Phenolic compounds profile, neuroprotective effect and antioxidant potential of a commercial Turkish coffee

Perfil do componente fenólico, efeito neuroprotector e potencıal antioxidante de um café comercial turco

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A B S T R A C T

Objective

The purpose of this study is to determine the phenolic and flavonoid contents, and antioxidant activities and neuroprotective effects of powdered coffee sample of a commercial coffee brand originated from Sivas, Turkey.

Methods

Total phenolic, flavonoid and antioxidant contents, enzymatic and non-enzymatic antioxidative activities based on 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity, metal chelating potential, reducing power, superoxide dismutase and catalase activity tests and lipid peroxidation inhibition potentials of the ethanolic and aqueous extracts of the coffee sample were assayed using the commonly preferred spectrophotometric methods. Furthermore the extracts' cholinesterase and tyrosinase inhibition potentials were evaluated. Phenolic profiles of the coffee sample were investigated using high performance liquid chromatography.

Results

Catechin was the most frequently detected phenolic acid. In addition, it was demonstrated that the water extract has a significant impact when compared with standard antioxidants. While the SC_{50} (sufficient concentration to obtain 50% of a maximum scavenging capacity) value for the scavenging activity of 2,2-diphenyl-1-picrylhydrazyl free radical was calculated as being 0.08mg/mL for water extract, the amount of chelating agents with half Fe^{2+} ions in the medium was found to be 0.271mg/mL. Additionally, it was shown that 0.1mg/mL concentration of both extracts prevents lipid peroxidation by 8%. Compared with standard drugs, inhibition potentials of cholinesterase and tyrosinase enzymes were considered as moderately acceptable in these samples.

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Conclusion

Besides the extracts' enzymatic antioxidant activity, their inhibition potential on cholinesterase and tyrosinase enzymes – which are important clinical enzymes – reveal that this natural source can be used as a valuable resource in different fields, especially in medicine.

Keywords: Anticholinesterase. Antioxidant effect. Free radicals. Lipid peroxidation.

R E S U M O

Objetivo

O objetivo deste estudo é determinar o conteúdo fenólico e flavonoide, bem como as atividades antioxidantes e os efeitos neuroprotetores de uma amostra de café em pó de uma promissora marca comercial proveniente de Sivas, Turquia.

Métodos

A partir dos métodos espectrofotométricos comumente utilizados, foram analisados os seguintes aspectos da amostra de café: teores de fenólicos totais, flavonoides e antioxidantes; atividades antioxidantes enzimáticas e não enzimáticas, baseadas na atividade de eliminação de radicais livres de 2,2-difenil-1-picrilhidrazila potencial quelante de metais; poder redutor; testes de atividade de superóxido dismutase e catalase; e potenciais de inibição da peroxidação lipídica dos extratos etanólicos e aquosos. Além disso, foram avaliados os potenciais de inibição da colinesterase e da tirosinase dos extratos. Os perfis fenólicos da amostra de café foram investigados por cromatografia líquida de alta eficiência.

Resultados

Entre os ácidos fenólicos estudados, o mais detectado foi a catequina. Especialmente, foi demonstrado que o extrato de água tem um impacto significativo quando comparado com os antioxidantes padrão. Determinou-se que o valor de SC_{50} (a concentração suficiente para obter 50% da capacidade máxima de eliminação) da atividade de eliminação do radical 2,2-difenil-1-picrilhidrazilab/para extrato de água era de 0,08mg/mL, enquanto a quantidade de agentes quelantes com metade de Fe^{2+} íons na média foi encontrada como 0,271mg/mL. Também foi demonstrado que a concentração de 0,1mg/mL de ambos os extratos inibe a peroxidação lipídica em cerca de 8%. Comparado com drogas padrão, os potenciais de inibição das amostras nas enzimas e tirosinase foram aceitáveis como moderados.

Conclusão

Os resultados mostram que, além de terem atividade antioxidante enzimática, os extratos apresentam potencial de inibição das enzimas colinesterase e tirosinase, que são importantes enzimas clínicas, o que revela que essa fonte natural pode ser usada como um recurso valioso em vários campos, principalmente na medicina.

Palavras-chave: *Anticolinesterase. Efeito antioxidante. Radicais livre. Peroxidação de lipídios.*

I N T R O D U C T I O N

Since coffee is one of the most important agricultural products in international trade and has a significant commercial and social importance, coffee analysis investigations are of great importance [1]. Recent studies emphasize that regular coffee consumption (about 2-4 cups per day) is associated with a low risk of death, colorectal cancer development, liver damage, and cirrhosis, as well as degenerative, progressive and chronic diseases (Alzheimer's and Parkinson's disease, Type II diabetes, Coronary heart disease) [2]. Coffee is consumed for a variety of reasons: thanks to its caffeine content it is a stimulant drink, it is beneficial to health and has an excellent taste and aroma. Taste, aroma and caffeine content play an important role in popularity, but coffee beans contain thousands of different chemical compounds, such as carbohydrates, lipids, nitrogen compounds, vitamins, minerals, alkaloids and phenolic compounds [3]. Although other foods contain more antioxidants

than coffee, the amount and frequency of consumption make this drink one of the main sources of dietary antioxidants [4].

Polyphenolic antioxidants have been used for centuries in preventive medicine, and human epidemiological studies support the concept that there is an inverse relationship between body antioxidant levels/consumption of coffee and the cognitive function as well as the development of neurodegenerative diseases [5]. Recently, studies have shown that progression of Alzheimer Disease (AD) is slowed by the consumption of foods containing components with antioxidant activity [6]. Furthermore a study on rabbit hippocampus revealed that caffeine prevents chronic, detrimental alterations by activating the brain's antioxidant systems [7].

Neurodegenerative diseases are among the deadly diseases affecting the elderly population. In particular, it is known that Alzheimer (AD) and Parkinson (PD) diseases have a multifactorial and progressive feature and there are no complete treatment strategies for both diseases. For this reason, intensive investigations are in progress to find new drug candidates of natural or synthetic origin for the treatment of these diseases with uncertain pathogenesis. The most accepted theory for Alzheimer's disease is the cholinergic hypothesis, represented by a significant decrease in the acetylcholine level in individuals who suffer from this disease. Therefore, the inhibition of Acetylcholinesterase Enzyme (AChE) which breaks down acetylcholine in the brain is a widely used treatment strategy against AD. Similarly, the inhibition of Butyrylcholine Estrerase (BuChE), which is capable of hydrolyzing various cholinesterases, is also important for the treatment of AD. Furthermore, since Phosphodiesterases (PDEs) are broadly expressed in the brain, Phosphodiesterases inhibitors (PDEIs) are considered to modulate neurodegenerative conditions through the regulation of Cyclic Adenosine Monophosphate (cAMP) and Cyclic Guanosine Monophosphate (cGMP) in the brain. The best-known PDEIs are Methylxanthines (*e.g.* caffeine) acting as non-selective inhibitors on single isoforms of PDEs [8].

Since dementia is also associated with Parkinson's disease, cholinesterase inhibitors are also of great interest in the treatment of PD [9]. Apart from this, tyrosinase plays a role in melanin biosynthesis, which takes place in two steps. Tyrosine is converted to 3,4-Dihydroxy Phenylalanine (DOPA), a process called tyrosinase monophenolase activity. The next step is called diphenolase activity and includes oxidation of DOPA to DOPA quinone. The reactive o-quinone, DOPA quinone, polymerises spontaneously and non-enzymatically to form high molecular weight melanin. In humans, melanin is often used to protect the skin from UV radiation, but overproduction of melanin results in hyperpigmentation of the skin characterized by age spots, melasma and chloasma. Furthermore, tyrosinase is known to oxidate dopamine in order to produce melanin in the brain. Thus, tyrosinase is also associated with pathogenesis of PD and related neurodegenerative disorders [10]. Therefore, inhibition of tyrosinase has also become a popular target in drug research for Parkinson's treatment. In addition, tyrosinase inhibitors are also useful for maintaining food quality, in the development of cancer drugs as well as in the treatment of skin hyperpigmentation [11].

A number of studies have emphasized that since coffee increases neuronal stability there is an inverse relationship between Parkinson's disease and the consumption of coffee and other sources of caffein [12]. Thus, it can be said that, the beneficial effects of coffee is mainly due to the compounds it contains, caffeine being the most important ingredient. In another study, the beneficial properties of coffee were associated with methylxanthine phytochemicals, which are involved in caffeine and are abundant in coffee. Among these methylxanthines, caffeine has been extensively studied and the effects of caffeine on the functioning of the neuronal network are clearly demonstrated by supporting continuous cognitive performance. Caffeine can protect neurons against dysfunction and death in stroke, Alzheimer's disease and Parkinson's disease animal models. The mechanism of action

of caffeine is thought to be based on antagonism of various subclasses of adenosine receptors. Apart from caffeine, metabolites such as theobromine and theophylline which are known as downstream xanthine metabolites also contribute to the beneficial properties of coffee, tea and cocoa on brain health [13]. It had been reported that levels of theobromine in the cerebral spinal fluid are inversely correlated with the amyloid beta 42 levels in Alzheimer's patients [14]. Several meta-analyses have shown that moderate coffee intake lowers one's risk of developing PD by 24-30%. It should be kept in mind that the effects of coffee consumption on these neurological diseases vary according to gender, dose and hormone levels [13]. Another 21-year follow-up study dealing with 1409 subjects (875 women and 534 men), revealed that moderate consumption of coffee reduced the AD risk up to 64% in later life as compared to low coffee drinkers [15].

Drinking Turkish coffee in Turkey is an important part of both cultural and outdoor and indoor social activities. Turkish coffee is not just a type of coffee, it also has a unique preparation method: sufficient quantity of coffee and desired amount of sugar are added to preferably cold water, measured with cups. Then, the ingredients are mixed thoroughly in a narrow-topped coffee pot called cezve until the coffee gets wet and the sugar is dissolved. At the end of the mixing process, the mixture is heated. After boiling it is poured into cups and served [16].

To demonstrate the coffee benefits mentioned above, many studies on commercially available coffee brands have been performed in many countries, including Turkey. However, due to variation in coffee type, dose, range of study, selection of biomarkers and lack of information about the composition of the bioactive substances of coffee, further studies are needed to evaluate the beneficial or harmful effects of coffee on human health [4,9]. From this point of view, after investigating phenolic compounds in ethanol and water extracts of Talas brand Turkish coffee, the total phenolic and flavonoid contents were determined by spectrophotometric methods and antioxidant activities were investigated using different methods. On the other hand, the critical role of lipid peroxides in inflammation progression and regulation of inflammation has long been known due to their importance in the production of secondary precursors. Therefore, due to their role in many diseases and cause of death, intensive efforts have been made in order to identify and develop compounds that eliminate the toxic effects of lipid peroxides [17]. For this purpose, the efficacy of coffee extracts to prevent lipid peroxidation was also tested. In addition, anticholinesterase and antityrosinase activities were investigated in order to reveal the neurobiological effects of coffee extracts.Thus, in addition to similar studies found in the literature, this specific coffee brand will be evaluated by performing different analyses to find out whether the use of a different brand of Turkish coffee would be beneficial to health in various aspects.

METHODS

All chemicals used for the analyses were analytical grade and purchased from Sigma Chemical Co. (Saint Louis, USA). All high purity standards used for High Performance Liquid Chromatography (HPLC) analysis were also obtained from Sigma. Acetylcholinesterase and butyrlcholinesterase were obtained from Electrophorus electricus (electric eel) and equine serum, respectively. Tyrosinase and catalase were also obtained from mushroom and bovine liver, respectively. Spectrophotometric measurements were performed using an UV-1800 spectrophotometer.

Fifty mililiters of ethanol absolute (*p.a.* ≥99.81) and/or distilled water were added to 1g of ground Talas brand Turkish coffee purchased commercially in the Sivas province and extracted using

a shaking water bath at 25°C for 24h. Subsequently, the mixture was centrifuged and extraction was performed two more times after removing the supernatant. All supernatants were mixed together and filtered using filter paper; the solvent of the ethanol extract was removed by evaporation, and the water of the water extract was removed using a lyophilizat or. After lyophilization, the ethanol and water extracts were prepared by adding ethanol and water up to a certain concentration [9].

Phenolics were analyzed using an HPLC equipment (Thermo Dionex Ultimate 3000) with a RS Diode Array detector. Analyses were carried out by using ODS Hypersil C18 column (150mmx4.6mm). Elution was carried out using 0.1% acetic acid as the solvent A and acetonitrile as the solvent B with a gradient program starting with 100% A, to reach 5% B after 2 min, 40% B after 20 min and 80% B after 30min. Optimization of the operation was performed by adjusting the injection volume of samples and standards to 10μL, flow rate to 0.9 mL/min, column temperature to 25°C [18,19].

The phenolic compounds detected in both extracts were identified through comparison of their retention times and spectra obtained by injection of the standard solution under the same conditions. The peak area was used for quantitation, using external calibration with standards [20].

Total Phenolic Content (TPC) of the coffee sample's ethanol and water extracts was determined spectrophotometrically as Gallic Acid Equivalent (mg GAE/g sample) using Folin-Ciocalteu reagent [21]. The Total Flavonoid Content (TFC) was also determined as Quercetin Equivalent (mg QE/g sample) by using the method based on the formation of flavonoid-aluminum chloride complex [22].

The antioxidant activity of the extracts was examined using different methods. Total Antioxidant Activity (TAA) of the extracts was calculated as the Ascorbic Acid Equivalent (AAE) by the phosphomolybdenum test, based on the reduction of Mo(VI) to Mo(V) in the presence of a tested antioxidant substance and the subsequent formation of a green phosphate/Mo(V) complex in acidic medium. For this purpose the appropriate amount of the sample was combined with the reagent solution (0.6M sulfuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate) in a tube. The content of the tube with a tightly closed mouth was incubated at 95°C for 90min. After the samples had cooled down to room temperature, the absorbance was measured at 695nm against a blank [23].

To determine 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities, commercially purchased stable DPPH radical was used. When DPPH interacts with an antioxidant contained in the extract, it loses hydrogen and turns into its reduced form. This change leads to a decrease in absorbance at 517nm [24]. In this procedure, methanolic DPPH solution was added to the extract at different concentrations. Only the mixture containing DPPH solution and the solvent used for sample preparation was used as control. After incubation for 30min in the dark, the absorbance values were recorded and DPPH free radical scavenging activities were calculated by using the following Equation 1.

$$\text{Scavenging ratio (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad \text{(Equation 1)}$$

The SC_{50} values (the extract concentration which eliminates half of the DPPH in the medium) for both extracts were calculated with the aid of a graph plotted using the scavenging ratio against concentration data.

The ability to form chelates with the iron ion was expressed by the rate of inhibition of complex formation with Ferrozine-Fe^{2+} and was compared with Ethylenediaminetetraacetic Acid (EDTA) [17]. The extract concentrate sufficient to reduce the concentration of Ferrozine-Fe^{2+} complex expected to be formed under the trial conditions was calculated by following the steps set for the in the DPPH test.

The appropriate amount of extracts was mixed with potassium ferricyanide (1.0%) in phosphate buffer (0.1M pH 6.6) and incubated for 20 minutes at 50°C in order to demonstrate the reducing power of ethanol and water extracts. After incubation, 10.0% trichloroacetic acid was added and after shaking, 0.1% $FeCl_3$ was added to the mixture. After 30 minutes, the conversion of Fe^{3+}-ferricyanide complex to Fe^{2+} was recorded by reading the absorbance at 700nm [25]. The greater the absorbance, the greater the reducing power.

Superoxide Dismutase (SOD) activities of the extracts were tested using the method based on the inhibition of Nitroblue Tetrazolium (NBT) reduction [26]. Extract concentrations (IC_{50}; mg/mL) that inhibit 50% of the SOD enzyme were calculated following the same procedure used in the DPPH assay.

The extracts' catalase activities were calculated by monitoring the dismutation of hydrogen peroxide solution (H_2O_2) (about 25mM) spectrophotometrically at 240nm after the addition of an appropriate amount of extract and/or catalase from bovine liver purchased commercially. The extinction coefficient for H_2O_2 was $42M^{-1}cm^{-1}$ [27]. 1U enzyme activity is defined as the amount of enzyme that catalyzes the decomposition of 1μmol H_2O_2 per minute.

To investigate the lipid peroxidation inhibition potential, the reaction mixtures were prepared in methanol to contain 0.26mM linoleic acid, 2.00mM 2,2-azobis-(2-amidinopropane)-dihydrochloride (ABAP) and the appropriate amount of extracts. After 10 minutes incubation of well-mixed mixtures, changes in absorbance were measured against methanol at 234nm. Extract-free mixture was used as a blank [19].

The spectrophotometric method modified by Ellman *et al.* [28] was followed to determine the inhibition degree of water and ethanol extracts on cholinesterase enzymes of a coffee sample at 1mg/mL concentration. Acetylcholinesterase (EC 3.1.1.7) obtained from Electrophorus electricus (electric eel) and butyrylcholinesterase (EC 3.1.1.8) from equine serum as enzyme source and acetylthiocholine iodide and butyrylthiocholine chloride as substrate were used in the reaction mixture. The drug galantamine (alkaloid type anticholinesterase) was used as reference [29]. Enzyme inhibition percentages were calculated by using the following Equation 2, comparing the reaction rate (A_{sample}) of the sample against the blank (A_{blank}) prepared to contain the buffer instead of the sample to be tested.

$$\text{Inhibition percentage (\%)} = (A_{blank} - A_{sample})/A_{blank} \times 100 \qquad \text{Equation 2}$$

In order to examine the tyrosinase inhibition of the extracts, mushroom tyrosinase was first incubated with 0.5mg/mL of extracts at 25°C for 10 minutes in 50mM phosphate buffer (pH 6.8). Subsequently, L-DOPA was added to this mixture and the enzymatic reaction was followed by monitoring the change in absorbance at 475nm due to DOPA chrome formation in 1 minute. Kojic acid was used as a standard inhibitor for comparison [23] and the tyrosinase enzyme inhibition potentials of 0.5mg/mL portions of both standard and extracts were calculated with the aid of the equation used above. Triplicate analysis was used in all the assays and the results are expressed as mean ± Standard Deviation (SD).

RESULTS

In order to determine the effect of solvents which have different polarity on the studied parameters, different biological experiments were carried out on Talas brand Turkish coffee ethanol and water extracts.

High performance liquid chromatography analysis of extracts revealed that the coffee sample contains phenolic acids, flavonoids, hydroxycinnamic acid and stilbene type compounds (Table 1). Although the species of phenolic compounds are different, both of the extracts contain almost the same number of phenolics. While gallic acid, homogentisic acid and coumaric acid could not be detected in the ethanol extract, caffeic acid and trans-sinapic acid could not be detected in the water extract. On the other hand, catechin as a flavonoid was the most conspicuous in terms of quantity for both extracts. Pyrogallol was significantly higher in ethanol extract than in water extract.

The calculated value of total phenolic content was much higher in the case of the water extract than alcohol extract, whereas flavonoid content is the opposite (Table 2). On the other hand, in the case of the water extract, 40.50mg GAE of phenolic acid per 1g of dry extract was obtained. This is a very valuable finding for coffee prepared with water to drink.

Table 1. Concentration (mg/kg) of phenolic compounds found in water and ethanol extracts of Talas branded Turkish coffee obtained from Sivas province. Ordu, Turkey, 2017.

Investigated Phenolics	Water Extract		Ethanol Extract	
	Calculated value	SD	Calculated value	SD
Pyrogallol	1.00	(±0.02)	133.44	(±7.59)
Protocatechuic acid	6.59	(±0.77)	2.63	(±0.95)
p-Hydroxybenzoic acid	4.06	(±1.03)	1.36	(±0.29)
Catechin	5098.57	(±10.58)	2614.21	(±30.17)
Vanillic acid	0.07	(±0.00)	3.56	(±1.25)
Caffeic acid	-	-	1.98	(±0.58)
Trans-sinapic acid	-	-	10.03	(±1.15)
Benzoic acid	3.52	(±1.00)	15.63	(±3.50)
Resveratrol	1.33	(±0.06)	1.12	(±0.30)
Trans-cinnamic acid	0.34	(±0.01)	0.27	(±0.09)
Gallic acid	4.71	(±1.06)	-	-
Homogentisic acid	43.64	(±5.30)	-	-
p-Coumaric acid	1.08	(±0.09)	-	-

Note: SD: Standard Deviation.

Table 2. Total phenolic, flavonoid and antioxidant contents, enzymatic and non-enzymatic antioxidant activities and lipid peroxidation inhibition potentials of water and ethanol extracts of Talas branded Turkish coffee obtained from Sivas province. Ordu, Turkey, 2017.

Assays	Water Extract		Ethanol Extract		Standard
	Calculated value	SD	Calculated value	SD	
Total Phenolic Content (mgGAE/g dry sample)	40.500	(±2.560)	5.560	(±1.050)	-
Total Flavonoid Content (mgQE/g dry sample)	2.170	(±0.010)	8.740	(±1.780)	-
Total Antioxidant Activity (mgAAE/g dry sample)	39.530	(±3.740)	20.540	(±3.400)	-
DPPH Radical Scavenging Activity (SC_{50}; mg/mL)	0.080	(±0.000)	0.735	(±0.075)	0.0032 for AA
Chelating Potential with Fe^{2+} (SC_{50}; mg/mL)	0.271	(±0.002)	8.170	(±2.560)	0.03 for EDTA
Reducing Power (Abs_{700} for 0.5mg/mL extract)	0.618	(±0.006)	0.408	(±0.004)	0.770 for AA
SOD activity (IC_{50}; mg/mL)	0.146	(±0.215)	1.018	(±0.056)	10^{-27} for commercial SOD
CAT activity (U for 0.05mg/mL extract)	0.033	(±0.002)	0.028	(±0.001)	1.74 for 0.01mg/mL commercial CAT
Potential on lipid peroxidation (%; for 0.1mg/mL extract)	8.550	(±1.250)	8.100	(±1.160)	-

Note: AA: Ascorbic Acid; AAE: Ascorbic Acid Equivalent; CAT: Catalase; GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent; SD: Standard Deviation; SOD: Superoxide Dismutase.

Total antioxidant activity of water extract (39.53mg AAE/g dry extract) is approximately 2 times higher than the ethanol extract (20.53mg AAE/g dry extract).

When the DPPH radical scavenging activities of water and ethanol extracts were calculated, 0.08mg/mL concentration of water extract could scavenge 50% of the DPPH radicals in the medium, which was proportional to the TAA and TPC. However, in the case of ethanol extract, 10 times more extract (0.735mg/mL) hardly exhibited similar activity. In this test, AA concentration, sufficient to sweep up to 50% of the radicals, was much smaller (0.0032mg/mL) than both extracts.

On the other hand, 0.271mg/mL concentration of the water extract had the potential to chelate half of the Fe^{2+} ions in the reaction medium. This value was also found to be much smaller (0.03mg/mL) for the positive control EDTA and much larger (8.17mg/mL) for the ethanolic extract.

A total of 0.5mg/mL of water extract, which resulted in an absorbance of 0.618 at 700nm, could have higher reducing power compared to the same amount of ethanolic extract with an absorbance of 0.408. Moreover, under the same conditions absorbance for the same concentration of AA was 0.77.

A total of 0.146mg/mL of the water extract is also sufficient to sweep 50% of the $O_2^{\bullet-}$ radicals present, whereas in the case of ethanol extract sweeping is about 10 times higher. When the activity test was performed under the same conditions in the presence of SOD obtained from bovine erythrocytes, the IC_{50} value was calculated as being 10^{-27} mg/mL, which is quite small.
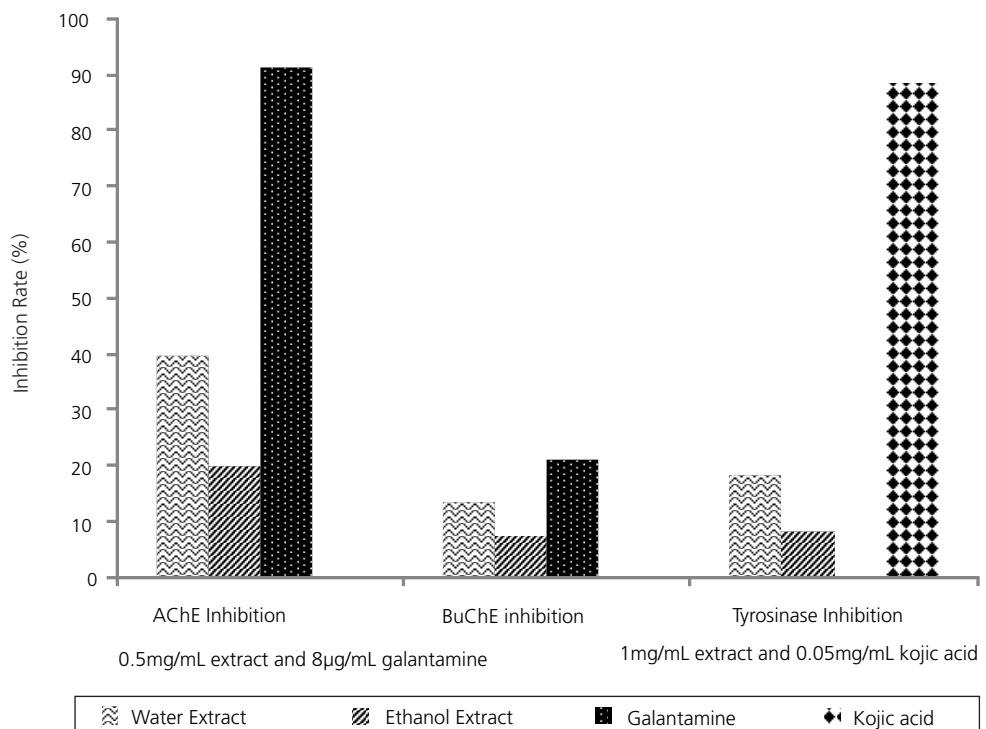

Figure 1. Anticholinesterase and antityrosinase activities of water and ethanol extracts of Talas brand Turkish coffee sample obtained from Sivas province. Ordu, Turkey, 2017.

Note: AChE: Acetylcholinesterase Enzyme; BuChE: Butrylcholinesterase Enzyme.

Catalase activities were calculated as 0.033 for water extract and 0.028U for ethanol extract. The activity of the commercially purchased enzyme is, no doubt, quite high as well as the activity of the extracts (1.74U for 0.01mg/mL catalase).

The degree of inhibition of ABAP, a well-known radical initiator, inducing lipid peroxidation of coffee extracts was evaluated. Both extracts at the concentration of 0.1mg/mL inhibited lipid peroxidation at the rate of 8%.

Acetylcholinesterase enzyme and Butyrylcholine estrerase inhibitions of 1mg/mL Talas brand ground coffee sample extracts were assessed and a moderate inhibition was observed. Although the degree of AChE inhibition is higher than BuChE, BuChE's inhibition is much more effective compared to galantamine (Figure 1).

Tyrosinase inhibition potentials at the concentration of 0.50mg/mL in ethanol and water extracts were 8.16% and 18.42%, respectively, and this value was calculated as being 88.50% for 0.05mg/mL of kojic acid.

DISCUSSION

It cannot be deduced that the unidentified phenolics detected in the HPLC analysis are not present in the extracts. This may mean that no effective amount of these phenolics was extracted. When the literature is examined, it is seen that the phenolic component analysis of coffee samples is concentrated on caffeine [30,31] and chlorogenic acid [9]. Not enough information is available on other compounds. Therefore, the present study provides important data for the literature.

The values obtained in terms of phenolic and flavonoid contents can be attributed to the fact that flavonoid-type components can be extracted in greater amounts in alcohol. When the results of the study of Köseoğlu Yılmaz *et al.* [32] were assessed, it was observed that the flavonoid content of the alcohol extract of the different roasted coffee samples was higher than the content in the water extract.

In a study conducted by Stelmach *et al.* [33] on green coffee infusions of 12 different coffe samples, total phenolic content was calculated to be 20.8-41.2mg GAE/g sample. Another study that demonstrates that our results are comparable with those obtained in studies conducted worldwide is the study of 58 different commercially available coffees (ground, soluble and caffeine-free) sold in Colombia [34].

According to those results, contribution of phenolic acids to total antioxidant activity can be considered to be much greater.

A concentration of 2mg/mL of 18 brands of Turkish coffee marketed in Turkey was shown to have a sweeping ability between 12.55% and 56.15% [9]. According to the results of the present study, it can be understood that the antioxidant activity of Talas brand Turkish coffee is much higher than that of the 18 other coffee brands.

It is estimated that extracts or compounds which prevent metal-induced free radical development by inhibiting the formation of complexes between ferrozine and iron can prevent lipid peroxidation. Due to their phenolic contents, they might have chelating activity and turn the transition metals stable [35]. From this point of view, it is very important to examine our extracts in terms of chelating activities and the ability of water and ethanol extracts to compete with ferrozine to form chelates with Fe^{2+} ions in the reaction medium compared with the standard chelating agent EDTA.

Most consumed coffee types in the western parts of Saudi Arabia [36] have been tested and it was found that the chelating potential of the tested Turkish coffee was much higher than that of the coffee types prepared with different methods

The reducing power of an extract is associated with its ability to transfer electrons and is another proof of the presence of antioxidant activity [37]. The reducing power of the water extract of the coffee sample examined is very close to the test standard ascorbic acid.

Compounds having a reducing power cause reduction of Fe3+/ferricyanide complex to Fe^{2+}; this reduction can be monitored by measuring absorbance at 700nm and the higher the absorbance the higher the reducing power. Erdem *et al.* [9] reported that the absorbance values at 700nm varied between 0.505 and 1.073 corresponding to the reducing power of 2mg/mL concentration of 18 different brands of Turkish coffee. It can be concluded that these values are lower than those observed for Talas brand Turkish coffee.

So far, studies on coffee antioxidative activity both in Turkey and in the world deal with non-enzymatic antioxidant activity. So, there is not enough data on the enzymatic antioxidant activity of coffee. However, hydroxycinnamic acid, one of the main coffee phenolics, is effective in sweeping $O_2^{\bullet-}$ radicals [38]. For this reason, Catalase and SOD activities were evaluated in order to reveal the enzymatic antioxidant activity of our coffee extracts. The SOD activities of extracts are quite low when compared to the commercial enzyme, but it should be remembered that we are working with raw extracts. On the other hand, the catalase activities of 0.05mg/mL of our water and alcohol extracts are quite similar, indicating that the extraction solvent has no effect.

Biological antioxidant capacity can also be measured by controlling induced lipid peroxidation [39]. Due to their role in causing disease and death, there is a strong interest in detecting and improving compounds that controlthe toxic effects of lipid peroxides [17]. Lipid peroxidation inhibition of the extracts may be considered to occur by presenting proton to the ABAP radicals of various phenolic substances in the extract composition. Several reports showed that caffeine as a coffee component inhibited lipid peroxidation induced by reactive oxygen species [31]. Contrary to what is expected, both tested extracts have an equally small potential for inhibition of lipid peroxidation without being affected by solvent differences. Because, in case of water extract, caffeic acid could not be detected during phenolic analysis.

Enzyme inhibitors are molecules that cause pharmacological effects through inhibition of certain enzymes and constitute half of the oral drugs in clinical use [40]. Although consumption of the Turkish coffee from Coffee Arabica beans is greatly appreciated in Anatolia and neighboring countries, there are only a few studies on the neuroprotective effects of coffee extracts. Studies on the cholinesterase and tyrosinase inhibition potentials of coffee samples are rare [9]. However, it is reported that the chlorogenic acid as a characteristic coffee component and the most abundant phenolic compound has a neuroprotective effect, which is an ester of caffeic and quinic acid [41].

The ethanol and water extracts of the coffee sample had relatively greater inhibition capability on acetylcholinesterase than butyrylcholinesterase. However, the effect of both extracts on these two enzymes is very little compared to galantamine.

Arendash & Cao [42] demonstrated the efficacy of moderate caffeine intake in the treatment of AD or for protection against AD in a mouse model. It is reported that potable water containing caffeine was given to mice suffering from AD and was effective to protect against memory weakness and couldreduce β-amyloid levels in the brain [32].

When we looked at the results of the study on the effects of enzyme inhibition on 18 different brands of Turkish coffee, it was observed that most of the samples at a concentration of 200μg/mL did not show AChE inhibition, but many showed BuChE inhibition at a very low concentration compared to galantamine.

Tyrosinase inhibition activity is useful in the suppression of melanogenesis which causes various dermatological disorders. It has also been reported that tyrosinase is associated with Parkinson's disease and other neurodegenerative diseases [40]. For these reasons, discovering tyrosinase inhibitors from natural sources is one of the most interesting research topics. However, there is not enough research on the antityrosinase activity of Turkish coffee, although there is information about other beneficial activities related to health.

Similar to the inhibition properties on cholinesterase, the water extract has a higher degree of tyrosinase inhibition compared to the alcohol extract. However, antityrosinase activity in both extracts is not very important besides kojic acid.

In the previous study on various coffee brands, antityrosinase activity was found in the range of 2.13%-15.72% for a concentration of 200μg/mL [9]. The coffee extracts we have examined in this context are also candidates for use in the cosmetic and health industry.

To summarize the results of the current study, it can be said that the ethanol and water extracts of Talas brand Turkish coffee contains significant phenolic substances and that it has a good antioxidant activity, which is thought to be due to such phenolic substances.

On the other hand, the present study brings a significant contribution to the very small number of studies available in the literature concerning the inhibition potential of Turkish coffee on some clinical enzymes. Furthermore, the fact that the water extract yielded a higher result for most of the investigated parameters is an important advantage for a resource consumed as a beverage.

CONCLUSION

As a result, it can be said that thanks to its antioxidative activity Turkish coffee, which is a beverage that can be consumed frequently, can be effective both in removing toxic effects of reagents formed after normal metabolic process and in the treatment and suppression of melanogenesis with diseases such as AD and PD that develop after various factors. Furthermore the consumption of coffee can contribute to the prevention and/or treatment of many diseases such as melanogenesis, AD, and PD.

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