, WEC and EEC had effective reducing effects by using Cu\(^{2+}\) and Fe\[(CN-)_6\]3 reducing methods. The Fe\[(CN-)_6\]3 reducing abilities of the WEC and EEC were determined according to the Oyaizu method.\[70\] As summarized in Table 2, EEC (r\(^2\): 0.9969) and WEC (r\(^2\): 0.9912) showed potent Fe\(^{3+}\) reducing profiles (p < .01). The Fe\(^{3+}\) reducing ability of WEC, EEC and standards decreased in following order: Trolox (1.651, r\(^2\): 0.9997) > α-Tocopherol (1.192, r\(^2\): 0.9987) > EEC (0.886, r\(^2\): 0.9969) ≥ WEC (0.719, r\(^2\): 0.9912). An increase in absorbance indicates an increased reducing ability due to increased complex formation (Figure 2a). The results demonstrated that both WEC and EEC could donate electrons and neutralize free radicals and ROS.

The Cu\(^{2+}\) reducing abilities of WEC and EEC were given in Figure 2a and Table 2. A good correlation was found between the Cu\(^{2+}\) reducing effect and the EEC (r\(^2\): 0.9942) and WEC (r\(^2\): 0.9825) samples (10–30 µg/mL). At the 30 µg/mL, the highest absorbance indicating the highest reducing power was displayed by EEC (1.251, r\(^2\):0.9942) and . The Cu\(^{2+}\) ion reducing abilities of the WEC, EEC and standards were as follows (Figure 2b): Trolox (1.644, r\(^2\): 0.9986) > α-Tocopherol (1.388, r\(^2\): 0.9877) > EEC (1.251, r\(^2\): 0.9942) > WEC (0.963, r\(^2\): 0.9825). The CUPRAC assay is rapid, stable, cheap, selective, and suitable.\[97\]

DPPH- and ABTS\(^{+}\) radical scavenging assays are the most putative and applicable methods to determine the antioxidant capacities and radical scavenging effects of beverages, foods, and plants. The DPPH- scavenging ability is the most common and oldest method for determining the antioxidant activities. In the radical scavenging assay, the antioxidant compounds and plant extracts can reduce DPPH- to DPPH\(_2\).\[70\] When an antioxidant component or plant extract reacts with DPPH-, they can give a hydrogen atom and consequently reduced DPPH. The color change was recorded at 517 nm. The IC\(_{50}\) values of DPPH- scavenging were found to be 21.25 µg/mL (r\(^2\): 0.9025) for EEC, 15.71 µg/mL (r\(^2\): 0.9394) for WEC, 16.78 µg/mL (r\(^2\): 0.9756) for α-Tocopherol and 9.60 µg/mL (r\(^2\): 0.9593) for Trolox (Figures 2c and 3). The results showed that EEC had strong DPPH- scavenging effect than α-Tocopherol.

| Antioxidants | \(\lambda_{700}\) reducing | \(\lambda_{450}\) reducing |
|-------------|-----------------------------|-----------------------------|
| α-Tocopherol| 1.192                       | 1.388                       |
| Trolox      | 1.651                       | 1.644                       |
| WEC         | 0.719                       | 0.963                       |
| EEC         | 0.886                       | 1.251                       |

Table 2. The reducing abilities of the WEC, EEC and standards including trolox and α-tocopherol at the same concentration (30 µg/mL) by Fe\(^{3+}\)-Fe\(^{2+}\) and Fe\(^{3+}\)-TPTZ transformation methods, as well as the Cu\(^{2+}\) reducing ability by the CUPRAC method (WEC: lyophilized water extract of cinnamon (*Cinnamomum verum*), EEC: evaporated ethanolic extract of cinnamon (*Cinnamomum verum*).
The ABTS·⁺ scavenging can be applicable for plant extracts including both hydrophilic and lipophilic compounds. In this assay, ABTS·⁺ has a maximum wavelength at 734 nm. [98] This assay can be used in a large pH range. [99] Both WEC and EEC exhibited effective radical scavenging effects against ABTS·⁺ (p > .001). The WEC and EEC effectively scavenged ABTS·⁺ in a dose-dependently (10–30 μg/mL), and these differences were found to be statistically significant (p < .001, Table 3). The EC₅₀ values for the WEC and EEC in this assay were calculated as 6.52 μg/mL (r²: 0.9715) and 5.79 μg/mL (r²: 0.9556). On the other hand, the EC₅₀ values were found to be 3.60 μg/mL (r²: 0.9996) for α-Tocopherol and 5.02 μg/mL (r²: 0.9784) for Trolox (Figures 2d and 3). The ABTS·⁺ scavenging effect of the WEC and EEC was found similar to standard antioxidants.

**Enzyme inhibition results**

The enzyme inhibition effects of the WEC and EEC were determined against α-amylase, α-glycosidase, AChE and BChE enzymes. Enzyme inhibition is the most studied therapeutic medium in the cosmetic, pharmaceutical and food industries. Also, enzyme inhibitors are clinically used as drugs for managing some health problems, including AD, obesity, and diabetes. [100] It was reported that there are side effects including gastrointestinal disturbances and hepatotoxicity of some synthetic inhibitors. Thus, there is great interest in
finding natural and novel inhibitors without side effects from natural products. The inhibition data of the WEC and EEC are summarized in Table 4. For the evaluation of the effects of the WEC and EEC on these enzymes, the following results have been described. The AChE and BChE inhibitory properties of the WEC and EEC were determined according to Ellman's procedure. The WEC and EEC had IC₅₀ values of 221.33 µM (r²: 0.9510) and 110.26 µM (r²: 0.9370) for AChE, respectively. These values were calculated as 461.69 µM (r²: 0.9880) and 94.93 µM (r²: 0.9651) for BChE, respectively. Additionally, tacrine as a clinically used inhibitor had Ki values of 0.124 µM (r²: 0.9804) and 0.101 µM (r²: 0.9698) against AChE and BChE.

Table 3. Determination of the half maximal concentrations (IC₅₀, µg/mL) of WEC and EEC standards for the DPPH⁺ and ABTS⁺⁺ scavenging activities (WEC: lyophilized water extract of cinnamon (Cinnamomum verum), EEC: evaporated ethanolic extract of cinnamon (Cinnamomum verum)).

| Compounds   | DPPH⁺scavenging | ABTS⁺⁺scavenging |
|-------------|-----------------|-----------------|
|             | IC₅₀⁺⁺ r²       | IC₅₀⁺⁺ r²       |
| α-Tocopherol| 16.78 0.9756    | 3.60 0.9996     |
| Trolox      | 9.60 0.9593     | 5.02 0.9784     |
| WEC         | 21.25 0.9025    | 6.52 0.9715     |
| EEC         | 15.71 0.9394    | 5.79 0.9556     |

Table 4. The enzyme inhibition results (IC₅₀ values; µg/mL) of the WEC and EEC on acetylcholinesterase, α-amylase, α-glycosidase, and butyrylcholinesterase enzymes (WEC: lyophilized water extract of cinnamon (Cinnamomum verum), EEC: evaporated ethanolic extract of cinnamon (Cinnamomum verum)).

| Enzymes           | WEC  | EEC  | Standards |
|-------------------|------|------|-----------|
|                   | IC₅₀ | r²  | IC₅₀ | r²  | IC₅₀ | r²  |
| α-Glycosidase *   | 206.86 0.9307 | 220.00 0.9218 | 22.80 0.9922 |
| α-Amylase*        | 189.86 0.9265 | 200.86 0.9486 | 10.01 0.9424 |
| Acetylcholinesterase** | 221.33 0.9510 | 110.26 0.9370 | 0.124 0.9804 |
| Butyrylcholinesterase** | 461.69 0.9880 | 94.93 0.9651 | 0.101 0.9698 |

*Acarbose was used as a standard for α-glycosidase and α-amylase enzymes.
**Tacrine was used as a standard for acetylcholinesterase and butyrylcholinesterase enzymes.
The both extracts demonstrated effective inhibitory profiles toward AChE and BChE, but the EEC showed a better inhibitory effect than that of the WEC against both cholinergic enzymes (Table 3 and Figure 3). AChE hydrolyzes acetylcholine (ACh) to choline and acetate. The AChE inhibition increases the level of ACh, and thus, AChE inhibition has been considered to be a useful therapeutic approach to treat neurological disorders including AD. As observed in the antioxidant effects, the WEC and EEC had effective AChE and BChE inhibitory capacities. We speculated that the major phenolics identified in the WEC and EEC act as AChE inhibitors. It is known that phenolic compounds are cholinergic enzyme inhibitors. It was reported that administration of cinnamon comparable to a human adult dose did not induce significant behavioral changes in terms of nervousness, excitement, dulness, ataxia, alertness or death in rats. α-Amylase and α-glycosidase exist on the cells lining the small intestine and hydrolyze polysaccharides to monosaccharide units for absorption through the intestine. For the reduction of body weight and control of the blood glucose level, specific inhibitors can inhibit the both digestive enzymes. The WEC and EEC exhibited IC₅₀ values of 189.86 µM (r²: 0.9265) and 200.86 µM (r²: 0.9486) for α-amylase as well as 206.86 µM (r²: 0.9307) and 220.00 µM (r²: 0.9218) for α-glycosidase, respectively (Table 4, Figure 3). The obtained results showed that the WEC and EEC had more affinity for α-amylase than for the α-glycosidase enzyme. The inhibition of both carbohydrate-hydrolyzing enzymes can reduce the postprandial blood glucose levels. Additionally, this can be a crucial therapeutic strategy to manage hyperglycemia associated with diabetes. Both WEC and EEC had greater inhibition on α-glycosidase effects than the acarbose molecule (IC₅₀: 22.800 mM). Also, it was reported that cinnamon bark is famed to cause drug interactions with hypoglycemic medicines, potential interactions with blood thinners such as warfarin and aspirin is also labeled as significant and may raise bleeding and bruising in patients taking warfarin and cinnamon bark, hence it should be monitored closely. However, it was known that cinnamon might improve anthropometric parameters, glycemic indices and lipid profile of patients with T2DM. Also, cinnamon extracts can also activate the glycogen synthase through stimulating glucose uptake, and inhibiting glycogen synthase kinase. These effects can be considered as the potential mechanism of the observed increases in lean mass in the cinnamon group.

Conclusions

The evaluation of the bioactivity and phytochemical screening of WEC and EEC had great importance. The WEC and EEC, as natural sources of phenolic compounds, were examined for their biological activities including antioxidant activities and some metabolic inhibitory properties. The WEC and EEC were found as having potent antioxidant properties in several bioanalytical assays including Fe³⁺ and Cu²⁺ reducing abilities, as well as DPPH- and ABTS⁺⁺ radical scavenging activities. In addition, both extracts were found as having powerful antioxidant activity and inhibitory effects on the indicated metabolic enzymes. Ethanol was efficient for the extraction of phenolics with the effective α-glycosidase, AChE, BChE, and α-amylase inhibition. This study proposed that cinnamon might be a promising potential source of beneficial phenolics to treat several diseases including postural tachycardia syndrome, myasthenia gravis, diabetes, and neurodegenerative diseases, including AD.

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