pISSN 1225-4339 eISSN 2287-4992
Korean J. Food Nutr. Vol. 35. No. 5, 313~323 (2022)
https://doi.org/10.9799/ksfan.2022.35.5.313

THE KOREAN JOURNAL OF
한국식품영양학회지
FOOD AND NUTRITION

Antioxidant Effect of Tea Tree Root Extracts using Various Extraction Methods

Hyun-suk Choi, Myung-ja Lee*, So-young Kwak** and †Dubok Choi***

Associate Professor, Dept. of Hotel Culinary Arts Patissier & Nutrition, Chungcheong University, Chungju 28171, Korea
**Director, Korean Tea Culture Federation (Incorporated Association), Changwon 51764, Korea*
***Doctor Course, Dept. of Medical Sciences, Graduate School of Nambu University, Gwangju 62271, Korea*
****Professer, Faculty of Advanced Industry Convergence, Chosun University, Gwangju 61452, Korea*

Abstract

To investigate antioxidant effects of tea tree root extracts using various extraction methods, cytotoxicity, DPPH and ABTS radical scavenging, SOD, nitrite scavenging activity and inhibitory activity of lipid peroxidation, reducing power, ferrous ion chelating activity were measured. Cytotoxicity for RAW 264.7 cells was not observed at concentrations treated with below 90 μg/mL in all extracts. The maximum DPPH radical, nitrite scavenging, SOD activity and inhibitory activity of lipid peroxidation were obtained at the ethylacetate and 70% ethanol extract. The maximum ABTS radical scavenging activity was obtained at the ethylacetate and hot water extract. However, in the case of reducing power and ferrous ion chelating activity, they were obtained at 70% ethanol and hexane extract, respectively. Nitrate scavenging activity showed the most excellent scavenging ability of 59.6% at 90 μg/mL of ethylacetate. The hexane extract had the highest ferrous ion chelating activity, showing 61.05% at 50 μg/mL, 66.07% at 70 μg/mL and 76.81% at 90 μg/mL, respectively. The results of this research show that the ethylacetate and 70% ethanol extracts of tea tree root can be used as a natural material for scavenging the radicals. However, future study is necessary to understand the mechanism of antioxidant activity by identification of substances.

Key words: tea tree root, cytotoxicity, antioxidant activity, nitrate scavenging activity, ferrous ion chelating activity

Introduction

The desire for healthy life and external beauty increases as the average lifespan of humans has been increased. Especially, the awareness of health and well-being increases as the standard of living increases, interest in disease prevention and aging suppression is increasing. Humans must produce energy through breathing, in which part of the oxygen is converted into reactive oxygen specifications (ROS) (Hwang et al. 2011). ROS is a general product produced under various conditions, such as habit or environmental factors, as well as breathing. In addition, ROS is a necessary substance, such as playing a role in allowing cells to grow and differentiate and protecting the human body from external invading antigens (Woo et al. 2010). However, if not removed, oxidative stress is caused in the human body by the remaining free radicals (Fiers et al. 1999).

Recently, active oxygen species including superoxide anion, hydroxy radical, and hydrogen peroxide have been overproduced due to external factors such as specific pollutants, organic solvents, pesticides, and cigarette smoke or internal factors of the human body (Seo et al. 2008). They cause lipid hyperoxidation, reducing cell membrane damage and antioxidant levels in cells, resulting in an imbalance between defense of antioxidant action in cells and oxidative production, causing many problems for human health (Rakhunde et al. 2014; Tobwala et al. 2014). In order to prevent diseases caused by such oxidative damage, the body has an antioxidant enzyme defense system such as superoxide dismutase, catalase, and glutathione peroxidase (Kang et al. 1996). However, structural and functional deformation occurs due to oxidative stress caused by excessive free radical production (Debnath et al. 2013). Oxidative stress is a major risk factor for chronic neurodegenerative diseases and various diseases

† Corresponding author: Dubok Choi, Professer, Faculty of Advanced Industry Convergence, Chosun University, Gwangju 61452, Korea. Tel: +82-62-230-7956, Fax: +82-62-608-5253, E-mail: choidb@chosun.ac.kr

(Shen et al. 2013). Antioxidants that inhibit active oxygen do not remove or absorb oxygen, but react with free radical to minimize loss of certain vitamins and essential amino acids, or to delay or prevent scattering of fat products (Sowndhararajan & Kang 2013).

Natural and synthetic antioxidants are being developed a lot for the development of antioxidants. Among them, butylated hydroxyanisole, butylated hydroxytoluene, propylgallate, a synthetic antioxidant, has been shown to induce liver damage and cancer in animal experiments, resulting in problems with side effects (Chipiti et al. 2013). However, the physiological active ingredients of natural products containing antioxidants have antioxidant, antibacterial, anti-inflammatory, anti-aging, and anti-cancer activity effects (An et al. 2017). Natural materials with excellent natural antioxidant efficacy, such as natural materials, contain active ingredients related to inflammation, detoxification, and antioxidation in stems, leaf berries, roots, flower seeds, and areas (Kim MK 2019). Substances widely known as natural antioxidants include phenol compounds, galic acid derivatives, tocopherols, flavone derivatives, phyllozurcins catechin, nordihydroguaiaretic acid, gospol, lignan glycosides, etc (Kikuzaki & Nakatani 1993). These days, functional products with efficacy such as antioxidant, whitening, anti-inflammatory, etc. through natural materials are being actively developed, and natural materials that compete with serious toxicity and side effects of synthetic materials are being developed (Seo et al. 2018).

The leaves of tea tree were rich in protein, carbohydrates, lipids, organic acids, vitamins, minerals, and various vegetable pigments in addition to the main ingredients such as catechin, caffeine, and amino acids (Zaveri NT 2006). Among them, caffeine is a natural ingredient contained in more than 60 kinds of plants and is contained in tea and coffee beans (Miura et al. 2000). Pharmacological effects include excitation of the vertebral nervous system, diuretic action, increased myocardial contraction power, bronchial joints, and relaxation of coronary vessels (Fujiki et al. 2002). In particular, the catechin in tea leaves is also called a polyphenolic substance and tastes bitter and bitter. More than 20 species of cars, including epigallocatechin, epigallocatechin, epigallocatechin, and epicatechate, known mainly as the four major catechin has been reported in tea leaves (Fujiki et al. 2002). These have been reported to have various physiological effects such as antioxidant, antimutant, anticancer, decreased blood cholesterol, platelet aggregation inhibition, and antibacterial (Zaveri NT 2006). In addition, various products such as antibacterial agents and food antioxidants using them are being commercially released. Since tea has such excellent ingredients, humans have developed various ways to fully utilize the ingredients of tea (Mohamadin et al. 2005). However, many studies on tea have been conducted using tea leaves, and there are few studies on tea tree roots.

In this study, cytotoxicity, DPPH radical scavenging activity, ABTS radical scavenging activity, and superoxide dismutase activity, nitrite scavenging activity, inhibitory activity of lipid peroxidation, reducing power, and ferrous ion chelating activity were investigated to examine antioxidant activity using solvent extracts of tea tree root.

Material and Method

1. Extracts preparation and reagents

The tea tree root used in this experiment was collected in autumn 2021 in Ungpo, Iksan-si, Jeollabuk-do. The soil and dust from the tea tree root was removed, washed, frozen at deep freezer −75℃ for 24 hours, and dried in a freeze dryer for 1 day. The dried sample was prepared with a powder to analyze the antioxidant activity. The hot water extract was extracted twice for 5 hours by adding 50 g of samples to 500 mL of hot water (60℃). For each solvent extraction, 500 mL of an extraction solvents (70% ethanol, ethylacetate, and hexane) was added to 50 g of the sample, respectively, and refluxed at room temperature for 5 hours. After extraction, the solids were separated using 0.2 micro filter paper (Sigma-aldrich, Tokyo, Japan), and the separated filtrate was concentrated under reduced pressure at 40℃ or less using a decompression concentrator (Eyela, Tokyo, Japan) to completely remove the solvent. Each extract was used in the experiment while being stored at −20℃ after filling with nitrogen.

2. Cell viability

It was measured by applying the Mosmann T (1983) method to determine the cytotoxicity of the extract by solvent. RAW 264.7 cells were divided into 96-well plates at a concentration of 5×10^4 cells/well, and the extracts were treated by concentration together with 100 μL of DMEM medium and cultured for 30 hours. A 3-[4,5-dimethylthiazol]-2-yl]-2,5-diphenyltetrazolium bromide (MTT) solution prepared by dissolving at a concentration of 5 μg/mL was added 50 μL to each well for 4-5 hours, and then the medium was removed. After dissolving formazan crystals

produced by adding 100 μL of DMSO solution to each well, the absorbance of color development was measured at 550 nm, and the relative cell survival rate of the drug treatment group was calculated based on the absorbance of blank.

3. Measurement of DPPH radical scavenging activity

Measurement of DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging activity was performed by applying the electron donating availability method (Blois MS 1958). 250 μL of the sample solution prepared by adding 70% ethanol to the extract and 750 μL of the 120 μM DPPH solution dissolved with 70% ethanol were mixed, reacted at room temperature for 40 minutes, and then absorbance was measured at 515 nm using a microplate reader (Model 680, Bio-Rad, Hercules, CA, USA). The DPPH radical scavenging activity of the extract was expressed as a percentage of the absorbance of the control group and the natural antioxidant, ascorbic acid (Sigma-Aldrich Co., St Louis, MO, USA) was used for activity comparison.

4. Measurement of ABTS radical scavenging activity

ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging activity was measured by applying Re et al. (1999). ABTS solution 14.8mM and potassium persulfate 5.28 mM were mixed in a 1:1 ratio to prepare an ABTS working solution and then the solution was reacted in a dark room for 24 hours to form ABTS radical. The absorbance of the ABTS working solution was measured, adjusted 0.7 to 0.8 and used in the experiment. 190 μL of the ABTS working solution and 10 μL of the diluted sample were mixed and reacted at room temperature and darkroom conditions for 20 minutes. The absorbance of this reaction solution was measured at 734 nm with a Microplate Reader.

5. Measurement of superoxide dismutase activity

The measurement of superoxide dismutase activity (SOD) activity was performed by modifying Marklund and Marklund's method (Marklund & Marklund 1974). The amount of pyrogallol that catalyzes the reaction of converting active oxygen species into hydrogen peroxide was measured and expressed as SOD activity. 120 μL of Tris-HCl buffer (50 mM trisamino-methane, 10 mM EDTA, pH 8.0) and 20 μL of pyrogallol (7.2 mM) were added to 40 μL of the sample, reacted for 10 minutes at 20℃ and then 20 μL of 1 N HCl was added to determine the reaction. The absorbance was measured at 420 nm using a UV-spectrophotometer (UV-1601PC, Shimadzu, Japan).

6. Measurement of reducing power

The reducing power was measured by the method of Mau et al. (2002). 250 μL of the extract was mixed with 250 μL of 0.2 M sodium phosphate buffer (pH 6.6) and 250 μL of 1% potassium ferricyanide ($K_3Fe(CN)_6$) respectively, reacted at 50°C for 20 minutes, and then 1% trichloroacetic acid was added. The reaction solution was centrifuged at 1,000 rpm for 10 minutes to mix 500 μL of distilled water with 500 μL of supernatant, and 0.1% ferric chloride was added. The absorbance of the reaction solution was measured at 700 nm.

7. Measurement of inhibitory activity on lipid peroxidation

The inhibitory activity on lipid peroxidation was measured by the thiocyanate method (Kikuzaki & Nakatani 1993). First, as a method of preparing a linoleic acid emulsion substrate, 2.51 g of linoleic acid was dissolved in 99.5% ethanol 100 mL, 2.05 mL of each extract was added to a conventional tube, and 2 mL of each extract was added, and 4 mL of 0.05 M phosphate buffer (pH 7.0) and 1 mL of distilled water were stored in a constant temperature. 0.1 mL of each sample and 0.1 mL of 30% ammonium thiocyanate were added to 4.7 mL of 75% ethanol, and after 3 minutes, 0.1 mL of a 3.5% HCl solution containing 0.02 M ferrous chloride was added to measure absorbance at 500 nm.

8. Measurement of ferrous ion chelating activity

Ferrous ion chelating activity was measured by modifying the method of Dinis et al. (1994). 1 mL of extract, 1 mM ferrozine 100 μL, 5 mM ferrozine 100 μL, and 3 mL of methanol were well mixed in 10mL of test tube, reacted at room temperature for 10 minutes, and then the absorbance of the reaction solution was measured at 562 nm.

9. Nitrite scavenging activity

The measurement of nitrite scavenging activity was performed by modifying the method of Kato et al. (1987). 1mL of extract was added to 2 mL of a 1mM $NaNO_2$ solution, and 0.1N HCl (pH 1.5) was used to make the volume of the reaction solution 10 mL. After heating the solution at 37℃ for 1 hour, 1 mL of each reaction solution was taken, 2 mL of 2% acetic acid solution and 0.4 mL of a grease reagent were added and mixed well, left at room temperature for 15 minutes, and measured

absorbance at 520nm to calculate the remaining amount of nitrous acid. The control sphere was performed in the same manner as above by adding 0.4 mL of distilled water instead of the grease reagent, and the nitrite elimination was expressed as a percentage of nitrite when the extract was added and when the extract was not added.

10. Statistical analysis

All experimental results were expressed as the average value ±standard deviation (Mean±S.D.) measured three times repeatedly. Statistical processing was performed using the Statistical Package for the Social Sciences (SPSS). One-way analysis of variance (ANOVA) was performed for the statistical significance test between each experimental group. If there was significance, a Duncan's test was conducted to determine that $p<0.05$ was significant, and the correlation was indicated using Pearson's correlation coefficient.

Result and Discussion

1. Cell viability

An MTT analysis, which measures cell survival, is a yellow water-soluble substance that is transformed into a dark blue formazan insoluble in water by mitochondria's reductase (Gross & Lapiere 1962). Since mitochondrial reductase is present in living cells, the amount of formazan produced when the cell is treated with MTT reagents can be quantified by measuring absorbance in proportion to the amount of living cells (Mosmann T 1983). To investigate the effect of tea root extract concentration on cell survival rate of RAW 264.7, 10, 30, 50, 70, 120, 150 and 180 μg/mL of hot water extract, 70% ethanol extract, ethyl acetate extract, and hexane extract were used and the result is shown in Fig. 1. The survival rate was 97% or higher at concentrations treated with 10, 30, 50, 70, and 90μg/mL of all extracts. However, hot water extract and 70% ethanol extract showed the survival rate of more than 92% even at the concentration of 180 μg/mL. In the case of ethylacetate extract and hexane extract, they were decreased to about 68-81%. Therefore, it was judged that each extract of tea tree root did not significantly affect RAW 264.7 cell survival rate and proliferation up to 90 μg/mL concentration, and 50 μg/mL, 70 μg/mL, and 90 μg/mL concentrations were set as experimental conditions. The above results show that the extracts using hot water extract, 70% ethanol extract, ethyl acetate extract, and hexane extract have high safety up to 90 μg/mL, so it is considered that it can be used at a high concentration when mixing cosmetics and functional food raw materials.

2. DPPH radical scavenging activity

DPPH radical scavenging activity is a widely used method to quickly and simply measure the antioxidant capacity of plant extracts. The organic nitrogen radical of DPPH represents a purple color and a maximum absorbance near a wavelength of 525 nm when dissolved in an organic solvent (Blois MS 1958). When it reacts with a substance with antioxidant activity of a plant extract, it receives electrons from hydrogen radical and is reduced to 2,2-diphenyl-1-picrylhydrazine, which is a stable compound state (Debnath et al. 2013). The reduced state of DPPH is bleached from purple to yellow, and the higher the degree of fading, the higher the DPPH radial erasure ability of the reactant. In general, it has been reported that if DPPH radical is effectively removed, the reaction with free radical may also

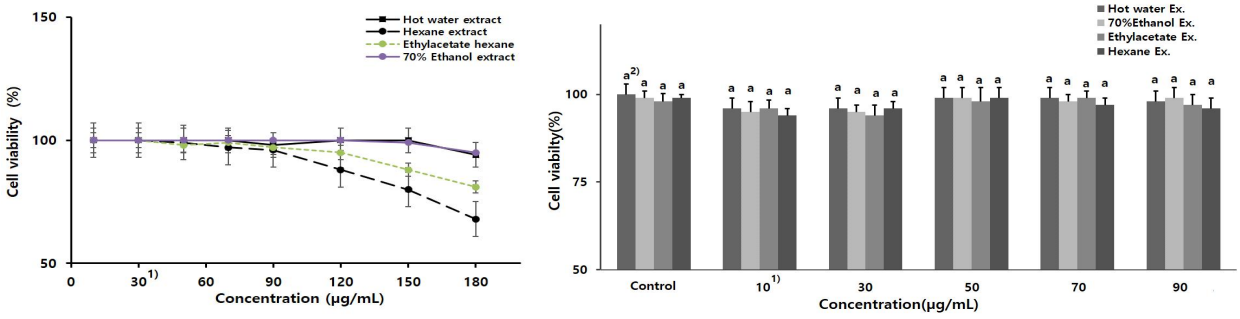

Fig. 1. Effect of various solvent extracts of tea tree root on the cell viability. [1)] Concentrations of various solvent extracts of tea tree root. [2)] Means with different letters ($^{a-c}$) within the same activity are significantly different ($p<0.05$) by Duncan's multiple range tests.

be high (Mau et al. 2002). Accordingly, the DPPH radical scavenging ability of the extracts of tea tree root by solvent was tested and the result is shown in Fig. 2. At 50, 70, and 90 μg/mL of extract, DPPH radical scavenging activities of ethyl acetate extract of tea tree root were 40, 43, and 46%, respectively, which was the highest compared to other extracts, and showed a high DPPH radical scavenging ability in order of hot water extract, 70% ethanol extract, and hexane extract. As the concentration of each extract of the tea tree root increased, the activity was higher in the order of ethyl acetate, 70% ethanol extract, hot water extract, and hexane extract, and there was no significant result compared to vitamin C value as the positive control group (p<0.05). Na et al. (2018) reported that the green tea root extract extracted with ethanol had a total pure saponin content of 54%, which contained more saponin than ginseng extract, and a number of studies have been reported by the glass radical scavenging effect using DPPH of saponin (Choi et al. 2006; Kim et al. 2008; Doh et al. 2010). It is believed that the result of the DPPH radical scavenging ability of the extract of tea tree root by solvent in this study was influenced by various antioxidants including saponin of the tea tree root extract.

3. ABTS radical scavenging activity

The measurement of antioxidant capacity using ABTS radical is a method using the removal of ABTS free radical produced by reaction with potassium persulfate by antioxidant in the extract, resulting in the discoloration of cyanide, a unique color of radical (Re et al. 1999). ABTS radial scavenging activity was measured for each concentration of extracts by various solvents using tea tree root and the result is shown in Fig. 3. Among the extracts, the ethylacetate extract showed the highest ABTS radial scavenging activity with 22% at 50 μg/mL, 34% at 70 μg/mL, and 40% at 90 μg/mL. Next, at 90 μg/mL of hot water extract, ABTS radial scavenging activity similar to ethyl acetate extract was shown. A study by Cha et al. (2008) reported that the total polyphenol content in the roots was three times higher than that in the seeds. The positive correlation between total polyphenol compounds and antioxidant activity of several plant extracts has been reported in various ways (Moreno et al. 2006). In our experiment, as the concentration of extracts for each solvent of tea tree roots increases, the total polyphenol content increases, which is considered to increase the ABTS radial scavenging activity.

4. Superoxide dismutase activity

Superoxide dismutase(SOD), one of the antioxidant enzymes, is a representative active oxygen inhibitor that catalyzes the production of hydrogen peroxide by reacting with highly reactive superoxide radials (Kang et al. 1996). It is currently used as an additive for anti-inflammatory materials or cosmetics as a cosmetic material for preventing skin aging by preventing the production of the most toxic hydroxyl radical (Grasbon et al. 1999; McKersie et al. 2000). In this study, the result of SOD activity measurement using different solvent extracts of the tea tree root is shown in Fig. 4. The SOD activity of the extract of tea tree root showed a tendency to depend on the

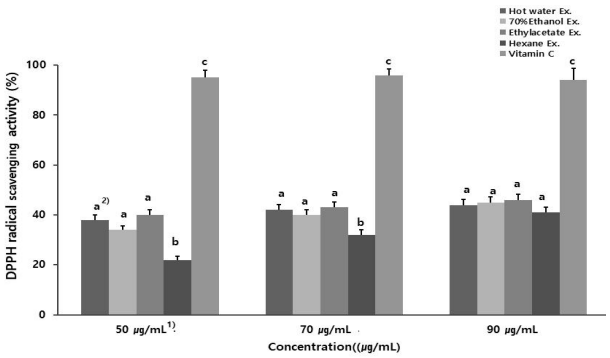

Fig. 2. Effect of various solvent extracts of tea tree root on the DPPH radical scavenging activity. 1) Concentrations of various solvent extracts of tea tree root. 2) Means with different letters ($^{a-c}$) within the same activity are significantly different (p<0.05) by Duncan's multiple range tests.

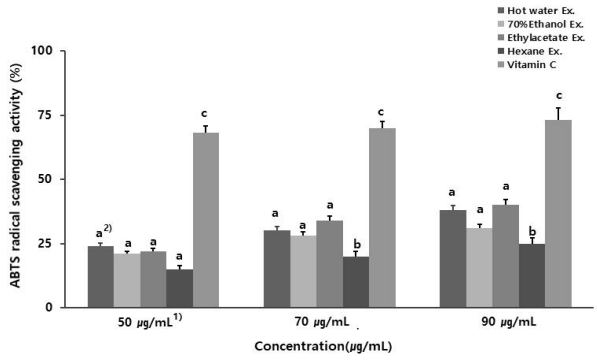

Fig. 3. Effect of various solvent extracts of tea tree root on the ABTS radical scavenging activity. 1) Concentrations of various solvent extracts of tea tree root. 2) Means with different letters ($^{a-c}$) within the same activity are significantly different (p<0.05) by Duncan's multiple range tests.

concentration of each extract, and the ethylacetate extract was the highest. Next, 70% ethanol extract, hot water extract, and hexane extract showed a high activity in the order. At 90 μg/mL, 71.4% of vitamin C as positive control was shown and its activity was about twice higher than that of hexane extract. During solvent extraction, polyphenols such as flavonoids and alkaloids, which are low-molecular-low polar materials, are separated (Dai & Mumper 2010). From this result, it is considered that the ethyl acetate extract of the tea tree root has a high content of total polyphenols compared to other solvent extracts. Because phenolic hydroxyl groups are widely distributed in plants, phenolic compounds are known to exhibit various biological activities in vivo, easily combined with proteins and other large molecules (Joung et al. 2007; Seo & Lee 2013).

5. Reducing power

Reducing power refers to the ability of the ferric-ferricyanide mixture to provide hydrogen at 700 nm to stabilize the free radicals and convert them to ferrous as absorbance values (Sa et al. 2010). In order to study effect of various solvent extracts of tea tree root on the reducing power, hot water extract, 70% ethanol extract, ethylacetate extract, and hexane extract were used and the result is shown in Fig. 5. The hexane extract showed lower absorbance values compared to other extracts at the same concentration. As the concentration of the tea tree root ethyl acetate extract increased, the absorbance value also increased, and at the concentration of 90 μg/mL, the absorbance value of 0.56 showed the highest activity among the extracts. This value showed a 70% absorbance value compared to 50 μg/mL of butylated hydroxytoluene (BHT) used as a positive control, but was not a significant result. According to a study by Choi et al. (2007), it is reported that the reduction force is closely related to the DPPH radical cation, and the reduction force test result of this experiment showed the same pattern as the DPPH radical elimination ability. This result is thought to be caused by the total polyphenol content of the green tea root ethylacetate extract. Paśko et al. (2009) and Dlamini et al. (2007) reported that the higher the total polyphenol content, the higher the antioxidant activity.

6. Lipid peroxidation inhibition

Lipid peroxidation is known to form an intermediate compound such as an alkyne compound, an alkyne compound, and etc., which is active through an initial reaction ($RH \rightarrow R\cdot + O_2 \rightarrow RO_2\cdot$), a chain reaction ($R\cdot + R\cdot \rightarrow RRR$, $R\cdot + ROO\cdot \rightarrow ROOR + O_2$) during autoxidation (Frankel EN 1980). It has been reported that increased viscosity of fat and decreased essential fatty acid content make absorption difficult in the body, reducing nutritional value (Yamamoto et al. 1987). Fig. 6 shows the results of comparing the extract concentration of each solvent extract of tea tree root and BHA, which is a positive control on the lipid peroxidation inhibition ability. The results of measuring lipid peroxidation inhibition at each concentration of 50, 70, and 90 μg/mL of solvent extracts by tea tree root are as follows. In the 70% ethanol extract, lipid peroxidation inhibition was the highest at 53.84% at a concentration of 50 μg/mL, 68.11% at a

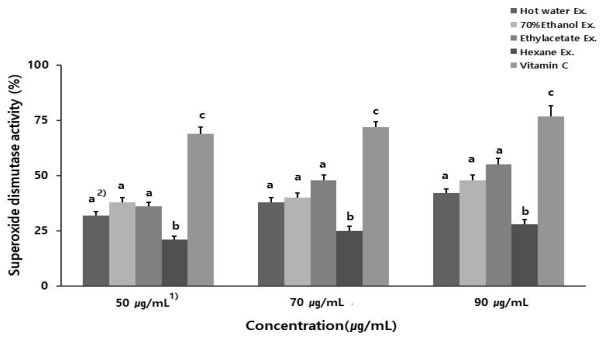

Fig. 4. Effect of various solvent extracts of tea tree root on the superoxide dismutase activity. [1] Concentrations of various solvent extracts of tea tree root. [2] Means with different letters ($^{a-c}$) within the same activity are significantly different ($p<0.05$) by Duncan's multiple range tests.

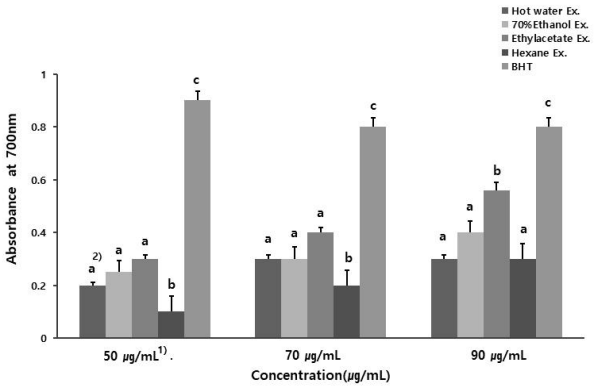

Fig. 5. Effect of various solvent extracts of tea tree root on the reducing power. [1] Concentrations of various solvent extracts of tea tree root. [2] Means with different letters ($^{a-c}$) within the same activity are significantly different ($p<0.05$) by Duncan's multiple range tests.

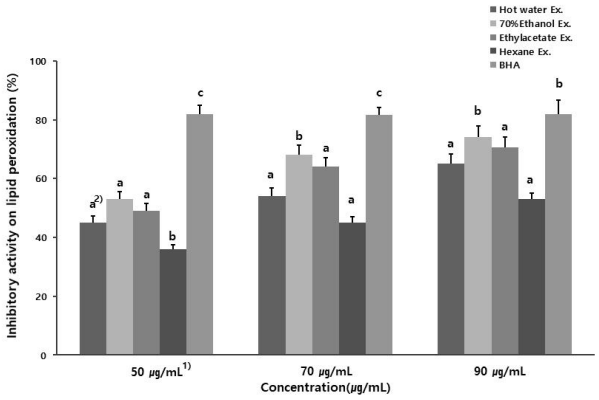

Fig. 6. Effect of various solvent extracts of tea tree root on the inhibitory activity on lipid peroxidation. [1] Concentrations of various solvent extracts of tea tree root. [2] Means with different letters ($^{a-c}$) within the same activity are significantly different (p<0.05) by Duncan's multiple range tests.

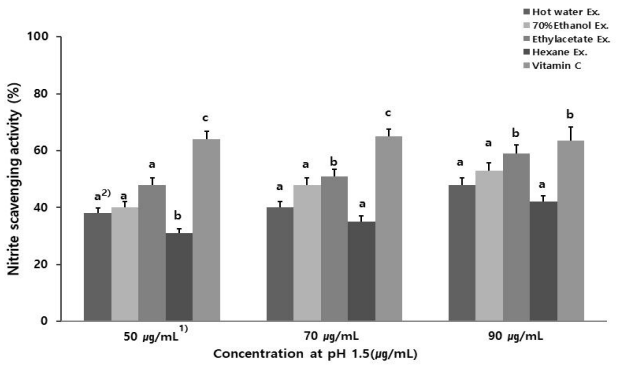

Fig. 7. Effect of various solvent extracts of tea tree root on the nitrate scavenging activity at pH 1.5. [1] Concentrations of various solvent extracts of tea tree root. [2] Means with different letters ($^{a-c}$) within the same activity are significantly different (p<0.05) by Duncan's multiple range tests.

concentration of 70 μg/mL, and 74.26% at a concentration of 90 μg/mL, respectively. It showed significant results at a concentration of 90 μg/mL compared to 82.32% of BHA (p<0.05). As the concentration of all extracts increased, the lipid peroxidation inhibition increased, followed by 70% ethanol extract, ethyl acetate extract, hot water extract, and hexane extract. As a result of this experiment, the ability to inhibit lipid peroxidation is confirmed in all tea tree root extracts, and the effect as a natural antioxidant in lipid components is expected. In particular, given that linoleic acid is a biomembrane- composed fatty acid based on the above research results, 70% ethanol extract suggests that it can be a good material for preventing several chronic diseases caused by oxidative stress *in vivo*.

7. Nitrate scavenging activity

Fig. 7 shows the nitrate scavenging activity of the solvent extract by tea tree root at pH 1.5 Like the test results of other antioxidant activities, the nitrate scavenging activity increased as the concentration of ethyl acetate extracts increased. At 90 μg/mL of ethyl acetate, it showed the most excellent ability of 59.6±1.3%. The hexane extract had the lowest scavenging ability at all concentrations. According to Kim et al. (2001), it was also consistent with reports that the lower the pH, the better the nitrate scavenging activity. This result is believed to contribute to preventing nitrosamine-induced cancer *in vivo* when the tea tree root extract is consumed with foods and processed foods that may contain amine and nitrites.

8. Ferrous ion chelating activity

Fe^{2+} promotes the oxidation of cell lipids and proteins in the body. Ferrozine forms a complex with Fe^{2+} and turns red, and if a substance with a chelate effect is present in the sample, the formation of the Fe^{2+}-ferrozine complex is hindered (Haber & Weiss 1932). In order to measure the chelating effect of Fe^{2+}, the solvent extract of the tea tree root was tested at different concentrations, and the result is shown in Fig. 8. Among various extracts, hexane extract had the highest ferrous ion chelating activity. The ferrous ion chelating effect of hexane extract increased as the concentration increased. Especially, the hexane extract had the highest activity, showing 61.05% at 50 μg/mL, 66.07% at 70 μg/mL and 76.81% at 90 μg/mL, respectively. However, all extracts had a lower chelating effect than EDTA used as a chelating agent, but showed significant result at a concentration of 90 μg/mL (p<0.05). The relatively low Fe^{2+} chelating activity while high DPPH and ABTS radical scavenging activity are different from those of metal ions (Seo et al. 2008) and it has been reported that the content of phenol compounds capable of erasing glass radical is high but the content of metal ions is low (Woo et al. 2010). As a result of this, ethylacetate extract from tea tree root is effective in removing radical substances that cause oxidation in the body. However, hexane extracts are effective in stabilizing ferrolysis, which produces secondary radical and promotes human oxidation, so it is considered that various solvent extracts

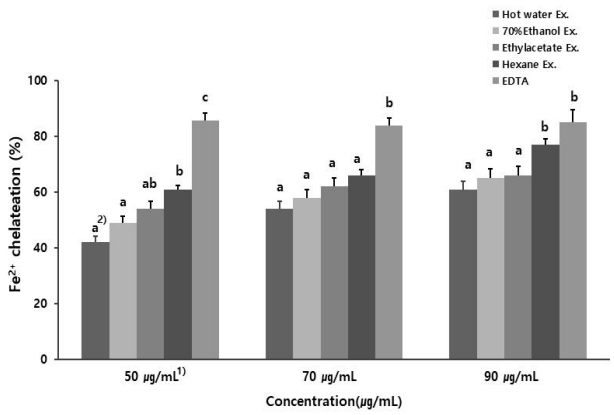

Fig. 8. Effect of various solvent extracts of tea tree root on the ferrous ion chelating activity. [1] Concentrations of various solvent extracts of tea tree root. [2] Means with different letters ([a-c]) within the same activity are significantly different (p<0.05) by Duncan's multiple range tests.

effective for each antioxidant should be used in combination when researching and developing antioxidants with natural substances.

9. Correlation

Table 1 shows the results of analyzing the correlation among antioxidant activity items of extracts by tea tree root solvent. The value of the correlation represents a positive correlation as it approaches 1 based on 0, and the closer it is to －1, the more negative it is. he correlation between antioxidant active items showed a positive (+) correlation in all items except for the ferrous ion chelating activity (p<0.01). According to a study by Cha et al. (2008), the polyphenol content of tea tree roots is reported to be three times higher than that of tea seeds, and the correlation between polyphenol contained in tea and antioxidant power is generally known to be high (Lu & Yeap Foo 2000). In this study, the correlation among antioxidant active items was examined, but it is considered necessary to examine the correlation among the items with the polyphenol content of the extract by tea tree root solvent.

Conclusion

The role of free radicals in disease and aging has increased interest in antioxidants, and accordingly, natural or synthetic antioxidants are being used in food and medicine. However, instability at high temperatures and harmful effects were found in some synthetic antioxidants, raising concerns about the use of synthetic antioxidants. Therefore, it is a global trend to find antioxidants from natural materials, and various plants with antioxidant effects are being studied to be used as medicinal and food additives. In this study, cytotoxicity, DPPH radical scavenging activity, ABTS radical scavenging activity, and superoxide dismutase activity, nitrite scavenging activity, inhibitory activity of lipid peroxidation, reducing power, and ferrous ion chelating activity of tea tree root extract using different extraction methods were investigated. The RAW 264.7 cell survival rate of the extract of tea tree root showed high safety up to 90 μg/mL of extract, irrespective of extraction methods. All the extracts exhibited dose dependent radical scavenging activity at each extract. However, the antioxidant activity at same concentration was different depending on the extraction method. In particular, ethyl acetate extract and 70% ethanol extract were the highest in the DPPH and superoxide radical scavenging activity, nitrite scavenging activity, and

Table 1. Correlation among antioxidants of various solvent extracts of tea tree root

Factors	DPPH[1)]	ABST	SOD	RP	LPI	NSA	FIC
DPPH	1						
ABST	0.872**	1					
SOD	0.828**	0.858*	1				
RP	0.772**	0.634*	0.706*	1			
LPI	0.820**	0.852**	0.909**	0.813**	1		
NSA	0.812**	0.771*	0.811*	0.499	0.721**	1	
FIC	0.157	0.224	－0.024	0.283	0.275	－0.080	1

[1)] DPPH: DPPH radical scavenging activity, ABST: $ABTS^+$ radical scavenging activity, SOD: superoxide radical scavenging activity, RP: reducing power, LPI: lipid peroxidation inhibition, NSA: nitrite scavenging activity, FIC: ferrous ion chelating.
[2)] Correlation is significantly different at $^*p$<0.05, $^{**}p$<0.01.

inactivity activity of lipid peroxidation experiments. In the ABTS radial scavenging activity experiment, ethylacetate extract and hot water extract were the highest. In the reducing power experiment, 70% ethanol extract was the highest. However, in the ferrous ion chelating activity, hexane extract was the highest. This research result shows that among different extracts, ethylacetate extract and 70% ethanol extract have high antioxidant activity, so it is expected to be used as basic data in researching functions and developing functional products using the same. The correlation among antioxidant activity items showed a positive correlation in all items (p<0.01). However, it is judged that detailed research on the separation identification and antioxidant mechanism of effective compounds should be conducted in the ethylacetate extract and 70% ethanol extract in the future.

Acknowledgment

This study was supported by research fund from Chosun University (2022).

References

An DS, Seo SJ, Kim NW, Lee YS. 2017. Anti-aging and anti-inflammatory activity of *Rhus javanica* branches extracts. *J Invest Cosmetol* 13:103-111

Blois MS. 1958. Antioxidant determinations by the use of a stable free radical. *Nature* 181:1199-1200

Cha WS, Cho MJ, Ding JL, Shin HJ. 2008. Nutritional component analysis of green tea tree's root and seed. *Korean J Biotechnol Bioeng* 23:387-391

Chipiti T, Ibrahim MA, Koorbanally NA, Islam MS. 2013. *In vitro* antioxidant activities of leaf and root extracts of *Albizia antunesiana* harms. *Acta Pol Pharm* 70:1035-1043

Choi CS, Kim KI, Hong HD, Choi SY, Lee YC, Kim KT, Rho JH, Kim SS, Kim YC. 2006. Phenolic acid composition and antioxidative activity of white ginseng (*Panax ginseng*, C. A. Meyer). *J Ginseng Res* 30:22-30

Choi Y, Jeong HS, Lee J. 2007. Antioxidant activity of methanolic extracts from some grains consumed in Korea. *Food Chem* 103:130-138

Dai J, Mumper RJ. 2010. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules* 15:7313-7352

Debnath T, Park SR, Kim DH, Jo JE, Lim BO. 2013. Anti-oxidant and anti-inflammatory activities of *Inonotus obliquus* and germinated brown rice extracts. *Molecules* 18:9293-9304

Dinis TCP, Madeira VMC, Almeida LM. 1994. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch Biochem Biophys* 315:161-169

Dlamini NR, Taylor JRN, Rooney LW. 2007. The effect of sorghum type and processing on the antioxidant properties of African sorghum-based foods. *Food Chem* 105:1412-1419

Doh ES, Chang JP, Lee KH, Seong NS. 2010. Ginsenoside change and antioxidation activity of fermented ginseng. *Korean J Med Crop Sci* 18:255-265

Frankel EN. 1980. Lipid oxidation. *Prog Lipid Res* 19:1-22

Fiers W, Beyaert R, Declercq W, Vandenabeele P. 1999. More than one way to die: Apoptosis, necrosis and reactive oxygen damage. *Oncogene* 18:7719-7730

Fujiki H, Suganuma M, Imai K, Nakachi K. 2002. Green tea: cancer preventive beverage and/or drug. *Cancer Lett* 188:9-13

Grasbon T, Grasbon-Frodl EM, Juliusson B, Epstein C, Brundin P, Kampik A, Ehinger B. 1999. CuZn superoxide dismutase transgenic retinal transplants. *Graefes Arch Clin Exp Ophthalmol* 237:336-341

Gross J, Lapiere CM. 1962. Collagenolytic activity in amphibian tissues: A tissue culture assay. *Proc Natl Acad Sci USA* 48:1014-1022

Haber F, Weiss J. 1932. Über die katalyse des hydroperoxydes. *Naturwissenschaften* 20:948-950

Hwang EY, Kim DH, Kim HJ, Hwang JY, Park TS, Lee IS, Son JH. 2011. Antioxidant activities and nitric oxide production of medicine plants in Gyeongsangbukdo (*Carthamus tinctorius* seed, *Cyperus rotundus*, *Schizonepeta tenuifolia*, *Polygonatum odoratum* var. *pluriflorum*, *Paeonia lactiflora*). *J Appl Biol Chem* 54:171-177

Joung YM, Park SJ, Lee KY, Lee JY, Suh JK, Hwang SY, Park KE, Kang MH. 2007. Antioxidative and antimicrobial activities of *Lilium* species extracts prepared from different aerial parts. *Korean J Food Sci Technol* 39:452-457

Kang YH, Park YK, Ha TY, Moon KD. 1996. Effects of pine needle extracts on enzyme activities of serum and liver, and

liver morphology in rats fed high fat diet. *J Korean Soc Food Nutr* 25:374-378

Kato H, Lee IE, Chuyen NV, Kim SB, Hayase F. 1987. Inhibition of nitrosamine formation by nondialyzable melanoidins. *Agric Biol Chem* 51:1333-1338

Kikuzaki H, Nakatani N. 1993. Antioxidant effects of some ginger constituents. *J Food Sci* 58:1407-1410

Kim AJ, Han MR, Woo N, Kang SJ, Lee GS, Kim MH. 2008. Physicochemical properties of Korean ginseng pickles with Chija and Omija. *Korean J Food Nutr* 21:524-529

Kim MK. 2019. Comparison of antioxidant, antimicrobial activity and anti-inflammatory effects of on neem (*Azadirachta indica*) extracts with different extraction methods. *J Korean Soc Beauty Art* 20:265-278

Kim SM, Kim KH, Ahn JK. 2001. The antioxidant ability and nitrite scavenging ability of plant extract. *Korean J Food Sci Technol* 33:623-632

Lu Y, Yeap Foo L. 2000. Antioxidant and radical scavenging activities of polyphenols from apple pomace. *Food Chem* 68:81-85

Marklund S, Marklund G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* 47:469-474

Mau JL, Lin HC, Song SF. 2002. Antioxidant properties of several specialty mushrooms. *Food Res Int* 35:519-526

McKersie BD, Murnaghan J, Jones KS, Bowley SR. 2000. Iron-superoxide dismutase expression in transgenic alfalfa increases winter survival without a detectable increase in photosynthetic oxidative stress tolerance. *Plant Physiol* 122:1427-1438

Miura Y, Chiba T, Miura S, Tomita I, Umegaki K, Ikeda M, Tomita T. 2000. Green tea polyphenols (flavan 3-ols) prevent oxidative modification of low density lipoproteins: An *ex vivo* study in humans. *J Nutr Biochem* 11:216-222

Mohamadin AM, El-Beshbishy HA, El-Mahdy MA. 2005. Green tea extract attenuates cyclosporine A-induced oxidative stress in rats. *Pharmacol Res* 51:51-57

Moreno S, Scheyer T, Romano CS, Vojnov AA. 2006. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radic Res* 40:223-231

Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods* 65:55-63

Na HW, Lee Y, Park JS, Lee TR, Kim HJ. 2018. Green tea root is a potential natural surfactant and is protective against the detrimental stimulant PM2.5 in human normal epidermal keratinocytes. *J Soc Cosmet Sci Korea* 44:67-72

Paśko P, Bartoń H, Zagrodzki P, Gorinstein S, Fołta M, Zachwieja Z. 2009. Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth. *Food Chem* 115:994-998

Rakhunde PB, Saher S, Ali SA. 2014. Neuroprotective effect of *Feronia limonia* on ischemia reperfusion induced brain injury in rats. *Indian J Pharmacol* 46:617-621

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 26:1231-1237

Sa YJ, Kim JS, Kim MO, Jeong HJ, Yu CY, Park DS, Kim MJ. 2010. Comparative study of electron donating ability, reducing power, antimicrobial activity and inhibition of α-glucosidase by Sorghum bicolor extracts. *Korean J Food Sci Technol* 42:598-604

Seo HM, Lee JH. 2013. Physicochemical and antioxidant properties of yanggaeng incorporated with black sesame powder. *J Korean Soc Food Sci Nutr* 42:143-147

Seo MS, Jang YA, Lee JT. 2018. The study of cosmeceutical activities from *Lentinula edodes* extracts and application a natural cosmetic material. *J Oil Appl Sci* 35:1003-1012

Seo SJ, Choi YM, Lee SM, Kong SH, Lee JS. 2008. Antioxidant activities and antioxidant compounds of some specialty rices. *J Korean Soc Food Sci Nutr* 37:129-135

Shen B, Truong J, Helliwell R, Govindaraghavan S, Sucher NJ. 2013. An *in vitro* study of neuroprotective properties of traditional Chinese herbal medicines thought to promote healthy ageing and longevity. *BMC Complement Altern Med* 13:373

Sowndhararajan K, Kang SC. 2013. Evaluation of *in vitro* free radical scavenging potential of *Streptomyces* sp. AM-S1 culture filtrate. *Saudi J Biol Sci* 20:227-233

Tobwala S, Fan W, Hines CJ, Folk WR, Ercal N. 2014. Antioxidant potential of *Sutherlandia frutescens* and its protective effects against oxidative stress in various cell cultures. *BMC Complement Altern Med* 14:271

Woo JH, Shin SL, Lee CH. 2010. Antioxidant effects of ethanol extracts from flower species of compositae plan. *J Korean*

Soc Food Sci Nutr 39:159-164

Yamamoto Y, Brodsky MH, Baker JC, Ames BN. 1987. Detection and characterization of lipid hydroperoxides at picomole levels by high-performance liquid chromatography. *Anal Biochem* 160:7-13

Zaveri NT. 2006. Green tea and its polyphenolic catechins: Medicinal uses in cancer and noncancer applications. *Life Sci* 78:2073-2080

Received 31 August, 2022
Revised 16 September, 2022
Accepted 23 September, 2022