1. Introduction

Free radicals are recognized as prime sources of aging and diseases over the years. These include superoxide radical ($O_2^{•−}$), hydroxyl radical ($•OH$), singlet oxygen ($^1O_2$), and hydrogen peroxide ($H_2O_2$), which lead to oxidative stress when tipping the scales between the free radicals and antioxidants. Antioxidants are compounds which can donate electrons or hydrogen to the free radicals in order to inhibit the oxidation processes significantly. Synthetic antioxidants, such as BHT, BHA, and ascorbic acid, have proven to be beneficial on anti-aging, anti-inflammatory, and anti-radiation, and natural antioxidants are currently gaining attention due to its efficacy and safety to humans.

*Rehmannia glutinosa*, also called Di Huang, is mainly distributed in China and Korea, and used as a traditional herbal medicine. It could be a cure for various injuries and diseases because of its ability to clear away the heat from the blood, stimulate the production of saliva, and dominate growth in order to regulate the body balance. Hot water and 80% MeOH extracts of *Rehmannia glutinosa* have shown a comparatively higher antioxidant activity as compared with other 29 herbs, according to. Furthermore, the hot water extract demonstrated higher phenolic component and antioxidant activities. Based on the study of regarding the anti-proliferative effect of the *Rehmannia glutinosa* water extract, they found that the extract inhibits the proliferation and induces apoptosis of the hepatocellular carcinoma cells. On the other hand, we found that there were only a number of researchers who focused on the extracted *Rehmannia glutinosa* by using ethyl alcohol. For this reason, our research purpose is to examine the antioxidant activities of the *Rehmannia glutinosa*.

2. Materials and Methods

2.1 Chemicals and reagents

The Folin-Ciocalteu reagent, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), sodium hydroxide (NaOH), Trichloroacetic Acid (TCA), BHA, tannic acid, and α-tocopherol were supplied by Sigma (St, Louis, MO, USA). In addition, the other chemicals or reagents were considered to be of analytical grade.

2.2 Material

In this study, the material used was the *Rehmannia glutinosa* that was acquired from Korea. A pin crusher...
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(Myungsung Machine, Seoul, Korea) was used to turn the dried Rehmannia glutinosa into powdered form, while ethyl alcohol was used to extract the powdered sample 3 times. The extracted Rehmannia glutinosa was filtered with the use of a No. 1 filter paper (Whatman, Maidstone, UK) and evaporated by a vacuum rotary (CCA-1110; Eyela, Tokyo, Japan).

2.3 Total phenolic and flavonoid constituent
The Folin Ciocalteu method was utilized to measure the total phenolic component. A 1.0 mL aqueous extract was mixed with a 1.0 mL FC reagent, and then kept in room temperature for 10 minutes. A 7.5% Na₂CO₃ 2.0 mL was added and then incubated. The tannic acid was used for the calibration curve and expressed as Tannic acid equivalent/g (mg TAE/100 g).

The measurement of the total flavonoids was based on the method described by, wherein an aliquot of 0.5 mL extract was added to 0.1 mL of 10% aluminium chloride hexahydrate, 0.1 mL of 1 M potassium acetate, 2.8 mL of sterile water, and 1.5 mL of 95% ethyl alcohol, and then kept in room temperature for 40 minutes. Quercetin was used to perform a calibration curve and it was expressed as mg Quercetin equivalent/g (mg QE/100 g).

2.4 DPPH Free Radical Scavenging Activity
According to, the DPPH free radical scavenging activity was used with a slight modification, wherein a 0.5 mL DPPH methanol solvent was added to 0.5 mL of extract solution at different concentrations. The mixture was kept in a dark container for 30 minutes, and then measured the absorbance at 515 nm by using a multiplate spectrophotometer reader (ELx800TM, BioTek, Winooski, VT, USA). In addition, α-Tocopherol was used as positive control.

2.5 Metal-Chelating Activity
As reported by, the chelating activity was measured via modified ferrozine assay. In short, 1 mL of various concentrations of extracts was mixed with 3.7 mL of absolute methanol and 0.1 mL of 1 mM FeCl₂. The reaction was followed by vigorously agitating and measuring at 562 nm with an additional 0.2 mL of 5 mM ferrozine. The EDTA was used as the standard for the preparation of the calibration curves.

2.6 Reducing Power Activity
According to the method that was reported by, the reducing power activity was measured with a slight modification. A 200 μL of sample solution was incorporated into the mixture of 500 μL 0.2 M phosphate buffer (pH 6.6) and 500 μL 1% (w/v) potassium ferricyanide, respectively. After pre-incubation at 50°C for 30 minutes, the solution was centrifuged at 3000 rpm for 10 minutes at room temperature after adding a 500 μL of 10% (w/v) TCA. The 500 μL of supernatant was removed, poured into a new tube, and then a 500 μL of sterile water and a 100 μL of 0.1% (w/v) ferrous chloride were added. The absorbance was observed at 700 nm.

2.7 Total antioxidant activity
In described the analysis of the modified total antioxidant activity of the extract. The 200 μL of extract solution (1 mg/mL), which was dissolved in ethyl alcohol, was mixed with a 600 μL of reagent (0.6 M sulphuric acid, 28 mM NaCO₃, and 4 mM ammonium molybdate) and incubated at 95°C for 90 minutes. Subsequently, the mixture was cooled to room temperature, and then it was measured at 695 nm. The ascorbic acid was used as a standard.

2.8 Statistical Analyses
The tests that were conducted in triplicate (n = 3) showed results that were expressed in terms of mean ± standard deviation. The statistical analysis was calculated by SPSS 21 (SPSS Institute, Cary, NC, USA) through a one-way Analysis of Variance (ANOVA), followed by the multiple comparison procedure of Duncan’s multiple-range test. The significant difference between the groups was found to be p < 0.05.

3. Results and Discussion

3.1 Total Phenolic and Flavonoid Constituent
The Rehmannia glutinosa extract contains a phenolic component of 1432.3±16.2 mg TAE/100 g as shown in Table 1. The data was similar to what reported in 2006, wherein it was also suggested that the aqueous extract contained more abundant phenolics than the alcohol extract. The flavonoids with the value of 97.6±14.1 mg QE/100 g existed in the Rehmannia glutinosa extract.
Flavonoids and phenolics are considered as beneficial antioxidants due to their ability to scavenge active oxygen species by donating electrons or hydrogens to harmful electrons as $O_2^{\bullet-}$, $H_2O_2$, •OH or $1O_2$ in order to form stable compounds.  

Table 1. Total phenolic, flavonoid content, and DPPH free radical scavenging activity

| Extracts         | Phenolics | Flavonoids | DPPH Scavenging Activity |
|------------------|-----------|------------|--------------------------|
|                  | mg TAE/100 g | mg QE/100 g | IC$_{50}$ μg/mL          |
| *Rehmannia glutinosa* | 1432.3±16.2    | 97.6±14.1   | 469.87±12.42             |

3.2 DPPH free radical scavenging activity

DPPH is a compound that has a deep purple color. It is considered to be a stable free radical that has been widely used in measuring the radical scavenging activity of the antioxidants. In addition, it has a maximum absorbance of approximately 515 nm. The antioxidants will turn the deep purple into colorless. The DPPH free radical scavenging activity was expressed through the effective concentration at which the DPPH radicals were scavenged by 50% (IC$_{50}$). Moreover, the *Rehmannia glutinosa* extract has an IC$_{50}$ value of 469.87±12.42 μg/mL, wherein the IC$_{50}$ of positive control α-tocopherol was 17.43 ± 0.06 μg/mL. It also showed free radical scavenging activity via other solvents. In research, the boiling water extract of *Rehmannia glutinosa* found a scavenging activity of DPPH free radical of approximately 1 mg/mL extract scavenging 40% DPPH radical, thereby indicating that the *Rehmannia glutinosa* could be a potential radical scavenger.

3.3 Metal-chelating activity

In order to measure the activity of the chelating irons, which have the ability to catalyze the hydrogen peroxide break down, a metal-chelating activity was carried out. The EDTA was an effective ingredient for enhancing the redox potential and decreasing the oxidation from the iron. Figure 1 demonstrated the chelating activity of *Rehmannia glutinosa*. The result suggested that when the concentration was below 1 mg/mL, a weak chelating activity was detected; however, an increase in concentration showed that the chelating activity increased sharply, and it also exhibited a dose-dependent manner.

3.4 Reducing Power Activity

Researchers have found that a direct correlation existed in the antioxidant activities and the reducing power of the plant extracts. The reducing power activity of the compound may be considered as an efficient indicator of antioxidant activity due to its reduction ability of Fe$^{3+}$ ability to Fe$^{2+}$. During the process, the yellow reagent changes to green, according to the reducing power of the sample extract, as shown in Figure 2. The ascorbic acid showed a high reducing power activity in 50 μg/mL, while the ethyl alcohol of *Rehmannia glutinosa* extract exhibited a low activity as compared with the positive control. When the concentration of the extract was increased, the reducing power activity also increased slightly.
3.5 Total Antioxidant Activity

The antioxidant protection activity is based on the reduction of Mo (VI) to Mo (V) through the existence of the extract. The blue color (isopoly-molybdenum blue) at 695 nm was used to measure the reduction ability. This assay is widely used to evaluate the oxidative stress combined with another antioxidant measurement. The total antioxidant activity of *Rehmannia glutinosa* was weaker than the positive control ascorbic acid, which was shown in Figure 3. Although the activity increased along with the concentration, it was still lower when the extract concentration was 800 μg/mL, as compared with 50 μg/mL ascorbic acid.

![Figure 3](image)

**Figure 3.** Total antioxidant activity of Rehmannia glutinosa ethanol extract in different concentrations. Ascorbic acid was used as a positive control. Each value was expressed by the mean ± SD.

4. Conclusions

In conclusion, the *Rehmannia glutinosa* has a total phenolics and flavonoids of 1432.3±16.2 mg TAE/100 g and 97.6±14.1 mg QE/100 g, respectively, and it may contribute to the DPPH scavenging activity with the IC₅₀ value of 469.87 μg/mL. However, the other method, which served to evaluate the antioxidant activity, suggested that the extracted *Rehmannia glutinosa* demonstrated a weak activity as an antioxidant. These findings revealed that *Rehmannia glutinosa* would be used as a free radical scavenger.

5. References

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