

## Analysis of Antioxidant Efficacy of *Ginkgo Biloba* Leaves and *Acer Palmatum* Leaves

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**Abstract: Background/Objectives:** 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.

**Methods/Statistical analysis:** An alternative method for solving the problem was selected, and *Ginkgo biloba* Leaves (hereinafter GBL) and *Acer palmatum* Leaves (hereinafter 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.

**Findings:** In the results of the study, GBL and APL showed DPPH activity at all concentrations ( $\mu\text{g}/\text{mL}$ ). 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.

**Improvements/Applications:** 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.

**Keywords:** *Ginkgo biloba* Leaves, *Acer palmatum* Leaves, Antioxidant, Aging, Oxygen free radical.

### 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 [Dr. Trisha 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 [Nimitha Kateel.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 . [A. J. Lau. 2013]. Total flavonoid content is used as an important quality 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 AA.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. And natural antioxidants have the efficacy to filter out free radicals and block cell damage in the human body [Millogo-Kone.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 efficacy evaluations 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

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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 Sanyo (Japan) autoclave and deep-freezer, ision scientific (Korea) clean bench and vortex mixer, rotary vacuum evaporator (EYELA FDU-540, Japan), platemaker (Lab-Line, U.S.A.), freeze dryer (Ilshin biobase, Korea), centrifuge (Hanil, Korea), micro platereader (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  $-80^{\circ}\text{C}$  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-siocalte phenol 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  $\text{Na}_2\text{CO}_3$  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%  $\text{Al}(\text{NO}_3)_3$ , 0.1 ml of 1M  $\text{CH}_3\text{COOK}$  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[Paul, S.2011] is a method of measuring antioxidant efficacy using stable free radical (DPPH). 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.[Sagar B.2011]. The final concentrations of GBL and APL were diluted to concentrations of 1, 10, 100, 1000  $\mu\text{g}/\text{ml}$ , and 150  $\mu\text{l}$  of the 0.2 mM DPPH solution dissolved in ethanol and 100  $\mu\text{l}$  of the sample were mixed and reacted at  $37^{\circ}\text{C}$  for 30 minutes. After the reaction, the absorbance was 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 F.1996]. Dilutions were made so that final concentrations of GBL and APL could be at concentrations of 1, 10, 100, 1000  $\mu\text{g}/\text{ml}$ . 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 ( $\text{ABTS}^+$ ). Absorbance was measured at 732 nm and diluted so that the absorbance value was 1.5 or less. 95  $\mu\text{l}$  of  $\text{ABTS}^+$  + diluted solution and 5  $\mu\text{l}$  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 Y. S.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 [Y. S. Kim.2015].

FRAP reagent is prepared by mixing 20 mM  $\text{FeCl}_3$  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  $\mu\text{l}$  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  $\text{FeSO}_4$  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  $38.96 \pm 2.81$  mg GAE/g and  $139.38 \pm 9.08$  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,  $46.44 \pm 3.05$

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 [Iris F. F. 1996]. However, it is quite remarkable that the finding of the higher flavonoid content of APL than that of GBL is remarkable.

**Table 1: Polyphenol and Flavonoid Contents of GBL and APL**

Sample	GBL	APL
Polyphenol content (mg GAE <sup>1</sup> /g)	38.96±2.81	139.38±9.08
Flavonoid content (mg QE <sup>2</sup> /g)	17.38±5.09	46.44±3.05

Experimental results were expressed as mean ± SD.

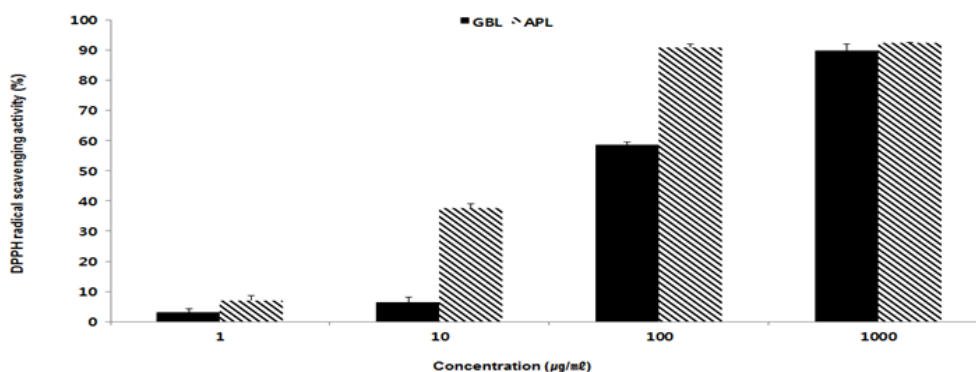
- 1) Total phenol content is expressed as gallic acid equivalent milligrams per gram of extract
- 2) Total flavonoid content is expressed in milligrams of quercetin equivalent per gram.

The DPPH test results are presented in Table 2 and Figure 2. It does not distinguish the free radical type, but it has been established that it is generally ideal for radical quenching capacity. As 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).

**Table 2: DPPH Activity of GBL and APL**

Concentration (µg/ml)	DPPH radical scavenging activity (%)	
	GBL	APL
1	3.09±1.26	7.01±1.74
10	6.26±1.75	37.77±1.30
100	58.47±1.17	91.00±0.95
1000	89.84±2.11	92.31±0.11



**Figure 1: DPPH radical scavenging activity of GBL and APL**

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.

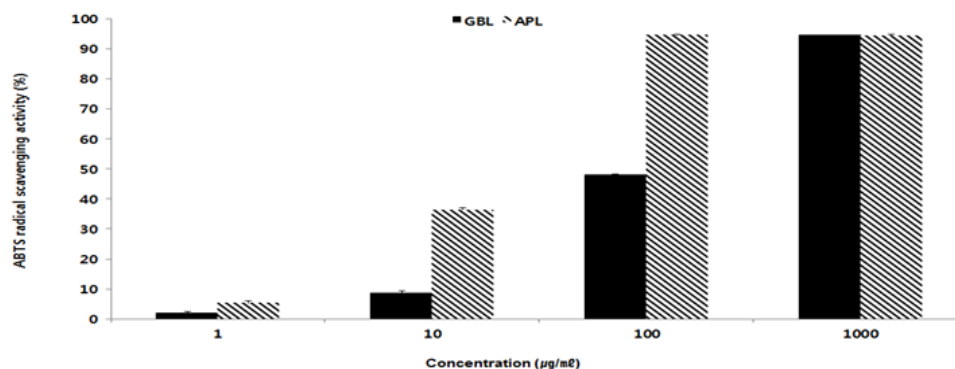
The results of measuring the ABTS ability are shown in Table 3 and Figure 2, and both GBL and APL showed a concentration-dependent increase.

At 1, 10 and 100 concentrations (µg/ml), APL showed significantly higher ABTS radical scavenging activity than GBL, but as GBL and APL showed 94.57±0.03 µg/ml and 94.54±0.17 at 1000 Concentration (µg/ml),

respectively, it was shown that ABTS radical scavenging activity was reversed. APL showed significant ( $p < 0.05$ ) ABTS values compared to GBL.

**Table 3. ABTS Activity of GBL and APL**

Concentration ( $\mu\text{g}/\text{m}\ell$ )	ABTS radical scavenging activity (%)	
	GBL	APL
1	2.04 $\pm$ 0.53	5.35 $\pm$ 0.80
10	8.72 $\pm$ 0.74	36.46 $\pm$ 0.47
100	47.96 $\pm$ 0.40	94.59 $\pm$ 0.14
1000	94.57 $\pm$ 0.03	94.54 $\pm$ 0.17



**Fig. 2. ABTS radical scavenging activity of GBL and APL.**

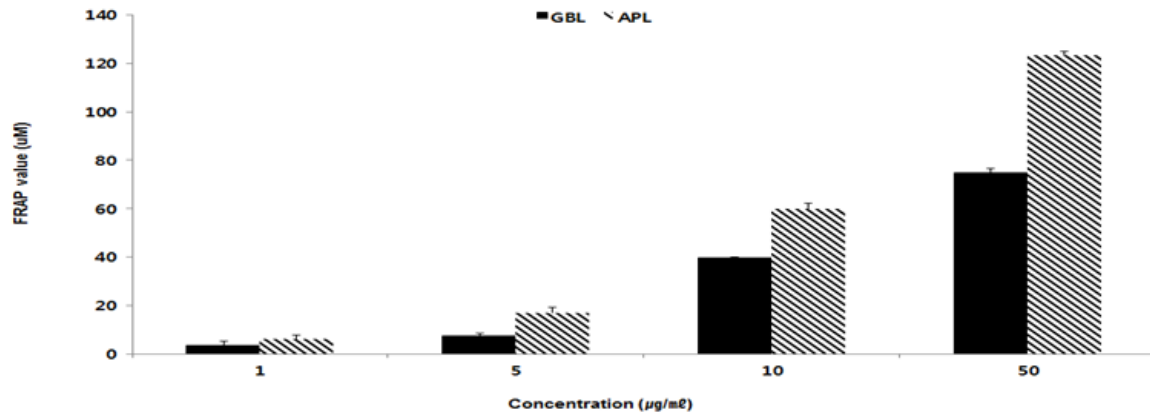
GBL and APL are incubated for 10 minutes with ABTS solution at 1, 10, 100 and 1000  $\mu\text{g} / \text{m}\ell$ , respectively. ABTS activity is determined by measuring absorbance at 732 nm.

As a result of measuring FRAP activity, as shown in Table 4 and Figure 3, both GBL and APL showed a concentration-dependent increase.

APL showed twice the FRAP value in all concentrations ( $\mu\text{g}/\text{m}\ell$ ) higher than GBL, and in particular, at 5 ( $\mu\text{g}/\text{m}\ell$ ) concentration, GBL and APL were 7.32 $\pm$ 1.01 and 17.01 $\pm$ 2.16  $\mu\text{M}$ , respectively, showing the largest difference in FRAP values. APL showed significant ( $p < 0.05$ ) FRAP values compared to GBL. As a result, it is shown that APL has much higher antioxidant capacity than GBL.

**Table 4. FRAP Value of GBL and APL**

Concentration ( $\mu\text{g}/\text{m}\ell$ )	FRAP value ( $\mu\text{M}$ )	
	GBL	APL
1	3.57 $\pm$ 1.76	6.01 $\pm$ 1.64
5	7.32 $\pm$ 1.01	17.01 $\pm$ 2.16
10	39.61 $\pm$ 0.35	59.94 $\pm$ 2.33
50	74.70 $\pm$ 1.71	123.45 $\pm$ 1.41



**Fig. 3. FRAP value of GBL and APL.**

GBL and APL were incubated at 1, 5, 10, and 50 µg/mL with FRAP reagent solution for 30 mins. FRAP activity is determined by measuring absorbance at 593 nm.

### Conclusion

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.

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