Comparison of Antioxidative Efficacy of Ginkgo Biloba Leaves and Acer Palmatum Leaves

Jang Soon Park
Department of Beauty Art, Song Won University, Gwangju-61756, Republic of South Korea

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ABSTRACT

Modern people pay attention to the antioxidant effects that protect human aging and damage, but active ingredients used in cosmetics or pharmaceutical raw materials of synthetic ingredients are exposed to a number of problems. An alternative method for solving the problem was selected, and Ginkgo Biloba Leaves (GBL) and Acer Palmatum Leaves (APL) were selected to compare and analyze antioxidant efficacy evaluations such as total polyphenol, total flavonoid, DPPH ability, ABTS radical scavenging ability, and FRAP activity measurement. In the results of the study, GBL and APL showed DPPH activity at all concentrations (μg/ml). ABTS activity and FRAP Value also showed antioxidant activity in both GBL and APL and in particular, APL has been found to be potent and is considered a rich antioxidant. ABTS activity and FRAP Value also showed antioxidant activity in both GBL and APL, and in particular, APL has been found to be more potent. Therefore, these species can be evaluated as rich antioxidants. This study hopes to provide GBL and APL extracts as data for the development of natural antioxidants in cosmetics and pharmaceuticals.

INTRODUCTION

Because modern people are threatened with health due to excessive stress on their busy daily life and work, interest in disease prevention and physiological efficacy is increasing. Due to this trend, modern people want to improve their lives as well as their families by managing and improving their health (Kumari, 2019). Through medical services provided by medical institutions, it is possible to improve the quality of life while improving the expected level of life (Kateel and Koodamara, 2018). On the other hand, modern people pay more attention to the antioxidant effect that protects the body from aging and damage caused by oxygen free radicals. However, as active ingredients used in cosmetics or pharmaceutical ingredients of synthetic ingredients are exposed to a number of problems, attention is focused on natural materials to replace them. Although natural materials have lower antioxidant efficacy compared to synthetic antioxidants, it is necessary to spur on accelerating research of effective efficacy by separating and purifying antioxidants from natural materials. Since decades ago, Ginkgo biloba L. has had a unique position as the most popular natural material because of its excellent biological activities. Along with GBL, the reddish APL is also excellent as a natural antioxidant because it has accumulated a large amount of anthocyanins. In some clinical trials, GBL has potent efficacy in cardiovascular or cerebrovascular activity, and it is also applied to Alzheimer's patients due to its natural antioxidant (Lau et al., 2013). Total flavonoid content is used as an important qual-
ity index for GBL and APL and flavonoids are the main chemical component of the standard extract of G. biloba leaf (EGB761) (more than 24% of the mass). Flavonoids, the primary natural material that acts as an antioxidant with tannins (Franke et al., 1998) have numerous physiological efficacies, such as treatment of asthma, bronchial and cardiovascular diseases, improved peripheral blood flow, and reduced brain dysfunction.

Natural plants contain antioxidant compounds with free radical scavenging function as reducing agents for single radical formation. Natural antioxidants have the efficacy to filter out free radicals and block cell damage in the human body (Millogo-Kone et al., 2009). Free radicals have been reported to cause molecular modifications associated with several degenerative diseases such as atherosclerosis, diabetes, asthma, Parkinson’s, Alzheimer’s, and immunodeficiency disease. Based on these previous studies on antioxidants, this study compared and analyzed antioxidant efficacies of GBL and APL such as total polyphenol and total flavonoid, DPPH and ABTS activity, and FRAP activity measurement. Throughout this study, we aim to provide the basic data for GBL and APL to be developed for natural antioxidants in cosmetics or pharmaceutical ingredients.

MATERIALS AND METHODS

GBL and APL samples used in the experiment were collected in Gwangju Metropolitan City at the end of November 2019, dried for about two weeks in a cool, well ventilated place and used after careful selection at TBRC-RIC of Daejeon University. The reagent supplied from Sigma (U.S.A.) has 1,1-diphenyl-2-picryl-hydrazyl, gallic acid, 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid, sodium acetate trihydrate, quercetin, aluminum nitrate nonahydrate, potassium acetate solution, glacial acetic acid, hydrochloric acid, 2,4,6-Tris-s-triazine, Iron(III) chloride hexahydrate, Iron(II) sulfate heptahydrate. The other reagent is folin ciocalteu’s phenol reagent (Merck, U.S.A.).

The experimental equipment used in this experiment was San yo (Japan) autoclave and deep freezer, vision scientific (Korea) clean bench and vortex mixer, rotary vacuum evaporator (EYELA FDU-540, Japan), plate shaker (Lab-Line, U.S.A.), freeze dryer (Ilsin biobase, Korea), centrifuge (Hanil, Korea), micro plate reader (Molecular Devices, U.S.A.), etc. After Ginkgo biloba Leaves and Acer palmatum Leaves were extracted with reflux for three hours after adding two liters of distilled water to 120 g of each, the filtered liquid is concentrated with rotary vacuum evaporator, and the concentrated liquid was dried with a freeze dryer. GBL obtained 22.03 g (18.35% yield) powder and APL obtained 22.09 g (18.40% yield) powder. It was stored in a low-temperature freezer at -80°C and diluted with distilled water and used at the concentration required for this experiment. As a measure of total polyphenol content, 0.5 ml of 50% foil siocaltephe- nol reagent was added to 1 ml of GBL and APL and reacted at room temperature for 3 minutes. After mixing 1 ml of 10% saturated Na2CO3 solution and 7.5 ml of distilled water in order to reaction solution and left to stand for 30 minutes, after centrifugation at 1200 rpm for 600 seconds, the supernatant was collected and measured for absorbance at 760 nm. The total phenol contents was decided according to the calibration curve prepared using gallic acid as a standard material.

As a measure of total flavonoid content, after 0.1 ml of 10% AlN3O9, 0.1 ml of 1M CH3COOK and 4.3 ml of 80% ethanol were mixed together with 0.5 ml of GBL and APL, and after leaving at room temperature for about 40 minutes, centrifugation at 1200 rpm for 10 minutes, the supernatant was taken and measured for absorbance at 415 nm. Total flavonoid content was determined according to a calibration curve using quercetin as a standard substance. The DPPH Radical Scavenging Capacity measurement developed by Blois is a method of measuring antioxidant efficacy using stable free radical (DPPH) (Paul et al., 2011).

Because radical compounds are stable and do not need to be produced, DPPH assay is very accurate, simple and economical to evaluates the free radical scavenging ability of antioxidants. DPPH activity experiments were carried out by Nanjo et al., (Kedare and Singh, 2011). The final concentrations of GBL and APL were diluted to concentrations of 1, 10, 100, 1000 μg/ml, and 150 μl of the 0.2 mm DPPH solution dissolved in ethanol and 100 μl of the sample were mixed and reacted at 37°C for 30 minutes. After the reaction, the absorbance was...
Table 1: Polyphenol and Flavonoid contents of GBL and APL.

| Sample                  | GBL       | APL       |
|-------------------------|-----------|-----------|
| Polyphenol content (mg GAE$^1$/g) | 38.96 ± 2.81 | 139.38 ± 9.08 |
| Flavonoid content (mg QE$^2$/g)     | 17.38 ± 5.09 | 46.44 ± 3.05 |

Table 2: DPPH activity of GBL and APL.

| Concentration (µg/ml) | DPPH radical scavenging activity (%) |
|-----------------------|--------------------------------------|
|                       | GBL                                  | APL                                  |
| 1                     | 03.09 ± 1.26                         | 07.01 ± 1.74                         |
| 10                    | 06.26 ± 1.75                         | 37.77 ± 1.30                         |
| 100                   | 58.47 ± 1.17                         | 91.00 ± 0.95                         |
| 1000                  | 89.84 ± 2.11                         | 92.31 ± 0.11                         |

accurately measured at a wavelength of 517 nm, and distilled water was additionally added to the control group of the experimental sample. The total antioxidant efficacy of GBL and APL extracts was measured using the ABTS radical scavenging ability assay (Nanjo et al., 1996). Dilutions were made so that final concentrations of GBL and APL could be at concentrations of 1, 10, 100, 1000 µg/ml.

Figure 2: ABTS radical scavenging activity of GBL and APL.

After preparing 7.4 mm ABTS, 2.6 mm potassium persulphate, ABTS solution was left in the dark for one day to form a cation (ABTS+). Absorbance was measured at 732 nm and diluted so that the absorbance value was 1.5 or less. 95 µl of ABTS + diluted solution and 5 µl of sample were mixed and reacted at room temperature for about 10 minutes, and measure the absorbance at a wavelength of 732 nm. Distilled water is added to the control sample. FRAP analysis measures the antioxidant activity capacity of a substance in a reaction medium based on its reducing capacity (Kim et al., 2012). Reducibility is usually closely related to the presence of a reducing agent, and by adding hydrogen atoms to stop radical chain reactions, it exerts antioxidant activity (Kim et al., 2015). FRAP reagent is prepared by mixing 20 mm FeCl3 and 10 mm TPTZ dissolved in 300 mm sodium acetate buffer in a 10:1:1 ratio, respectively. Thereafter, 100 ml of concentration specific samples and 900 µl of distilled water were added to a 15 ml tube and 2 ml of FRAP reagent was added to the mixed solution, and reacted for 30 minutes in the dark. After reacting with each other, absorbance measurements were accurately performed at 593 nm wavelength, and the antioxidant activity ability was measured according to the calibration curve prepared using FeSO4 as a standard material.

RESULTS AND DISCUSSION

Table 1 shows the results of measuring the total polyphenol content using gallic acid as a standard. GBL and APL were found to be 38.96±2.81 mg GAE/g and 139.38±9.08 mg GAE/g, respectively, showing that the polyphenol content of APL was higher. From the results of measuring the total flavonoid content by taking Quercetin as a standard material, 46.44±3.05 mg QE/g in APL was found to be much higher than GBL which showed 17.38±5.09 mg QE/g Table 1. In recent years, flavonoids of GBL have received considerable attention in a variety of literature due to the well-known free radical scavenging activity (Benzie and Strain, 1996). However, it is quite remarkable that the finding of the higher flavonoid content of APL than that of GBL is remarkable.

Experimental results were expressed as mean ± SD.  
1) Total phenol content is expressed as gallic acid equivalent milligrams per gram of extract  
2) Total flavonoid content is expressed in milligrams of quercetin equivalent per gram.

The DPPH test results are presented in Table 2 and Figure 1. It does not distinguish the free radical type, but it has been established that it is generally ideal for radical quenching capacity. A result of measuring
DPPH ability, both GBL and APL showed a concentration-dependent increase. At all concentrations, APL’s DPPH radical scavenging capacity was found to be higher than that of GBL. GBL showed a trend of increasing DPPH radical scavenging capacity at all concentrations while the concentration of DPPH ability slowed down at the concentration of APL above 100 (μg/ml). GBL and APL are incubated with DPPH solution at 1, 10, 100, 1000 μg/ml for 30 minutes. The absorbance is determined by measuring at 517 nm. DPPH experiment results are expressed as mean ± S.D values derived from three independent experiments.

The obtained results showed DPPH activity of GBL and APL at all concentrations tested (μg/ml). ABTS activity and FRAP Value also showed antioxidant activity in both GBL and APL, and in particular, APL has been found to be more potent. Therefore, these species can be evaluated as rich antioxidants. This study hopes to provide GBL and APL extracts as data for the development of natural antioxidants in cosmetics and pharmaceuticals. And based on this experiment and also based on the results of this study, it is possible to find the possibility of utilization as a useful antioxidant material in the field of hair beauty, such as ginkgo biloba and maple leaf materials, other than pharmaceuticals, such as alleviating damaged hair or dyeing hair, or skin soothing effect or allergy relief.

### Table 3: ABTS activity of GBL and APL.

| Concentration (μg/ml) | ABTS radical scavenging activity (%) |
|-----------------------|--------------------------------------|
|                       | GBL                                  | APL                                  |
| 1                     | 02.04 ± 0.53                         | 05.35 ± 0.80                         |
| 10                    | 08.72 ± 0.74                         | 36.46 ± 0.47                         |
| 100                   | 47.96 ± 0.40                         | 94.59 ± 0.14                         |
| 1000                  | 94.57 ± 0.03                         | 94.54 ± 0.17                         |

### Table 4: FRAP value of GBL and APL.

| Concentration (μg/ml) | FRAP value (uM) |
|-----------------------|-----------------|
|                       | GBL             | APL             |
| 1                     | 03.57 ± 1.76    | 006.01 ± 1.64   |
| 5                     | 07.32 ± 1.01    | 017.01 ± 2.16   |
| 10                    | 39.61 ± 0.35    | 059.94 ± 2.33   |
| 50                    | 74.70 ± 1.71    | 123.45 ± 1.41   |

CONCLUSIONS

The obtained results showed DPPH activity of GBL and APL at all concentrations tested (μg/ml). ABTS activity and FRAP Value also showed antioxidant activity in both GBL and APL, and in particular, APL has been found to be more potent. Therefore, these species can be evaluated as rich antioxidants. This study hopes to provide GBL and APL extracts as data for the development of natural antioxidants in cosmetics and pharmaceuticals. And based on this experiment and also based on the results of this study, it is possible to find the possibility of utilization as a useful antioxidant material in the field of hair beauty, such as ginkgo biloba and maple leaf materials, other than pharmaceuticals, such as alleviating damaged hair or dyeing hair, or skin soothing effect or allergy relief.
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Conflict of Interest
The authors declare that they have no conflict of interest for this study.

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