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Comparative Evaluation of Antimicrobial and Antioxidant Potential of Bark and Leaf of *Magnolia obovata* THUNB. and *Machilus thunbergii* S. et Z.

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Abstract
The bark of *Magnolia obovata* has been used in traditional medicine resources for digestive diseases and the bark of *Machilus thunbergii* has been used as a substitute of the bark of *Magnolia obovata* in Korea. This study is the first to compare the antimicrobial activity and antioxidant activity of bark and leaf of the two plants. The ethyl acetate and water fraction of hydro-methanol extract from the two plants was evaluated by the disc diffusion method to determine the minimal inhibition concentration (MIC) against nine different bacterial strains. The ethyl acetate fraction was the more effective against the bacterial strains than water fraction. The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity and xanthine oxidase inhibitory activity was evaluated to measure antioxidant activity. The DPPH scavenging activity and xanthine oxidase inhibitory activity of ethyl acetate fraction was the more effective as compared to that of water fraction like antimicrobial activity. The bark fraction exhibited stronger antimicrobial and antioxidant activity than leaf fraction and Gram-positive bacteria were more sensitive to the tested fraction than Gram-negative bacteria. Thus, the results obtained in the present study indicate that bark of *Machilus thunbergii* as substitute of bark of *Magnolia obovata* for antimicrobial and antioxidant resources.
Keywords: *Magnolia obovata*, *Machilus thunbergii*, Antimicrobial activity, Antioxidant activity

INTRODUCTION

The antimicrobial and antioxidant activity of plant is particular interest to food/drug industry which is looking for plant with antimicrobial and antioxidant activity to be used as preservatives [1]. The plants are used traditionally as medicine for the treatment of microbial infections and other diseases and plants used in folk medicine have been reputed as an indispensible source in new drug discovery and development [2]. Currently, there is an increasing interest for production of biologically active compounds that have antimicrobial and antioxidant actions from natural sources [3].

Microorganisms are causative factors for the pathogens of various diseases as well as for the spoilage and deterioration of food, pharmaceutical and cosmetic products. And the mistrust of synthetic antimicrobial agents due to their potential toxicity has intensified the efforts for discovering natural antimicrobial agents [4]. Antioxidant act as free radical scavengers and are thus helping to mitigate the oxidate stress in a variety of disease. Many studies have demonstrated the efficacy of plant derived products as good sources of antioxidants[5, 6].

Magnolia of the Magnoliaceae family have large showy flowers, which are solitary on the ends of shoots, large deciduous, alternate and simple leaves. The bark of *Magnolia obovata* has been used in traditional medicine resources for digestive diseases in Korea. *Machilus* of the Lauraceae family have evergreen/deciduous, small spike or umbel inflorescence and simple leaves. The bark of *Machilus thunbergii* has been used as a substitute of the bark of *Magnolia obovata* [7].

Although there are many studies showing that medicinal plants have antimicrobial and antioxidant activities [8-11], no information is available regarding antimicrobial and antioxidant activities of *Magnolia obovata* and *Machilus thunbergii in vitro*. The aim of this study was the *in vitro* evaluation and comparison of antimicrobial and antioxidant activities of the two plants used *Magnolia obovata* mixed with *Machilus thunbergii* as HuòPò (厚朴) in Korea.

MATERIALS AND METHODS

Plant materials

The fresh bark and leaf of *Magnolia obovata* was collected from Suncheon-si, southern area of Korea, and the fresh bark and leaf of *Machilus thunbergii* was collected from Goheung-gun, southern coast of Korea. They were authenticated by one of authors, Prof. K.W.Yun. The vouther specimen was deposited in Herbarium of Sunchon National University. The bark and leaf was air-dried in shade for two weeks and powdered.

Tested microorganisms

The tested microorganisms included four Gram-positive bacteria (*Bacillus cereus* ATCC 27348, *Bacillus subtilis* ATCC 9327, *Staphylococcus aureus* ATCC 13301 and MRSA Methicillin resistant *Staphylococcus aureus* ATCC 33593) and four Gram-negative bacteria (*Escherichia coli* ATCC 15489, *Pseudomonas aeruginosa* ATCC 9027, *Pseudomonas fluorescens* ATCC 11250 and *Salmonella typimurium* KCCM 11862) and one lactic acid bacterium (*Lactobacillus brevis* ATCC 13648). The Gram-positive and Gram-negative bacteria were cultured on a nutrient broth agar, while the lactic acid bacterium was on MRS agar.

Extract Preparation

The powdered samples (100 g) were soaked in methanol/water (80:20, v/v, 1,000 ml) for 24hrs at room temperature, the solution was filtered through Whatman No.2 filter paper. The crude extract was partitioned with 300 ml n-hexane and then the layer was concentrated (hexane fraction). The remaining layer was successively fractionated with 300 ml ethyl ether and then ethyl acetate (ether and ethyl acetate fraction). The remaining residue was water fraction. Each fraction was concentrated *in vacuo* to 30 ml at 30℃ and tested antimicrobial and antioxidant activity. Antimicrobial and antioxidant activity was measured only with the ethyl acetate and water fraction. The other fractions revealed no activity.

Determination of antimicrobial activity

Agar diffusion method was followed to determine the antimicrobial activity of the ethyl acetate and water fraction. In brief, each tested strains were individually inoculated in nutrient broth at 30 °C for 18~24 hrs prior to testing, and subcultured three

times for another 18~24 hrs. The turbidity of bacterial suspensions was brought to 0.3 optimal density (OD) at 660 nm by adding sterile broth and was then used for the test. Next, 200 µl of the bacterial suspension was uniformly spread on agar plates. Sterilized paper disks (8.0 mm diameter) impregnated with the extracts (ethyl acetate fraction and water fraction) were carefully placed on the inoculated agar. The diameters of the resulting inhibition zone were measured after incubation of plates was performed for 24 hrs or 48 hrs at 37℃. The minimum inhibitory concentration (MIC) was determined as the lowest concentration that caused an inhibition zone.

Determination of antioxidant activity

DPPH free radical scavenging activity The DPPH free radical scavenging activity was evaluated using the Blois method [12], with slight modification. Briefly, each sample fraction and DPPH were dissolved in methanol, after which 160 µl of each fraction at various concentrations (100 µM as the final concentration) were added to 40 µl of DPPH solution (1.5×10^{-4} M). The solutions were then gently mixed and allowed to stand at room temperature for 30 min, after which the optical density was measured at 520 nm using a microplate spectrophotometer reader (Molecular Devices). The activity of each fraction was expressed in terms of IC_{50} values (the concentration required to inhibit DPPH radical formation by 50%). Ascorbic acid was used as a positive control.

Xanthine oxidase inhibitory activity Bovine milk xanthine oxidase (XO) activity was measured based on uric acid production at 290 nm using a UV-vis spectrophotometer at 25℃. The reaction mixture in the sample wells consisted of xanthine oxidase (0.04 unit/ml, final concentration) in potassium buffer (50 mM, 0.14 ml, pH 7.5, 33mM final concentration), xanthine solution (0.15 mM, 0.1 ml, pH 7.5, 0.05 mM final concentration). The stock solutions of sample extracts (12 g/ml) were prepared in DMSO solution. Sample extracts were added in appropriate volumes so that the final concentration of DMSO in the assay did not exceed 3.3%. The reaction was initiated by the automatic addition of 0.05 ml of XO solution to a final concentration of 0.006 units/ml. For blank, xanthine was omitted from the samples and ascorbic acid was used as positive control. Each extracts were tested in triplicate. The IC_{50} values were calculated by analysing the inhibitory percentage of each fraction.

Statistical analysis

All experiments were performed in triplicate. The data were recorded mean±standard deviations and analyzed using statistical SPSS software (SPSS Inc., Chicago, USA. Version 21.0). The significance of difference was calculated by Duncan's multiple range test, and values $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Antimicrobial activity

The antimicrobial activity of ethyl acetate and water fraction of *Magnolia obovata* was evaluated against 4 Gram-positive, 4 Gram-negative and 1 lactic acid bacteria (Table 1). The results from the disc diffusion method, followed by measurement of minimum inhibitory concentration (MIC), indicated that ethyl acetate fraction of both bark and leaf exhibited more antimicrobial activity as compared to that of water fraction. And bark fraction exhibited more antimicrobial activity as compared to leaf fraction. MIC values for ethyl acetate fraction of bark were 0.1mg/ml, 0.3mg/ml, 0.5 mg/ml, 1.0 mg/ml, 0.2 mg/ml and 0.2 mg/ml against *Bacillus cereus*, *Bacillus subtilis*, MRSA, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Salmonella typimurium*. However, water fraction of *Magnolia obovata* leaf was not detected MIC against any tested bacteria.

The antimicrobial activity of ethyl acetate and water fraction of *Machilus thunbergii* on the tested bacteria is presented in Table 2. Both the ethyl acetate fraction and water fraction of bark and leaf showed antimicrobial activity against all the tested Gram-positive bacteria. In special, MIC value for ethyl acetate fraction of bark and leaf of *Machilus thunbergii* on *Staphylococcus aureus* were 0.1 mg/ml and 0.5 mg/ml, while no detection of MIC of the two fractions of *Magnolia obovata* was observed. *Escherichia coli* and *Lactobacillus brevis* was not detected MIC by any feaction of *Machilus thunbergii*. However, Khaled-Khodja et al. showed that *Escherichia coli* was sensitive to methanolic extracts of some Lamiaceae [4], and Rizwana et al. stated that *Escherichia coli* and MRSA was resistant to the extracts of *Matricaria aurea*, while *Bacillus subtilis* was shown the lowest MIC [13].

Among all the fractions tested, ethyl acetate fraction exhibited higher antimicrobial activity. Earlier report suggested similar observation [14]. In general, Gram-negative bacteria were more resistance to the tested fractions than Gram-positive bacteria. This result is in agreement with that of Rizwana et al. [12]. The resistance of Gram-negative bacteria is because they possess an outer membrane made of lipopolysaccharide, which is impermeable to the antimicrobial compounds present in the plant extracts [15].

Antioxidant activity

DPPH free radical scavenging activity Medicinal plants have been used to treat human diseases for thousands of years. The health benefits of medicinal plants are thought to arise partly from potential antioxidants on the reactive oxygen species produced in human body.[16] The free radical scavenging activity of ethyl acetate and water fraction was determined using DPPH assay. As it known, the lower the IC_{50} value the higher the antioxidant. The IC_{50} values for ethyl acetate fraction of bark and leaf of *Magnolia obovata* was 35.59±0.38 and 42.15±1.01 µg/ml, respectively. The water fraction exerted weaker DPPH radical scavenging activity than ethyl acetate fraction.. The free radical scavenging activity for ethyl acetate and water fractions of *Machilus thunbergii* was higher than those of *Magnolia obovata*. It means that *Machilus thunbergii* exhibited stronger antioxidant activity than *Magnolia obovata*.

Xanthine oxidase inhibitory activity Xanthine oxidase has been known as a key enzyme associated oxidation of purines hypoxanthine and xanthine to form uric acid and it is responsible for the medical condition known as gout, characterized by hyperuricemia and causing oxidative damage to living tissues.[17] IC_{50} values determined by xanthine oxidase inhibitory activity was 40.79±1.65 and 56.85±2.47 µg/ml for bark and leaf ethyl acetate fraction of *Magnolia obovata*. This activity was lower in comparison with ascorbic acid (12.79±0.12 µg/ml), but much higher than bark and leaf water fraction (152.98±2.10 and 155.13±2.10 µg/ml). The ethyl acetate fraction of *Machilus thunbergii* bark showed the highest antioxidant activity with IC_{50} value of 11.44±0.01 µg/ml. While, the lowest activity was observed for water fraction from *Machilus thunbergii* bark. The IC_{50} values of ethyl acetate and water fraction of leaf were 94.87±0.47 and 123.69±0.14 µg/ml.

The antioxidant activity in the bark used as a oriental medicine resource was higher than that in the leaf. Deng et al. reported that antioxidant and antimicrobial acticity of ths leaf and bark of *Solidago canadensis* varied with ripeness stage, tissue type and extraction [18]. And the higher antioxidant was shown in ethyl acetate fraction, regardless the method according to which it was determined (DPPH or xanthine oxidase), was found to be the same with the antimicrobial activity of the two tested plants. This result was in agreement with that reported in previous paper on antioxidant and antimicrobial properties of *Tetraclinis articulata* [14].

Table 1 Antimicrobial activity of fractions of hydro-methanol extract of *Magnolia obovata* (MIC in mg/ml) against tested bacteria.

Microorganisms	*Fractions*			
	Ethyl acetate fraction		*Water fraction*	
	Bark	*Leaf*	*Bark*	*Leaf*
Gram(+) bacteria				
Bacillus cereus	0.1	0.5	2.0	-
Bacillus subtilis	0.3	-	3.0	-
Staphylococcus aureus	-	-	2.0	-
MRSA	0.5	0.5	3.0	-
Gram(-) bacteria				
Escherichia coli	-	-	-	-
Psendomonas aeruginosa	1.0	-	5.0	-
Psendomonas fluorecens	0.2	1.0	2.0	-
Salmonella typhimurium	0.2	1.0	3.0	-
Lactic acid bacteria				
Lactobacillus brevis	0.5	-	-	-

- : not detected

Table 2 Antimicrobial activity of fractions of hydro-methanol extract of *Machilus thunbergii* (MIC in mg/ml) against tested bacteria.

Microorganisms	*Fractions*			
	Ethyl acetate fraction		*Water fraction*	
	Bark	*Leaf*	*Bark*	*Leaf*
Gram(+) bacteria				
Bacillus cereus	0.2	0.5	0.3	2.0
Bacillus subtilis	0.5	1.0	2.0	3.0
Staphylococcus aureus	0.1	0.5	0.5	3.0
MRSA	0.3	0.5	1.0	3.0
Gram(-) bacteria				
Escherichia coli	-	-	-	-
Psendomonas aeruginosa	3.0	1.0	-	-
Psendomonas fluorecens	0.5	1.0	1.0	3.0
Salmonella typhimurium	0.5	1.0	2.0	3.0
Lactic acid bacteria				
Lactobacillus brevis	-	-	-	-

- : not detected

Table 3 IC_{50} values of fractions of bark and leaf of *Magnolia obovata* and *Machilus thunbergii*.

Tested Plant	Antioxidant assay	Half maximal inhibitory concentration (IC_{50}, ug/ml)[a]				
		Ascorbic acid	Ethyl acetate fraction		Water fraction	
			Bark	Leaf	Bark	Leaf
Magnolia obovata	DDPH[b]	26.05±2.59[c]	35.59±0.38[b]	42.15±1.01[b]	46.59±3.34[b]	77.49±1.65[a]
	XO[c]	12.79±0212[c]	40.79±1.65[b]	56.85±2.47[b]	152.98±2.10[a]	155.13±2.10[a]
Machilus thunbergii	DDPH[b]	26.05±2.59[b]	27.23±069[b]	26.62±0.69[b]	33.36±0.62[ab]	41.87±0.44[a]
	XO[c]	12.79±0.12[c]	11.44±0.01[c]	94.87±0.47[b]	214.67±2.74[a]	123.69±0.14[ab]

[a] These values are the mean values of three replicates ± standard deviation. Different letters in each line exhibit significant difference in mean values at $p < 0.05$ according to Duncan's multiple range test.
[b] DPPH(2,2-diphenyl-1-picrylhydrazyl) in ug/ml
[c] Xanthine oxidase assay (XO) in ug/ml. Lower the value of IC_{50}, more is antioxidant activity.

CONCLUSION

In the present study, antimicrobial and antioxidant activity of ethyl acetate and water fraction was compared in bark and leaf of *Magnolia obovata* and *Machilus thunbergii*. The ethyl acetate fraction enhanced its antimicrobial and antioxidant activity, indicating that the active principles might be more concentrated in ethyl acetate as compared to water fraction. These activities were comparatively higher in bark fractions as compared to leaf fractions. Therefore, this study recommends the use of bark of *Machilus thunbergii* as substitute of bark of *Magnolia obovata* for antimicrobial and antioxidant resources.

REFERENCES

1. Skott, E., Anastasaki, E., Kanellou, G., Polissiou, M., *Industrial Crops and Products* 2014, 53, 46-54.
2. Balkan, I. A., Taskin, T., Dogan, H. T., Deniz, I., Akaydin, G., *Industrial Crops and Products* 2017, 95, 695-703.
3. Nain, P., Kumar, A., Sharma, S., Nain, J., *Asian Pacific Journal of Tropical Medicine* 2011, 1, 804-807.
4. Khaled-Khodja, N., Boulekbache-Makhlouf, L., Madani, K., *Industrial Crops and Products* 2014, 61, 41-48.
5. Karuna, D. S., Dey, P., Kundu, A., Bhakta, T., *Journal of Traditional and Complementary Medicine* 2018, 8, 60-65.
6. Kumar, V., Sharma, N., Sourirajan, A., Khosla, P. K., *Journal of Traditional and Complementary Medicine* 2017, 7, 1-7.
7. Lee, G. S., Kim, J. H., Choi, G., Kang, D. H., Hwang, S. Y., Jeong, S. I., Kim, H. J., Ju, Y. S., *Korean Journal of Herbology* 2008, 23, 21-29.

8. Bora, K. S., Kumar, A., Bisht, G., *Journal of Ayurveda and Intergrative Medicine* 2017, xxx, 1-5.
9. Chan, L. W., Cheah, E. L. C., Saw, C. L. L., Weng, W., Heng, P. W., *Chinese Medicine* 2008, 3, 1-10.
10. Mahmoudi, S., Khali, M., Benkhaled, A., Benamirouche, K., Baiti, I., *Asian Pacific Journal of Tropical Biomedicine* 2016, 6, 239-245.
11. Stankovic, N., Mihajilov-Krstev, T., Zlatkovic, B., Stankov-Jovanovic, V., Mitic, V., Jovi, C. J., Comic, L., Kocic, B,, Bernstein, N., *NJAS - Wageningen Journal of Life Sciences* 2016, 78, 21-28.
12. Blois, M. S., *Nature* 1958, 26, 1199-1202.
13. Rizwana, H., Alwhibi, M. S., Soliman, D. A., *International Journal of Pharmacology* 2016, 12(6), 576-586.
14. Rached, W., Zeghaba, F. Z., Bennaceur, M., Barros, L., Calhelha, R. C., Heleno, S., Alves, M. J., Carvalho, A. M., Marouf, A., Ferreira, I. C. F. R., *Industrial Crops & Products* 2018, 112, 460-466.
15. Silhavy, T. J., Kahne, D., Walker, S., *Cold Spring Harbor Perspectives in Biology* 2010, 2, 1-17.
16. Li, S., Li, S. K., Gan, R. Y., Song, F. L., Kuang, L, Li, H. B., *Industrial Crops and Products* 2017, 51, 289-298.
17. Santi, M. D., Zunini, M. P., Vera, B., Bouzidi, C., Dumontet, V., Abin-Carriquiry, A., Grougnet, R., Ortega, M. G., *European Journal of Medicinal Chemistry* 2018, 143, 577-582.
18. Deng, Y., Zhao, Y., Padilla-Zakour, O., Yang, G., *Industrial Crops and Products* 2015, 74, 803-809.