Comparison of Antioxidant and Antimicrobial Activities of Tilia (*Tilia Argentea Desf Ex DC*), Sage (*Salvia Triloba L.*), and Black Tea (*Camellia Sinensis*) Extracts

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The antioxidant activity of the water extract of *Tilia argentea Desf ex DC* was determined by the thiocyanate method. The antioxidant activity of the water extract increased with the increasing amount of lyophilized extract (50−400 μg) added into the linoleic acid emulsion. Statistically significant effect was determined in 100 μg and higher amounts. Antioxidant activities of water extracts of tilia (*Tilia argentea Desf ex DC*), sage (*Salvia triloba L.*), and two Turkish black teas commercially called Rize tea and young shoot tea *(Camellia sinensis*) were compared. For comparison studies, 100 μg portions of extracts were added into test samples. All samples were able to show statistically significant antioxidant effect. Both of the tea extracts showed highest antioxidant activities, nevertheless, differences between tilia and sage and tilia and tea were not statistically significant (for both cases $p > 0.05$). Like antioxidant activity, the reducing power of water extract of *Tilia argentea Desf ex DC* was also concentration dependent. Even in the presence of 50 μg of extract, the reducing power was significantly higher than that of the control ($p < 0.05$) in which there was no extract. Unlike antioxidant activity, the highest reducing power activity was shown by sage extract. Among the tea extracts, young shoot extract was the most effective one, however, it had significantly lower activity than sage ($p < 0.05$). Although tea flower had the lowest reducing power activity, it was higher than that of tilia. But this difference was not statistically significant ($p > 0.05$). From these results, we could suggest that although the reducing power of a substance may be an indicator of its potential antioxidant activity, there may not always be a linear correlation between these two activities. In addition, antimicrobial activities of each of the above extracts were studied by disk diffusion methods on different test microorganisms. None of the extracts showed antibacterial activity on the studied microorganisms.

Keywords: *Antioxidant activity; reducing power; antimicrobial activity; tilia; sage; black tea*

INTRODUCTION

Reactive oxygen species (ROS), sometimes called active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions ($O_2^{\bullet -}$) and hydroxyl radicals ($OH^{\bullet}$), as well as nonfree-radical species such as hydrogen peroxide (H_2O_2) (Halliwell, 1995; Squadriato et al., 1998). In living organisms various ROS can form by different ways. Normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides (Halliwell and Gutteridge, 1989; Halliwell, 1994; Davies, 1994; Robinson et al., 1997). Free radicals can cause lipid peroxidation in foods which leads to the deterioration of the food (Sasaki et al., 1996; Miller et al., 1995). In addition, reactive oxygen species have been implicated in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes, and cancer (Alho and Leinonen, 1999; Tanizawa et al., 1992; Duh, 1998; Hertog, 1993). When produced in excess ROS can cause tissue injury. However, tissue injury can itself cause ROS generation (Auroma, 1998). Nevertheless, all aerobic organisms, including human beings, have antioxidant defenses that protect against oxidative damages, and numerous damage removal and repair enzymes are present to remove or repair damaged molecules (Davies, 1994; Granelli et al., 1995; Fridowich, 1995; Sun et al., 1998). However, this natural antioxidant mechanism can be inefficient; hence, dietary intake of antioxidant compounds becomes important (Halliwell, 1994; Duh, 1998; Terao et al., 1994). Although there are some synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which are commonly used in processed foods, it has been reported that these compounds have some side effects (Branien, 1975; Ito et al., 1983). In addition, it has been suggested that there is an inverse relationship between dietary intake of antioxidant-rich foods and the

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incidence of number of human diseases (Rice-Evans et al., 1997). Therefore, research for the determination of the natural antioxidants source is important.

In Turkish folk medicine, Tilia species are traditional medicinal plants which have been used for various purposes, such as sedatives, tranquilizers, diuretics, and expectorants, and for diaphoretic activities. For this purpose, the infusion of their inflorescence is used to prepare a tea. Some reports indicate that tilia also has more activities such as anxiolytic (Viola et al., 1994) and antistress (Aydin et al., 1992); and in addition it promotes intestinal iron absorption (Elshobaki et al., 1990) and helps digestion as well (Sokolov 1986). However, so far, there is no report related to the antioxidant activities of *Tilia argentea Desf ex DC*. In contrast, there are some reports about the antioxidative activities of *Salvia officinalis L*. (Cuvelier et al., 1994; Wang et al., 1999). Tea (*Camellia sinensis*) is the most widely consumed beverage worldwide. However, the type and quantity of tea taken varies in different countries and races. Black (fermented) tea is popular in the West; oolong type (semifermented) tea is commonly drunk in Taiwan and parts of China; green (nonfermented) tea is favored in the rest of China, Northern Africa, and Japan (Benzie and Szeto 1999). There are several reports related to the antioxidant activities of various teas (Robinson et al., 1997; Yen et al., 1997; Chung et al., 1998; Lin et al., 1998).

In the present study, antioxidant activity of the infusion of inflorescence of *Tilia argentea Desf ex DC*, one of the species found in the market, was determined. In addition, antioxidant activities of *Salvia triloba L* and some of the Turkish black teas, which are sold in markets under different commercial names, were compared with the above tilia. Also, it was of interest to determine antimicrobial activities of these extracts and this was carried out by the disk diffusion method.

MATERIALS AND METHODS

Preparation of Extracts. Linden flowers (*Tilia argentea Desf. ex DC*), sage (*Salvia triloba L.*), and studied black tea samples were purchased from a market. A 15 g dried sample esd chopped into small parts in a blender and then extracted with 300 mL of boiled water by stirring for 30 min. The extraction was followed by filtration. Afterward, the filtrate was freeze-dried in a freeze-dryer at 5 μHg pressure at −50 °C. Different amounts of freeze-dried yields were obtained for the different extracts.

Antioxidant activity. Antioxidant activity was determined according to the thiocyanate method. Briefly, each sample (containing 50−400 μg of extract) in 0.5 mL of distilled water was mixed with 2.5 mL of linoleic acid (Sigma) emulsion (0.02M, in 0.02 M pH 7.0 phosphate-buffered saline, Sigma) and 2 mL of phosphate-buffered saline (0.02M, pH 7.0) in a test tube and incubated in darkness at 37 °C. The amount of peroxide was determined by reading absorbance at 500 nm after coloring with $FeCl_2$ and thiocyanate at intervals during incubation (Yen and Chen, 1995).

Reducing Power. Extracts (50−500 μg) in 1 mL of distilled water were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide [$K_3Fe(CN)_6$] (1%), then the mixture was incubated at 50 °C for 30 min. Afterward, 2.5 mL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL of the upper layer solution was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%), and the absorbance was measured at 700 nm (Yen and Chen, 1995). Increased absorbance of the reaction mixture indicated increased reducing power.

Antimicrobial Activity. To be able to determine antimicrobial activities, *S. aureus* ATCC 25923, *C. albicans* ATCC 60193, *E. coli* ATCC 25922, *B. subtilis* ATCC 6633, and *P. aeruginosa* ATCC 10145 were used. These were obtained from the Karadeniz Technical University, Medical School, Department of Microbiology and Clinical Microbiology, located in Trabzon, Turkey.

Antimicrobial activities were determined by the disk diffusion method (Ingolfsdottir et al., 1997). Briefly, 50 mg of freeze-dried sample was dissolved in 50 mL of distilled water, and then 10 μL, 50 μL, and 100 μL aliquots of this solution were transferred into separate 6-mm diameter paper disks (Whatman 1). Afterward, disks were left at room temperature to dry. Distilled water absorbed paper disks were used as control. As a standard, penicillin G (Oxoid) antibiotic absorbed disks were used.

Test microorganisms grown on nutrient agar (Oxoid) (for bacteria) or on potatoes dextrose agar (Oxoid) (for fungus) for 24 h were transferred into 12-cm diameter Petri dishes (containing solid media) by using a sterile, cotton-wool-covered wand. Subsequently these microorganisms were spread over the surface of the solid media as a thin film. Finally, Petri dishes were incubated at 37 °C for 48 and 72 hours for bacteria and fungus, respectively, and then inhibition zones were observed.

Statistical Analysis. Statistical calculations were done by using SPSS 9.0 software. To be able to determine the statistical significance of antioxidant activity results first, one-way variance analysis was applied, which showed that there was a statistically significant difference ($P < 0.01$), then, multiple comparison was carried out by LSD test. Results related to the reducing power activities were statistically analyzed by applying the Student *t*-test. Values of $p < 0.05$ were considered to be significant and values of $p < 0.01$ very significant.

RESULTS AND DISCUSSION

Antioxidant Activity. In the present study, antioxidant activity of water extracts of various black teas, sage, and tilia (linden flowers) was determined by the thiocyanate method: the amount of peroxides formed in emulsion during incubation is determined spectrophotometrically by measuring absorbance at 500 nm. High absorbance is the indication of high concentration of formed peroxides.

The yields of freeze-dried extract were varied. Highest yield was obtained from bud tea, whereas the lowest yield was obtained from linden flowers: 2.42 and 1.25 g, respectively.

Antioxidant activity of water extract of *Tilia argentea Desf ex DC* is increased with increasing amount of extract. As it can be seen in Figure 1, addition of even 50 μg of lyophilized extract into the linoleic acid emulsion was able to reduce the formation of peroxides. However, this effect was not statistically significant: $p > 0.05$ between control and 50 μg of extract containing sample. In the presence of 100 or 200 μg of dried extract, the formation of superoxide was suppressed at about 18 h, and after that absorption values started to increase. The difference between these extracts and the control was statistically highly significant, $p < 0.01$. Addition of 300 or 400 μg of extract into the linoleic acid emulsion system was able to prevent the increase of absorbance even after 33 h of incubation, in that absorbance of the control started to decline. There was no statistically significant difference between 100 and 300 μg or 100 and 400 μg, $p > 0.05$. Hence, addition of higher than 400 μg extract was not tested.

There were not statistically significant differences between antioxidant activities of black tea with various commercial names (data are not shown). Hence, to be

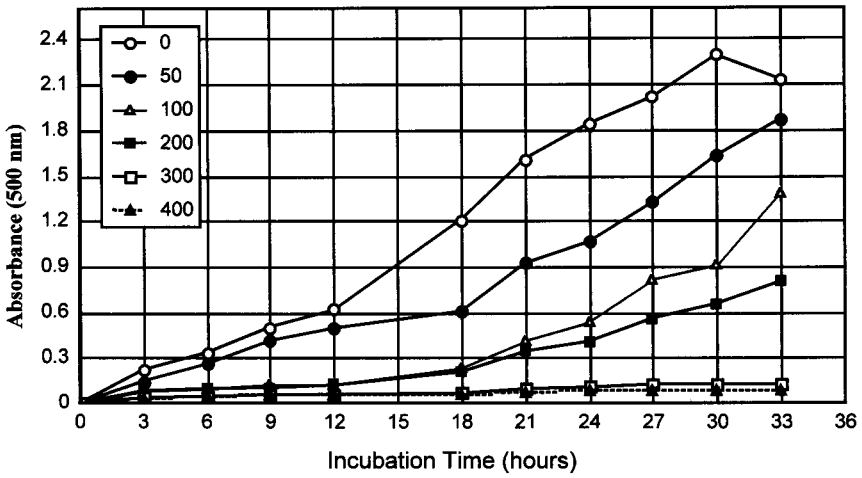

Figure 1. Antioxidant activities of water extracts of *Tilia argentea Desf ex DC*. Indicated amounts of extracts were added into each sample.

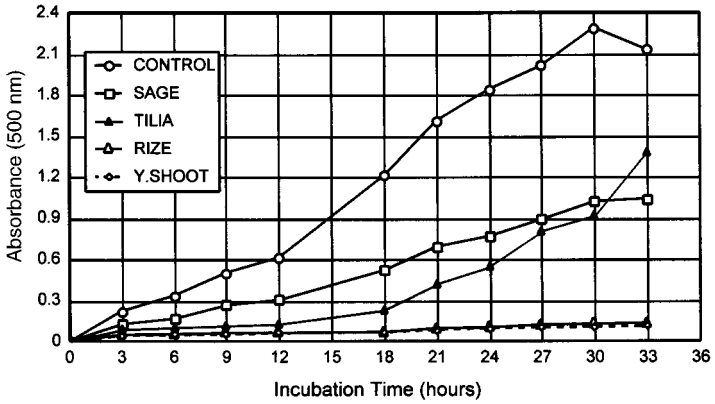

Figure 2. Antioxidant activities of water extracts of tilia (*Tilia argentea Desf ex DC)*, sage (*Salvia triloba L.*), and two Turkish black teas commercially called Rize tea and young shoot tea *(Camellia sinensis*). There was 100 μg of indicated extract in each sample; the control contained no extract.

able to compare the antioxidant activity of the water extract of *Tilia argentea Desf ex DC* with water extracts of *Salvia triloba L.*, two black teas commercially called Rize tea and young shoot tea were chosen. For comparison studies, 100-μg extracts were added into test samples; the lowest amount of tilia extract showed significant antioxidant activity. Both of the tea extracts showed highest antioxidant activity (Figure 2). Although tilia had shown lower antioxidant activity than tea, it was more effective than sage (Figure 2). However, differences between tilia and sage and tilia and tea were not statistically significant (for both cases $p > 0.05$). Nevertheless, the difference between the control and the sage extracts was statistically highly significant ($p < 0.01$), however, sage was significantly different from tea ($p < 0.05$).

Reducing Power. Like antioxidant activity, the reducing power of the water extract of *Tilia argentea Desf ex DC* was also concentration dependent. Hence, the reducing power of the extract is increased as the amount of extract is increased (Figure 3). Even in the presence of 50 μg of extract, reducing power was significantly higher than it was for the control ($p < 0.05$) in which there was no extract. The reducing power of 300 μg of extract was significantly higher than that of the 50, 100, and 200 μg extracts, but there was no significant difference between 300 μg of extract and 400 μg of extract ($p > 0.05$).

To compare reducing powers of various black teas, sage, and tilia, 100 μg extracts were added into the test mixture. Unlike antioxidant activity, *Salvia triloba L.* extract showed the highest reducing power activity (Figure 4). Although the reducing power of *Tilia argentea Desf ex DC* was the lowest of the samples, its activity was highly significantly different from that of the control ($p < 0.01$) Among the tea extracts, the young shoot extract was most effective, however, it was significantly lower than that of sage ($p < 0.05$). Although tea flower showed the lowest reducing power, its activity was higher than tilia, but the difference was not statistically significant ($p > 0.05$).

It was interesting to find that although the sage extract had the lowest antioxidant activity, it was the most effective one in reducing power. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir et al., 1995). However, we have previously found that this may not always be the case (Yıldırım et al., in press). Furthermore, the antioxidant activities of putative antioxidants have been attributed to various mechanisms, among these are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging (Diplock ,1997). Hence, we can suggest that there may not always be linear correlation between total antioxidant activity and reducing power activity.

In the present study it was found that the water

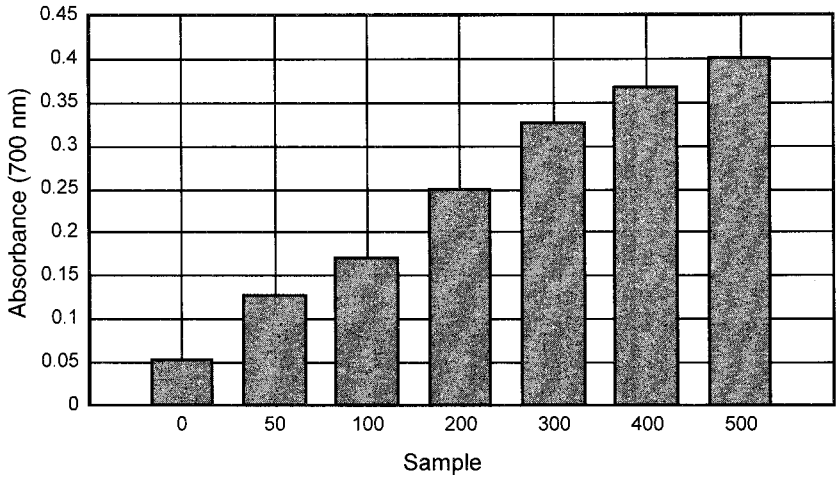

Figure 3. Reducing powers of water extracts of *Tilia argentea Desf ex DC*. Indicated amount of dried extract was added into test sample.

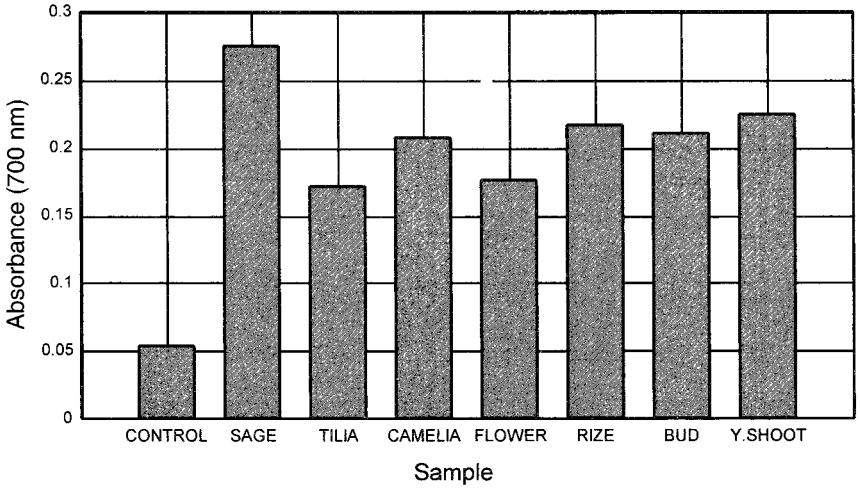

Figure 4. Reducing powers of water extracts of tilia (*Tilia argentea Desf ex DC)*, sage (*Salvia triloba L.*), and some of Turkish black teas *(Camellia sinensis*) commercially called different names. There was 100 μg of indicated extract in each sample; the control contained no extract.

extract of *Tilia argentea Desf ex DC* has antioxidant activity which is higher than that of sage, which is known to have antioxidant activity (Cuvelier et al., 1994; Wang et al., 1999). Some Turkish black teas commonly consumed in Turkey had high antioxidant activities and they were higher than those of sage and tilia extracts. It has been reported that there is an inverse relationship between dietary intake of antioxidant-rich foods and incidence of number of human diseases (Rice-Evans et al., 1997). In addition, antioxidant compounds, which are responsible for this activity, could be isolated and then used as food additives to delay deterioration of food due to oxidation. Therefore, research to identify antioxidant-rich foods is important.

Antimicrobial Activity. Positive and negative controls were used in this study and no activity was found for the highest concentration of extract on any of the test organisms. In contrast to this absence of antimicrobial activity, the extracts of tea, sage, and tilia had shown high antioxidant activity. It has previously been reported that phagocytic cells generate reactive oxygen species as part of their armory against invading microorganisms (Rice-Evans et al., 1994). Hence, we could speculate that as the above extracts had shown antioxidant activity which suppresses the activities of reactive oxygen species, thus, one can expect that these extracts could have no antimicrobial activity.

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