Pharmacologia 3 (4): 113-120, 2012
ISSN 2044-4648 / DOI: 10.5567/pharmacologia.2012.113.120
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Evaluation of Phytochemicals and Antioxidant Activity of *Ginkgo biloba* from Turkey

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Abstract: Back ground: *Ginkgo biloba* has been described as a living fossil, being last remaining member of the Ginkgoaceae family. **Method:** Antioxidant activity of different extracts from *Ginkgo biloba* grown in Turkey was evaluated by β-carotene-linoleic acid model system, 2,2-diphenyl-1-picrylhydrazyl (DPPH) analysis and inhibition of H_2O_2. Total phenolic and flavonoid contents of the extracts were determined by Folin-Ciocalteu and flavonoid-aluminium chelating method. Several phytochemicals in the extracts were analysed by using high performance liquid chromatography. **Results:** The results indicated that methanolic extract had strong antioxidant activity than acetone and hexane extracts and also highest phenolic and flavonoid contents. The main components were catechin hydrate in methanol extract, rutin in acetone extract and quercetin in hexane extract. **Conclusion:** *Ginkgo biloba* grown in Turkey was also a potential source as antioxidant comparison with endemic *Ginkgo biloba* from China, Japan and America.

Key words: Antioxidant activity, *Ginkgo biloba,* phytochemical, HPLC, free radical

INTRODUCTION

The antioxidant activity of the plant extracts is mainly attributed to their phenolic constituents such as flavonoids, phenolic acids and polyphenolic compounds which neutralize free radicals including hydrogen peroxide (H_2O_2), superoxide (O^{-1}), hydroxyl (OH), peroxyl (ROO) by different mechanism including metal chelation and electron donation as reducing agent. Free radicals called Reactive Oxygen Species (ROS) are normal products of human metabolism (Vaya and Aviram, 2001; Wada and Ou, 2002). But in excess, they can cause oxidative damage to DNA, proteins and lipids (Fritz *et al*., 2003). Human metabolism has several mechanisms carried out by antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase to scavenge reactive oxygen species (Andersen and Markham, 2006). However, antioxidant defense mechanism does’t meet demand in the case of increase of ROS level in several diseases. Damage on the tissues occurs by the increase in the content of aldehydes or isoprostanes generated by lipid peroxidation, protein carbonyls produced from protein oxidation and oxidized base adducts generated from DNA oxidation (Boveris *et al*., 2007). A large number of plants naturally occuring substances have antioxidant activities, thus result into increase interest of herbal drug formed on major part of complementary and alternative medicine or traditional medicine in the world. Many researchers have focused on many plants as drug supplement. Most important one is *Ginkgo biloba*, largely demands on the markets in the world due to its phytochemical constitutes.

Ginkgo biloba has been described as a living fossil, being last remaining member of the Ginkgoaceae family. It dates back 250 million year in China. *Ginkgo biloba* leaves are used as traditional Chinese herbal medicine to treat asthma and chilblains and prevent to drunkeness for thousand years (Nakanishi, 2005). In recent years, *Ginkgo biloba* extract has been extensively studied due to its various medicinal properties in the world. It is known that *Ginkgo biloba* is widespread among elderly people as memory enhancing supplement. However, it is used in treating cardiac and cerebral diseases. The main reason of medicinal effect of *Ginkgo biloba* is to contain phytochemicals which have been reported to has protective effect on cardiovascular diseases, diabetes, aging and several cancer types (Saw *et al*., 2006; Boveris *et al*., 2007; Chan *et al*., 2007). This protective effect is attributed to antioxidant activity of *Ginkgo biloba* leaves (Pietta *et al*., 2000; Maltas *et al*., 2011).

There are a lot of reports on chemical analysis of *Ginkgo biloba* leaves and clinical approaches interested in *Ginkgo biloba* extracts and, particularly EGb 761, in the literature. EGb 761, extract from *Ginkgo biloba* leaves (IPSEN, Paris), is a standardized extract to contain 24% flavonoids, 7% proanthocyanidins and 6% terpenoids (Goh and Barlow, 2002). The flavonoids are primarily flavonol-glycosides of kaempherol, quercetin and isohamnetin with glucose or rhamnose. The terpenoid

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fraction consists of a unique group of diterpenes (ginkgolides A, B, C and J) and the sesquiterpene, bilobalide. The EGb-761 extract also contains a number of organic acids including kinurenic, hydroxykinurenic and vanillic acids. The specific concentration of ginkgolide B found in *Ginkgo biloba* leaves has been shown to vary depending on the season and the origin of the tree. EGb 761 is among the most commonly prescribed drugs in European countries, especially in Germany and France. However, there is no study on *Ginkgo biloba* grown in Turkey. It's known that climatical changes lead to carry out mutation or modification on genomic structure of the plants, led to changes on morphological and chemical structures (Perry *et al*., 1999). The global trend about such an important plant, *Ginkgo biloba,* is an opportunity for Turkish trade, too.

In this study, we aimed: (1) to compare the antioxidant activity of different extracts of *Ginkgo biloba* grown in Turkey using DPPH and β-carotene-linoleic acid assay (2) to identify the phytochemicals of *Ginkgo biloba* using HPLC (3) to find out the proper solvent to extract the antioxidant compounds from *Gingko biloba* leaves since antioxidant activity could be affected by the extracting solvents (4) to compare phytochemicals of *Gingko biloba* leaves from Turkey with previous studies on Ginkgo from different origins.

MATERIALS AND METHODS

Plant materials: *Ginkgo biloba* L. (Ginkgoaceae) leaves were collected from garden of Antalya Directorate of Foresty (Southern Turkey), June 2008. The plant was identified by Dr. Osman Tugay. A voucher specimen (O. Tugay 5658 and E. Maltas) is deposited in KNYA Herbarium of the Faculty of Science, Department of Biology, Selcuk University. Leaves were dried and powdered before experiments.

Chemicals: Gallic acid, rutin, quercetin, Butylated Hydroxytoluen (BHT), Butylated Hydroxyanisol (BHA), β-carotene, lineolic acid, Tween 20, Trichloroacetic Acid (TCA), trolox, α-tocopherol, chloroform, methanol, n-hexane and acetone were purchased by Merck. Folin-Coalcetau reagent and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) were obtained from Sigma Aldrich. All chemicals and reagents were of analytical grade. Double distilled water was used in the experiments.

Preparation of extract: Dried *Ginkgo biloba* leaves were extracted with three solvents, methanol, acetone and n-hexane, having different properties. Extraction procedures were same for all solvents. Each 50 g *Ginkgo biloba* leaves were extracted with each solvent at 30°C for 6 h. After resulting extracts were evaporated under vacuum to 10 mL, extract solutions were dried at -50°C in a lyophiliser. Yields of each extract including methanol, acetone and hexane were determined as 13, 7 and 6% (w/w), respectively. All extracts were kept in the dark at +4°C until used.

Determination of total phenolic content: Total phenolic content was measured by the method described by Singleton and Rossi (1965). All samples were analysed in three replications.

Determination of total flavonoid content: Total flavonoid content was measured by a spectrophotometric assay described by Ebrahimzadeh *et al.* (2008). All samples were analysed in three replications.

β-Carotene-linoleic acid assay: In β-carotene-linoleic acid assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Kartal *et al*., 2007). The antioxidant activity of *Gingko biloba* extracts was determined according to the method described by Ismail *et al.* (2004). For this purpose, a stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0.2 mg of β-carotene in chloroform was added to mixture including 200 mg of Tween 20 and 0.02 mL of linolenic acid. After the chloroform was evaporated, 100 mL of distilled water saturated with oxygen had been added vigorously. The 0.2 mL of this reaction mixture was dispensed to test tubes and 5 mL portions of the extracts prepared at concentration of 2 mg mL^{-1} were added and the emulsion system was incubated at 40°C. The same procedure was repeated with synthetic antioxidants, BHA and BHT, as positive control and control. Absorbance of the mixtures was measured at 470 nm during 120 min. Degradation Rate (DR) was calculated using the following equation based on Al-Saikhan *et al.* (1995):

$$DR = \ln\left(\frac{a}{b}\right)$$

where, a is the absorbance at initial time; b is the absorbance at 120 min. Antioxidant Activity (AA) was expressed as percent of inhibition relative to the control, using the following equation:

$$AA = \left[\frac{(DR_{control} - DR_{sample}}{DR_{control}}\right] \times 100$$

where, $DR_{control}$ is degradation rate of the control, DR_{sample} is degradation rate of the extracts or standard antioxidants.

Tests were carried out in triplicate and Butylated Hydroxytoluene (BHT) and Butylated Hydroxyansol (BHA) were also used as positive controls.

Scavenging activity of DPPH: The hydrogen atom or electron donation ability of the corresponding extracts and some pure antioxidants was measured from the bleaching of methanol solution of DPPH. The scavenging activity of DPPH free radical by *Gingko biloba* extracts was determined according to the method reported by Sanchez-Moreno *et al.* (1998). Inhibition by DPPH (I%) was calculated as follows:

$$I\% = \left[\frac{(A_{blank} - A_{sample}}{A_{blank}}\right] \times 100$$

where, A_{blank} is the absorbance of the blank (containing all reagents except the extract or standard), A_{sample} is the absorbance of the extracts or standard antioxidants.

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentrations. Tests were carried out in triplicate and Butylated Hydroxytoluene (BHT) and Butylated Hydroxyansol (BHA) were also used as positive controls.

Scavenging activity of hydrogen peroxide: The ability of the extracts to scavenge hydrogen peroxide (H_2O_2) was assessed by the method of Benkeblia (2005). The scavenging of hydrogen peroxide was determined as follows:

$$I\% = \left[\frac{(A_{blank} - A_{sample}}{A_{blank}}\right] \times 100$$

where, A_{blank} is absorbance of blank (extract in PBS without H_2O_2), A_{sample} is absorbance of the extract or standard with H_2O_2. Tests were carried out in triplicate and rutin and quercetin were also used as comparative standards.

Phytochemical analysis: *Ginkgo biloba* extracts used for antioxidant analysis were also used to determine antioxidant components. Several phytochemicals of *Ginkgo biloba* extracts such as phenolic acids and flavonoids were analysed using high performance liquid chromatography. These were gallic acid, catechin, caffeic acid, epicatechin, p-coumaric acid, ferulic acid, vitexin, rutin, naringin, hesperidin, apigenin, rosmarinic acid, eriodiktyol, quercetin, naringenin, luteolin, apigenin and also carvacrol as standards.

The HPLC system consisted of on an Shimadzu 1100 series HPLC equipped with a SIL-10AD vp autosampler and LC-10Advp pump system, Diode Array Detector (DAD) and an Inertsil Agilent Eclipse XDB column (240 mm×4.60 mm 5 μm particle size). The mobile phase was mixture of methanol (solvent A) and 3% aqueous acetic acid (solvent B). The mobile phase program was a linear gradient from 5 to 66.5% solvent A. An over 75 min at 0.8 mL min^{-1} and UV detection was at 278 nm. Twenty microliter of the standards and extracts solutions were injected. Amount of each antioxidant component given in Table 3 was calculated on the basis of the calibration curve for each one (Sigma).

Statistical analysis: The statistical analysis was carried out by using OriginPro 7.5 software. One way ANOVA was applied to data and results were compared by using Tukey test. The p-value lower than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Total flavonoid and phenolic contents of the extracts of *Ginkgo biloba*: Phenolic compounds such as phenolic acids and flavonoids which are widely distributed in fruits, vegetables and plants have the ability to scavenge active oxygen, superoxide and hydroxyl radicals by single-electron transfer. However, flavonoids are known to show antioxidant activity and to act as metal chelator. Flavonoids exert their action by scavenging or chelating process in antioxidant mechanism (Vaya and Aviram, 2001).

In our experiments, we determined total flavonoid and phenolic content of three extracts of *Ginkgo biloba.* The flavonoid contents of the extracts were measured in terms of quercetin equivalent, QE (the standard curve equation of quercetin with $y = 6.299x+0.088$, $R^2 = 0.995$). As shown in Table 1, total flavonoid contents of *Ginkgo biloba* extracts exhibited differences depending on extraction solvents. The flavonoid content in the methanolic extract of *Ginkgo biloba* (98.15±4.31 mg g^{-1}) was higher than those in the hexane (60.95±1.76 mg g^{-1}) and acetone extracts (28.30±1.13 mg g^{-1}). Total flavonoid contents of *Ginkgo biloba* extracts were in the order of methanol>hexane>acetone. There were significant differences among the extracts ($p<0.05$).

Main constitutes of the plant extracts are phenolic compounds that have important role in stabilizing lipid oxidation associated with antioxidant activity (Pourmorad *et al.*, 2006). Table 1 also show the total phenolic content that was measured by Folin Ciocalteu method in terms of gallic acid equivalent, GAE (Standard

Table 1: Total phenolic and flavonoid contents of different extracts from *Ginkgo biloba*

Extracts	Total phenolic[1] (mg GAE/g)	Total flavonoid[2] (mg QE/g)
Hexane	27.6±3.1	60.95±1.76
Acetone	59.4±1.6	28.30±1.13
Methanol	76.0±2.2	98.50±4.31

[1]Total phenolic content was expressed as Gallic Acid Equivalent (GAE). [2]Total flavonoid content was expressed as Quercetin Equivalent (QE). Data expressed (p = 0.05) as Means±Standard deviation (n = 3)

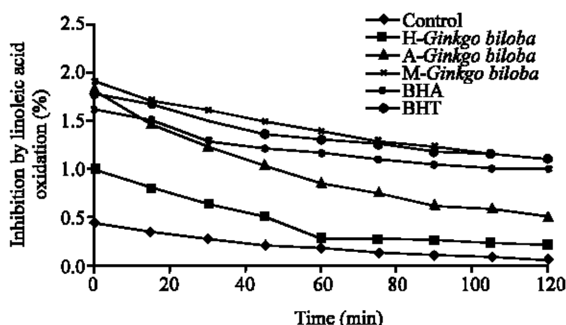

Fig. 1: Absorbance change of β-carotene at 490 nm in the presence of *Ginkgo biloba* extracts, M-*Ginkgo biloba*: methanolic extract, A-*Ginkgo biloba*: acetone extract H-*Ginkgo biloba*: hexane extract, control and positive control (BHA and BHT) during 120 min

curve equation of gallic acid; $y = 4.256x+0.065$, $R^2 = 0.984$). The highest level of total phenolic contents of the extracts was found to be 76.0±2.2 mg g^{-1} in the methanol extract. Total phenolic contents of hexane (27.6±3.1 mg g^{-1}) and acetone (59.4±1.6 mg g^{-1}) were lower than that of the methanolic extracts. There were significant differences among the extracts ($p<0.05$). Zheng and Wang (2001) have reported that total phenolic content of phosphate buffer extract (75 Mm, pH,7.5) of *Ginkgo biloba* leaves from Chicago, America was 1.57 mg GAE per g extract. Ellnain-Wojtaszek *et al.* (2001) collected *Ginkgo biloba* leaves from Poland during vegetative cycle and they measured total flavonoid content of *Ginkgo biloba* leaves collected in July as 8.9 mg QE per g extract. In our study, the results showed that the methanolic extract of *Ginkgo biloba* contains significantly more phenolics and flavonoids than those of the other extracts, hexane and acetone extracts and measured total phenolic and flavonoid contents of previous studies.

Antioxidant capacity in β-carotene-linoleic acid system: Antioxidant compounds inhibit volatile organic compounds and conjugated diene hydroperoxides arising from linoleic acid oxidation (Kartal *et al.*, 2007). In this method, as shown in Table 2, the highest inhibition was provided by the methanolic extract of *Gingko biloba* with

Table 2: DPPH scavenging activity, antioxidant activity by β-caroten-linoleic acid system of extracts from *Ginkgo biloba* and standards

Extracts	DPPH[1] (%)	AA[2] (%)
Hexane	31.2±3.2[a]	31.4±2.7[a]
Acetone	30.5±1.8[a]	38.2±1.3[a]
Methanol	85.0±3.0[b]	74.5±2.3[c]
BHT	95.8±1.0[b]	78.8±2.1[c]
BHA	98±0.6[b]	79.5±2.0[c]

[1]DPPH scavenging activity (%) at 1 mg mL^{-1} of extract or standard concentration. [2]AA, Antioxidant activity by β-caroten system (%) at 2 mg mL^{-1} of extract or standard concentration. Data expressed as Mean±Standard deviation (n = 3). Means within each column with different letters (a-c) differ significantly (p = 0.05)

74.5±2.3% followed by the acetone extract with 38.2±1.3% and the hexane extract with 31.4±2.7% at extract concentration of 2 mg mL^{-1}. However, the inhibition of the methanolic extract was approximately same value as BHA (79.5±2.0%) and BHT (78.8±2.1%) at the end of 120 min (Fig. 1). Shrifar *et al.* (2003) has been reported similar value (77.20±1.38%) for antioxidant capacity of the methanolic extract of *Ginkgo biloba* from Iran by β-caroten-linoleic acid system.

The higher amount of phytochemicals such as flavonoid and phenolic compounds in different extracts of *Gingko biloba* might contribute to their higher antioxidant activity in β-carotene-linoleic acid system. However, the methanolic extract having polar phenolics due to polar property of methanol was also effective in licoleic acid oxidation. Non-polar phenolics in the acetone and hexane extracts might show low inhibiting effect on the system.

Scavenging activity of DPPH: Free radicals such as oxygen, superoxide and hydroxyl are biologically important substances which naturally release from human tissues. The highly reactive radicals can cause oxidative damage to DNA, lipids and proteins (Boveris *et al.*, 2007; Fritz *et al.*, 2003). Therefore, free radicals result in many disorders like cancer, cardiovascular diseases and diabetes mellitus (Velioglu *et al.*, 1998; Vaya and Aviram, 2001). Main compounds carried out free radical scavenging are substances having antioxidant activity such as flavonoid and phenolic compounds or phenolic-rich plant extracts. The method of DPPH free radical scavenging can be used to evaluate the antioxidant activity of specific compounds or extracts. Figure 2 showed that inhibition percentage of methanol, acetone and hexane extracts of *Ginkgo biloba* ranged from 0.1-1 mg mL^{-1} and BHA and BHT as standards. Inhibition percentage of scavenging activity by DPPH for all extracts and standards was given in Table 2.

As shown in Table 2, the highest inhibition was provided by the methanolic extract of *Gingko biloba* with 85.0±3.0% in DPPH method. Inhibition was approximately

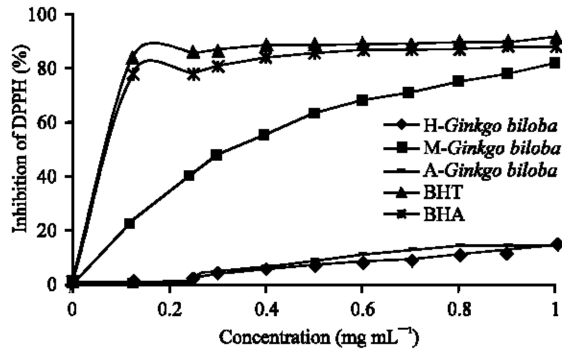

Fig. 2: Free radical scavenging ratio (%) against increasing concentration of extracts from *Ginkgo biloba*, M-*Ginkgo biloba*: methanolic extract, A-*Ginkgo biloba*: acetone extract, H-*Ginkgo biloba*: hexane extract and positive controls (BHA and BHT) on 1,1-diphenyl-2-picrylhydrazyl radicals

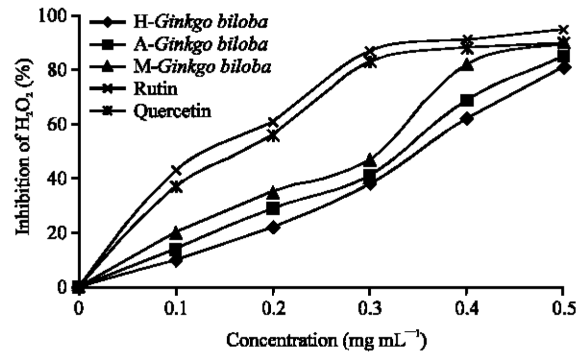

Fig. 3: Inhibition ratio (%) against increasing concentration of extracts from *Ginkgo biloba*, M-*Ginkgo biloba*: methanolic extract, A-*Ginkgo biloba*: acetone extract H-*Ginkgo biloba*: hexane extract and positive controls (rutin, quercetin on 1,1-diphenyl-2-picrylhydrazyl radicals

Table 3: IC_{50} values of extracts from *Ginkgo biloba* and standards

Extract	Methanol extract	Acetone extract	Hexane extract	BHT	BHA
IC_{50} (mg)	2.36±0.01	3.55±0.49	4.30±0.14	0.020±0.001	0.035±0.007

Data expressed (p = 0.05) as Means±Standard deviation (n = 3)

Table 4: Inhibition by H_2O_2 of extracts from *Ginkgo biloba* and standards

Extract	Methanol extract	Acetone extract	Hexane extract	Rutin	Quercetin
Inhibition(%)	89.1±1.1	48.1±0.8	20.5±0.9	89.9±0.8	96.1±0.5

Inhibition by H_2O_2 (%) at 0.5 mg mL^{-1} of extract or standard concentration. Data expressed (p = 0.05) as Means±Standard deviation (n = 3)

same values in the acetone extract with 30.5±1.8% and the hexane extract with 31.2±3.2%. However, the inhibition of the methanolic extract was lower than BHA (98±0.6%) and BHT (95.8±1.0%) at extract concentration of 1 mg mL^{-1} (Fig. 2). 50% inhibition (IC_{50}) of each extract and standard compound was also given in Table 3. The highest radical scavenging activity was observed by the metanolic extract with 2.36±0.01 mg mL^{-1}. It was observed that IC_{50} value of the methanolic extract was higher than those of acetone and hexane extracts but, it was lower than those of BHT with 0.020±0.001 mg mL^{-1} and BHA with 0.035±0.007 mg mL^{-1} ($p<0.05$). There were no significant difference among the hexane extract with 4.30±0.1 mg mL^{-1} and the acetone extract with 3.55±0.49 mg mL^{-1}.

In the DPPH method, the methanolic extract with high content of phenolic and flavonoid exhibited a significant higher scavenging effect compared with the other extracts. DPPH scavenging ability of the hexane extract by DPPH is approximately equal to that of the acetone extract depending on their total phenolic and flavonoid contents. However, the scavenging ability of BHT and BHA as standards was very close to that of the methanolic extract of *Ginkgo biloba* at a concentration of 1 mg mL^{-1}. Present results indicated that high scavenging ability on the DPPH radicals could be due to the phenolic and flavonoid contents in the extracts (Maltas *et al*., 2011).

Scavenging activity of hydrogen peroxide: Hydrogen peroxide is one of the product from normal metabolic pathway of human organs. It is not very reactive itself. It is known that hydrogen peroxide causes to release hydoxyl radical in the cell. Therefore, it can be toxic for human cell and tissue (Benkeblia, 2005). However, in the human metabolism, removing reactive hydrogen species is provided by enzymes such as superoxide dismutase, catalase and glutathione peroxidase by transforming them into relatively stable compounds (Vaya and Aviram, 2001). In the excess of hydrogen peroxide, enzyme is sufficient to scavenge hydroxyl radical releasing through H_2O_2. Antioxidants compounds such as flavonoids and phenolic acids inhibit significantly oxidative processes while often being oxidized themselves. In our experiments, the scavenging activity of *Gingko biloba* extracts on hydrogen peroxide was shown in Fig. 3 and compared with those of rutin and quercetin as standards. As shown in Table 4, scavenging activity of *Ginkgo biloba* extracts on H_2O_2 was in the order of methanolic (89.1±1.1%)> acetone (48.1±0.8%)>hexane (20.5±0.9%) at extract concentration of 0.5 mg mL^{-1}. There were significant differences ($p<0.05$) among the extracts. Where scavenging activity of the methanolic extract was the highest in all extracts of *Ginkgo biloba,* it was lower than that of quercetin (96.1±0.5%) and same with that of rutin (89.9±0.8%).

Table 5: Content of several phenolic and flavonoid compounds of *Ginkgo biloba* extracts ($\mu g\ g^{-1}$)

Compound	Methanol extract	Acetone extract	Hexane extract
Gallic acid	-	-	-
Catechin hydrate	935.4±1.97	-	-
Caffeic acid	49.25±0.07	18.9±0.7	-
Epicatechin	-	-	-
p-coumaric acid	-	9.85±0.21	-
Ferulic acid	6.75±1.34	-	-
Vitexin	-	-	-
Rutin	-	903.75±21.70	28.25±0.21
Naringin	-	-	-
Hesperidin	-	-	-
Rosmarinic acid	-	-	-
Eriodictyol	136.75±1.06	49.80±0.84	-
Quercetin	-	6.30±0.42	470.01±0.01
Naringenin	-	4.55±0.07	-
Carvacrol	-	-	-
Σ Total	1160,79	992,34	498.25

*Data expressed ($p = 0.05$) as Means±Standard deviation ($n = 3$)

The results indicated that scavenging activity by H_2O_2 was correlated with the scavenging activity by DPPH and linoleic inhibition for three extracts of *Ginkgo biloba*. The methanolic extract of *Gingko biloba* had highest antioxidant activity, followed by the acetone and hexane extracts in all antioxidant activity methods, respectively. The results showed that there was a good correlation between the antioxidant activity and total phenolic contents of each extract. It was concluded that high antioxidant activity was attributed to high total flavonoid and phenolic contents.

Phytochemical analysis: Several phenolic acids and flavonoid contents of the extracts were compared qualitatively and quantitatively depending on fifteen standard antioxidant compounds by HPLC analysis. The main compounds were catechin hydrate (935.4 $\mu g\ g^{-1}$) in the methanolic extract, rutin (903.75±21.70 $\mu g\ g^{-1}$) in the acetone extract and quercetin (470±0.01 $\mu g\ g^{-1}$) in the hexane extract. The highest amount of the total phenolic contents in three extracts of *Ginkgo biloba* might be attributed their more effective in antioxidant properties. Sum of them in methanolic, acetone and hexane extracts were 1160.79, 992.34 and 498.25 $\mu g\ g^{-1}$, respectively (Table 5). However, several compounds of the standards which were not observed in any extract of *Ginkgo biloba* were naringin, hesperidin, rosmarinic acid, vitexin, carvacrol and gallic acid. As a result of chromatographic analysis, catechin hydrate, quercetin and rutin were mainly contributed to the antioxidant activity of *Ginkgo biloba* grown in Turkey. There are many studies on chemical compositions of *Ginkgo biloba* leaves grown in Japan, China and America that were origin of *Ginkgo biloba* (Yang *et al*., 2002; Lichtblau *et al*., 2002). Jiang *et al*. (2008) analysed rutin, apigenin and luteolin in the methanolic extract of *Ginkgo biloba* from China. However, they have reported that eriodictyol was absent in their extract. Ginkgo leaves from different sources, USA, China and Columbia, were extracted with mixture of methanol:water (60:40, v/v) by Lin *et al*. (2008). And also phytochemicals of Ginkgo leaves were identified by LC-MS and the results were compared. Data indicated that there were no differences in the phytochemicals of *Gingko biloba* from different sources. All extracts contained myrcetin, quercetin, kaempferol, isorhammetin and luteolin. Li *et al*. (2004) has been identified flavonol aglycones of methanolic extract of *Ginkgo biloba* from Taiwan by H^1 nuclear magnetic resonance method. Major flavonol aglycones in Ginkgo leaves were found to be kaempferol, quercetin and isorhamnetin. In our study, the methanolic extract of *Ginkgo biloba* showed different results in comparison of previous studies. We have identified eriodictyol, ferulic acid, caffeic acid and catechin. However, Zheng and Wang (2001) have reported that caffeic acid was found to be in the phosphate buffer extract (75 Mm, pH,7.5) of Ginkgo leaves from Chicago, America. Colchicine in the methanolic extract of *Ginkgo biloba* was identified with LC-MS by Li *et al*. (2002). Several phenolic structures of acetone extract of *Ginkgo biloba* leaves (Nutrasource, USA) have been reported to be as quercetin and apigenin that were identified by NMR (Bedir *et al*., 2002). In comparison of the previous studies of *Ginkgo biloba*, the phytochemicals of *Ginkgo biloba* grown in Turkey showed significantly difference than those of *Ginkgo biloba* grown in China, America, Columbia and Taiwan. Also different extraction solvents resulted in differences on phytochemical distributions of *Ginkgo biloba* leaves according to the solvent properties, led to the different antioxidant properties for each extract.

Many studies on health benefits have been linked to the catechin, quercetin and rutin in *Ginkgo biloba* leaves grown in Turkey. Rutin and catechin are effective flavonoid component in most of medicinal plants. Rutin has been reported to have clinically relevant functions including antihypertensive, anti-inflammatory, antihemorrhagic activity and strengthen of the capillary permeability and stabilization of platelets (Guo and Wei, 2008). The pharmaceutical effects can be attributed to strong antioxidative and free-radical-scavenging activities of rutin (Yang *et al*., 2001). However, rutin's polyphenol structure makes it very sensitive to changes in the surroundings which can alter the planarity, hydrophobicity and electrostatic components and eventually can lead to changes in its antioxidant properties. Catechins have also been shown to possess antibiotic properties due to their role in disrupting a specific stage of the bacterial DNA replication process

(Gradisar *et al*., 2007). Quercetin is major flavonoid as a bioflavonoid in the human diet and its daily intake with foods is estimated to be 50-500 mg (Deschner *et al*., 1991). It is known to inhibit free radical processes in cells (Afanasev *et al*., 1989). It is able to protect cutaneous tissue-type cell populations, fibroblasts/keratinocytes and endothelial cells of human origin, from cytotoxic oxidative stress induced by protracted depletion of cellular glutathione (Katsarou *et al*., 2000; Coskun *et al*., 2004).

CONCLUSION

This study is the first to identify the phytochemical structures and to determine antioxidant activity of different extracts of *Ginkgo biloba* from Turkey. Data indicated significant difference on chemical profiles of *Ginkgo biloba* from Turkey comparison with previous studies. Depending on the phytochemical distribution in the extracts, antioxidant activity of the methanolic extract was the most effective by β-carotene-linoleic acid model system, 2,2-diphenyl-1-picrylhydrazyl (DPPH) analysis and inhibition of H_2O_2. The results indicated that the highest phenolic and flavonoid contents were in the methanolic extract. Consequently, phenolic acids and flavonoids are moderately associated with antioxidant properties. Antioxidant activity of the extracts were mainly attributed to catechin hydrate, rutin and quercetin. In conclusion, obtained results from this study suggest that *Ginkgo biloba* from Turkey is a potential source of natural antioxidants. In addition, analysis of the other phytochemicals and the antioxidant mechanisms of the extracts of *Ginkgo biloba* have been continued to study to gain more understanding of their antioxidant activity in our laboratory.

ACKNOWLEDGMENTS

We thank Scientific Research Foundation of Selcuk University (BAP) for providing foundation with 08101019 of project number. This study is a part of Esra Maltas's Ph.D. thesis being continued.

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