



***In Vitro* Antimicrobial and Antioxidant Properties of Extracts from *Magnolia denudata* and *Magnolia kobus* Flower Bud and Flower**

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Abstract

The flower bud of *Magnolia denudata* has been used as a traditional medicine to treat rhinitis and gastrointestinal disorder, but the flower has not been used for medicine in Korea. This study evaluated the extracts of *Magnolia denudata* and *Magnolia kobus* flower and flower bud for antimicrobial and antioxidant properties. The MIC by disk diffusion assay was used for the antimicrobial activity against three Gram-positive bacteria, four Gram-negative bacteria strains and one yeast. Antioxidant activity for three solvent extracts (hot water, ethanol 70%, ethanol 100%) of the two *Magnolia* plants was evaluated by using EDA and ABTS radical scavenging activity. The water fraction of the two *Magnolia* flower bud was shown to have the highest antimicrobial activity, with the MIC value of 5.0 mg/ml against *B. cereus* and *S. typhimurium*. The hot water extract of *Magnolia denudata* (flower bud) showed the highest antioxidant activity, with the lowest IC₅₀ values of 301.82±7.92 µg/mL in the EDA assay and 219.48±6.32 µg/mL in the ABTS assay. In addition, the hot water extract of the four samples revealed the highest TPC and TFC as compared to the other extracts and the order was flower bud of *M. denudata* > flower of *M. denudata* > flower of *M. kobus* > flower bud of *M. kobus*. There appears to be a relationship between the TPC (+TFC) and the antioxidant properties of *Magnolia denudata* and *Magnolia kobus*. This is first report on *in vitro* antimicrobial and antioxidant properties of the two *Magnolia* plants, these data imply that the two *Magnolia* plants might to be useful for future development in pharmaceutical, food and cosmetic industries.



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Abbreviations

MIC	Minimal Inhibitory Concentration
EDA	Electron Donating Activity
DPPH	1,1-diphenyl-2-picrylhydrazyl
ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
TPC	Total Polyphenolic Content
TFC	Total Flavonoid Content

Introduction

The rise in infectious diseases caused by harmful microbial strains is increasing the use of antibiotics. Extensive use of antimicrobial chemotherapy has led to microorganism's resistance to the antimicrobial chemicals and the increase of antimicrobial agents derived from plant sources have been developed plant-based medicine production. The secondary metabolite of plant, such as phenolic, terpenoid, flavonoid and tannin have antimicrobial activity.¹ Antioxidants are important substances that able to protect the human body from damage caused by free radicals induces oxidative stress. Consumption of medicinal plants is increasing as phenolic compounds contained in natural plant extract is expected to prevent the risk of reactive oxygen species-induced diseases.^{2,4}

Magnolia is a genus of flowering plant belong to the *Magnoliaceae* family. *Magnolia denudata* and *Magnolia kobus* is deciduous tree planted in garden or farm of Korea. The flower of *Magnolia denudata* has no sepals and the petals do not turn over, on the other hand, the flower of *Magnolia kobus* has sepals and petals that are turn over.

The plants with antimicrobial, antioxidant, and anti-inflammatory activities have been widely used in the food, cosmetic and pharmaceutical industries. *Magnolia* bark and flower has been used for treatment of anxiety and allergic disease and gastrointestinal disorder in Chinese and Japanese traditional medicine. Magnolol (MAG) and honokiol (HON) is phenolic compounds obtained from *Magnolia* bark. MAG, HON and *Magnolia* bark extract have antioxidant, antimicrobial, antidiabetic, antipyretic and anti-inflammatory activity.^{5,6} In general, the flower bud of *Magnolia denudata* has been used as a traditional medicine to treat rhinitis and gastrointestinal disorder in Korea.

The aim of the present study is to assess the antimicrobial and antioxidant properties of *Magnolia denudata* and *Magnolia kobus*. This is important study since it is the first time to compare the activities of flower and flower bud of the two *Magnolia* plants and these results suggest that the economic profits can be increased by using flowers, not just flower buds.

Materials and Methods

Material

The flower bud and flower of *Magnolia denudata* and *Magnolia kobus* were collected from a cultivated population in Namwon-si, Jellabuk-do, Korea at early April and late April 2024 and air-dried in shadow for two weeks. The air-dried sample was pulverized using an electric mill.

Extraction solvents were obtained from Daejung Chemicals and Metals Co. (Shiheung, Korea) and the other chemicals and reagents were sourced from Sigma-Aldrich Co. (St. Louis, USA) and BD (Becton, Dickinson and Company, Sparks, MD, USA).

Antimicrobial Activity

Test Microorganisms

The antimicrobial activity was evaluated using the agar diffusion method to determine the MIC against microorganisms obtained from Korean Culture Center of Microorganisms Patents (KCCM). These included three Gram-positive strains, *Bacillus cereus* KCCM 11204, *Bacillus subtilis* KCCM 11778 and *Staphylococcus aureus* KCCM 11335, four Gram-negative strains, *Escherichia coli* KCCM 11234, *Pseudomonas aeruginosa* KCCM 11266, *Pseudomonas fluorescens* KCCM 41709 and *Salmonella typhimurium* KCCM 40253 and one yeast, *Saccharomyces cerevisiae* KCCM 50712.

Preparation of Extract for Antimicrobial Activity

The finely powdered material (100 g each) was macerated in 1,000 mL ethanol (100%) at room temperature for 24 h. The mixture was filtered using Whatman No.2 paper to obtain the ethanol extract. The crude extract was subsequently subjected to liquid-liquid partitioning in a separating funnel with 500 mL of n-hexane. The upper hexane layer was collected and concentrated to yield the hexane fraction. The remaining aqueous-ethanol layer was further partitioned with 500 mL each of diethyl ether, followed by ethyl acetate, and finally water, resulting in the diethyl ether, ethyl acetate, and water fraction, respectively. All of the fractions were evaporated to approximately 30 mL using a rotary evaporator set at 30 °C. The fractions were stored at 5 °C for antimicrobial assay.

Determination of Antimicrobial Activity

Prior to the assay, each strain was cultured in nutrient broth and incubated at 30 °C for 18–24 h. The cultures were then subculture three additional times under the same conditions. The optical density of the resulting cell suspensions was adjusted to approximately 0.3 at 660 nm by adding sterile nutrient. For the assay, 0.1 mL of the adjusted microbial suspension was poured uniformly on nutrient broth agar plates. Sterile paper disks impregnated with the tested fraction were gently placed onto the inoculated agar surfaces. Plates were incubated at 37 °C for 24 h. The diameter (in mm) of the clear zone surrounding each disk were measured to assess antimicrobial activity. MIC was defined as the lowest concentration of the extract that produced a visible clear zone.⁷ MIC was measured only with water fraction showed the activity in preliminary experiment.

Antioxidant Activity**Preparation of Extract for Antioxidant Activity**

The powder of flower and flower bud of the two *Magnolia* plants (5 g) was mixed separately with 100 mL of distilled water, 70% ethanol and 100% ethanol. The samples mixed with ethanol were kept at room temperature and the sample mixed with water was kept at 80 °C on rotary shaker for 3 h. The filtration of the extracts was done through Whatman filter paper No. 41. The filtrate was further concentrated at 56 °C and freeze dried (Freeze Dryer PVTFD 10R, iShinBioBase Co., Ltd., Korea) and then stored at -20 °C for performing experiments.

Electron Donating Activity

The EDA of the extracts (hot water, ethanol 70% and ethanol 100%) of the two *Magnolia* plants was assessed based on their ability to scavenge the stable DPPH free radical. The assay followed a modified version of the method described by Blois. In brief, 160 µL of each extract at different concentrations (100 µM as the final concentration) was mixed with 40 µL of DPPH solution (1.5×10^{-4} M). The mixture was vortexed gently and incubated in the dark at room temperature for 30 min. Subsequently, the absorbance was recorded at 520 nm using a microplate spectrophotometer reader (EL800; Biotek, USA).⁸ The DPPH scavenging activity was expressed in term of IC_{50} value.

ABTS Radical Scavenging Activity

The ABTS scavenging activity was based on the protocol originally developed by Re *et al.* The ABTS cation stock solution was obtained by reacting 2.4 mmol/L potassium persulfate solution with 7 mmol/L ABTS. The working solution was kept to stand in the dark for 12 h. The resulting solution was further diluted with ethanol to attain the appropriate concentration to an absorbance of 0.70 ± 0.02 at 732 nm. 50 µL of each extract at various concentrations was added to 2.0 mL of the diluted ABTS working solution and then the absorbance was measured at 732 nm using a microplate spectrophotometer reader (EL800; Biotek, USA).⁸ The ABTS scavenging activity of each extract was expressed in term of IC_{50} value.

Quantification of Total Polyphenolic and Flavonoid Contents

TPC was quantified using a slightly modified Folin–Denis method. 0.5 mL of the extract (hot water, ethanol 70% and ethanol 100%) was treated with 0.025 mL of Folin-Ciocalteu reagent (1/1000 diluted). After reacting the mixture for 6 min, 0.1 mL of saturated sodium carbonate solution (10% Na_2CO_3 in distilled water) was added and the solution was incubated in the dark (22 °C). The absorbance was recorded at 765 nm using a UV-visible spectrophotometer (HP-8453, USA). For the assay, the standard was gallic acid (100–500 µg / mL), and the result was manifested as the TPC of the tested extracts as milligrams gallic acid per gram dry weight (mg GAE g^{-1} of dry weight).⁹ The calibration equation for gallic acid obtained was $y=0.0244x+0.0361$ ($R^2=0.9948$), where y is

the absorbance and x is the concentration of gallic acid in µg/mL.

TFC was quantified using the procedure described by Moreno *et al.* with minor modification. Briefly, 100 µL of each extract (hot water, ethanol 70% and ethanol 100%) was mixed with 20 µL of 10% aluminum nitrate, 20 µL of 1 M aqueous potassium acetate, and 860 µL of 80% ethanol. Afterwards, the mixture was incubated for 40 min at room temperature. The absorbance was recorded at 415nm using a UV-visible spectrophotometer (HP-8453, USA). For the assay, the standard was quercetin (50-300 µg / mL), and the result was

manifested as the TFC of the tested extracts as milligrams quercetin per gram dry weight (mg QE g⁻¹ of dry weight).⁹ The calibration equation for quercetin obtained was $y=0.001x+0.0441$ ($R^2=0.9916$), where y is the absorbance and x is the concentration of quercetin in µg/mL.

Statistical Analysis

All experimental data were subjected to one-way ANOVA and Duncan’s multiple range test was used to compare means based on three replicates (n=3). Statistical analyses were conducted using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

Table 1: MIC of water fraction of ethanol extract from *Magnolia denudata* and *M. kobus* against the tested bacteria

Plant		MIC (mg/mL)						
		Gram-positive			Gram-negative			
		<i>B. cereus</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>P. fluorescens</i>	<i>S. typhimurium</i>
<i>Magnolia denudata</i>	Flower bud	10.0	15.0	15.0	15.0	20.0	15.0	5.0
	Flower	15.0	15.0	15.0	15.0	20.0	15.0	15.0
<i>Magnolia kobus</i>	Flower bud	5.0	10.0	10.0	10.0	10.0	15.0	10.0
	Flower	15.0	10.0	10.0	10.0	10.0	15.0	10.0

Results

Antimicrobial Activity

As shown in Table 1, the MIC of the water fraction of *Magnolia kobus* flower against the five tested bacteria was identical with the MIC of the flower bud fraction. The antimicrobial activity of *Magnolia kobus* flower and flower bud fractions was higher than that of *Magnolia denudata* flower and flower bud fractions. *B. cereus* and *S. typhimurium* exhibited the highest sensitivity with MIC values of 5.0 mg/mL for the flower bud fraction of *M. kobus* and *M. denudata*. For *Saccharomyces cerevisiae*, any tested fraction was not generated an inhibition zone.

Antioxidant Activity

Electron Donating Activity

The EDA of the extracts (hot water, ethanol 70% and ethanol 100%) of the two *Magnolia* plants was

measured on the basis of the DPPH scavenging activity is shown in Table 2. A lower IC₅₀ value shows a higher antioxidant activity. The EDA of hot water extract was the highest, followed ethanol 70% extract and ethanol 100% extract. The IC₅₀ values of hot water extract of *Magnolia denudata* flower bud was found superior to other extracts.

ABTS Scavenging Activity

The results also show that ABTS scavenging activity of the hot water extracts of the two *Magnolia* plants was higher than that of the ethanol 70% extracts and ethanol 100% extracts (Table 3). The results also revealed that the hot water extract of *Magnolia denudata* flower bud has higher ABTS scavenging activity than the other extracts, which is consistent with the DPPH scavenging activity.

Table 2: DPPH scavenging activity of the extracts from *Magnolia denudata* and *M. kobus*

Solvents	DPPH scavenging activity (IC ₅₀ , µg/g, Mean±SD)			
	<i>Magnolia denudata</i>		<i>Magnolia kobus</i>	
	Flower bud	Flower	Flower bud	Flower
Hot water	301.82±7.92 ^b	340.23±12.26 ^b	384.01±14.22 ^{bc}	364.01±9.78 ^b
Ethanol 70%	348.03±10.32 ^b	385.76±7.92 ^{ab}	411.01±8.31 ^b	389.18±9.24 ^b
Ethanol 100%	468.82±11.35 ^a	500.81±16.84 ^a	676.18±15.26 ^a	570.66±12.45 ^a
Ascorbic acid	159.21±4.37			

Values with different superscripts within same column are significantly different ($p < 0.05$).

Table 3: ABTS scavenging activity of the extracts from *Magnolia denudata* and *M. kobus*

Solvents	ABTS scavenging activity (IC ₅₀ , µg/g, Mean±SD)			
	<i>Magnolia denudata</i>		<i>Magnolia kobus</i>	
	Flower bud	Flower	Flower bud	Flower
Hot water	219.48±6.32 ^b	241.91±5.26 ^b	258.86±8.01 ^b	255.47±7.34 ^b
Ethanol 70%	259.46±7.08 ^b	266.41±5.87 ^b	327.21±9.63 ^b	281.18±8.36 ^b
Ethanol 100%	514.48±21.74 ^a	508.34±19.46 ^a	1201.93±37.74 ^a	704.43±24.75 ^a
Ascorbic acid	155.21±4.37			

Values with different superscripts within same column are significantly different ($p < 0.05$).

Table 4: Total polyphenol content of the extracts from *Magnolia denudata* and *M. kobus*

Solvents	Total polyphenol content (mg GAE/g, Mean±SD)			
	<i>Magnolia denudata</i>		<i>Magnolia kobus</i>	
	Flower bud	Flower	Flower bud	Flower
Hot water	11.81±0.10 ^a	9.63±0.08 ^a	7.69±0.08 ^a	8.14±0.12 ^a
Ethanol 70%	9.91±0.13a ^b	8.77±0.06 ^{ab}	6.01±0.09 ^a	8.04±0.01 ^a
Ethanol 100%	4.88±0.01 ^b	3.97±0.03 ^b	1.92±0.01 ^b	3.71±0.09 ^b

Values with different superscripts within same column are significantly different ($p < 0.05$).

As for TFC, hot water extract had the highest amount compared with other extracts. The order of TFC in hot water extract revealed such as, flower bud of *M. denudata* > flower of *M. denudata* > flower of *M. kobus* > flower bud of *M. kobus* (Table 5).

Total Polyphenolic and Flavonoid Contents

The TPC of the extracts (hot water, ethanol 70%, ethanol 100%) of the two *Magnolia* plants was shown in Table 4. The results presented that the TPC in hot water extract of four samples were the highest as

compared to the other extracts. The order of TPC in hot water extract revealed such as, flower bud of *M. denudata* > flower of *M. denudata* > flower of *M. kobus* > flower bud of *M. kobus*.

Table 5: Total flavonoid content of the extracts from *Magnolia denudata* and *M. kobus*

Solvents	Total flavonoids content (mg QE/g, Mean±SD)			
	<i>Magnolia denudata</i>		<i>Magnolia kobus</i>	
	Flower bud	Flower	Flower bud	Flower
Hot water	7.38±0.07 ^a	6.86±0.04 ^a	4.50±0.15 ^a	5.57±0.05 ^a
Ethanol 70%	5.42±0.10 ^{ab}	5.17±0.05 ^{ab}	3.31±0.01 ^{ab}	4.42±0.11 ^{ab}
Ethanol 100%	2.21±0.01 ^b	1.90±0.04 ^b	0.73±0.01 ^b	1.31±0.01 ^b

Values with different superscripts within same column are significantly different ($p < 0.05$).

Discussion

Several plant extracts have been used as traditional medicines for microbial infections, suggest that naturals are the major material of antimicrobial agents.¹⁰ In this study, four different fractions, namely, hexane, ether, ethyl acetate and water of *Magnolia denudata* and *M. kobus*, were prepared. Only the water fraction was shown the antimicrobial activity. According to Afzal *et al.*, the choice of extraction solvent for plant bioactive chemicals is important to reliable for antibacterial activity.¹¹ And the compounds from the plant material depend on the extraction solvent.¹² The difference of antibacterial activity between Gram-positive bacteria and Gram-negative bacteria was not shown. In general, the Gram-positive bacteria were more sensitive to the antibacterial activity of tested extracts than the Gram-negative bacteria.¹³

Phenolic compounds have received an attention because of the antioxidative potential and commonly extracted by organic solvents from plants.¹⁴ Moreover, the EDA in different solvent extracts is shown to related with its TPC and TFC. Antioxidant chemicals, such as polyphenolic compounds and flavonoids, are efficient oxygen radical scavengers and they are associated with its medicinal values and help human body to fight against diseases.¹⁵ And several studies have showed the positive relationship between the antioxidant activity of plant extracts and their plant secondary metabolites.¹² On the other side, Morais *et al.* verified that there was no strong relation between the antioxidant activity and

phenolic compounds of pollen from five Portuguese Natural-Parks.¹⁶ Antioxidant activity does not only arise from the phenolic content but also because of other phytochemicals.¹⁷

Polyphenolic compounds are one of the major components with antioxidative potential and can play roles in absorbing and neutralizing free radicals. The organic extracts of the plants are considered more beneficial and less deleterious side effects than synthetic drugs.² The qualitative and quantitative differences of components from *Magnolia* bark are proposed to be due to different analytic methods, solvents, or growing areas.^{18,19}

The high ABTS scavenging activity of methanol extracts from the two *Magnolia* plants might be correlated with the amount of total phenolic content.²⁰ Extraction is the most important step in the purification of bioactive compounds from natural products and the content of a chemical in different solvent extracts was related to the antioxidant activity.⁶

Qualitative analysis of *Cibotium barometz* (rhizome hair) indicated that the higher total phenolic contained in more polar extracts than the two less polar extracts and the highest TFC was found in the ethanol extract.²¹ The phenolic and flavonoid antioxidants are correlates highly with the free radical scavenging property, which impart human health benefits as medicinal constituents.^{22,23} Quantitative analysis of leaves and flowers of eight *Magnolia*

plants showed that the concentration of magnol, honokiol, obovatol and neolignans was different the tested plants, which correlates with the inhibition of *Staphylococcus aureus*.²⁴

This study on the antimicrobial and antioxidant activity of flower bud and flower extracts from *Magnolia denudata* and *M. kobus*, the activities are affected by extraction solvent. These results are useful to further studies on the substances affecting antibacterial or antioxidant activities.

Conclusion

The results of the present study showed for the first time on the comparative antimicrobial and antioxidant potential of *Magnolia denudata* and *Magnolia kobus*. The water fraction of ethanol extract from two *Magnolia* plants was shown similar antimicrobial activity. The present findings also demonstrate that antioxidant activity of three solvent extracts of the two *Magnolia* plants. The EDA, ABTS radical scavenging activity and TPC and TFC of the hot water extract was higher than other two solvent extracts, it might be a correlation between TPC and TFC and antioxidant activity of the *Magnolia* plants. These results demonstrate that flower of the two *Magnolia* plants, like their flower bud, can be considered as sources of natural antimicrobial and antioxidants in food, pharmaceutical, and cosmetic industries.

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Conflicts of Interest

The authors do not have any conflict of interest.

Data Availability Statement

The manuscript incorporates all datasets produced or examined throughout this research study.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to Reproduce Materials from Other Sources

Not applicable.

Author Contributions

- **Kyeong Won Yun:** Conceptualization, Methodology, Data Collection & Analysis, Writing – Original Draft and Editing, Supervision.
- **Kyoung-Sun Seo:** Conceptualization, Methodology, Data Collection & Analysis, Writing – Original Draft and Editing.

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