IJPSR (2023), Volume 14, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 06 April 2023; received in revised form, 10 June 2023; accepted 04 July 2023; published 01 December 2023

IN-VITRO ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF *ARALIA CORDATA* AND *KALOPANAX SEPTEMLOBUS*

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Keywords:

Aralia cordata, Kalopanax septemlobus, Antibacterial activity, Antioxidant activity

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ABSTRACT: The present study is aimed to compare the *in-vitro* antibacterial and antioxidant activities of *Aralia cordata* Thunberg and *Kalopanax septemlobus* (Thunb.) Koidzumi (Araliaceae). The antibacterial activity of the two plants was evaluated disc diffusion and minimal inhibition concentration (MIC) method against two Gram-positive bacteria and two Gram-negative bacteria. The tested ether and ethyl acetate fraction of ethanol extract from the two species showed weak antibacterial activity against the tested Gram-positive and Gram-negative bacteria. The ether fraction of the two species exhibited relatively higher antibacterial activity against the tested bacteria as compared to the ethyl acetate fraction. The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging assay was applied for antioxidant activity. Results showed that the DPPH activity (IC_{50}) of ethanol extracts of the two species ranged from 0.05 ± 0.01 to 0.28 ± 0.07 mg/g and the corresponding values for the ABTS activity (IC_{50}) of ethanol extracts of two species ranged from 0.25 ± 0.01 to 1.80 ± 0.23mg/g. The antioxidant activity of ethanol extracts was higher than that of the hot water extracts, while the results of total polyphenol and flavonoid contents were not consistent exactly with the results of antioxidant activity. The results will be supported as a good information on the use of the two species in the food and pharmaceutical industries.

INTRODUCTION: The demand for vegetables in national and international markets has continuously grown, perhaps by the publication that shows the traditional medicine based on herbal therapies. According to the World Health Organization (WHO), over 80% of the world's population relies on traditional medicine, largely plant-based, to meet primary health care needs [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(12).5613-18

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.14(12).5613-18

The collection and processing of medicinal plants and plant products contribute to Korea's health care and national economy. Aralia cordata and *Kalopanax septemlobus* sprout has been used as a functional food or vegetable in early spring to restore appetite, vitality and lifespan in Korea.

The cortex of *Aralia cordata* has been used in folk medicine of Korea and China as a tonic and diuretic to cure cough, diabetes, hepatitis and rheumatoid arthritis [2]. The cortex of *Kalopanax septemlobus* is used for treating neuralgia, rheumatoid arthritis and diarrhea and known a variety of pharmacological activities, including

anti-diabetic, cytotoxic, antifungal and anti-inflammatory activities [3, 4].

Plant by-products could be used as antimicrobial or antioxidant agents derived from the phenolic compounds. Research have been promoted by need to find natural substitutes for synthetic antimicrobials or antioxidants [5]. Antimicrobial activity of plants is determined by measuring the growth response of microorganisms to plant extracts. Antioxidant activity is associated with reducing the risk of diseases by stabilizing or deactivating free radicals.

The antioxidant activity is evaluated by measuring the content of total phenolic compounds and the uptake of radicals 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) [6, 7]. The present study aims to evaluate the antibacterial and antioxidant activities of *Aralia cordata* and *Kalopanax septemlobus*.

MATERIALS AND METHODS:

Plant Materials: The stem cortex and sprout of *Aralia cordata*and *Kalopanax septemlobus* was collected from a farm in Suncheon-si, Jellanam-do Province of Korea in October 2019 and April 2020. One of the others (K. W. Yun) authenticated the plant, and voucher specimens (SCNU 2019 408 and SCNU 2020 42, respectively) were also collected and deposited in the herbarium of Sunchon National University. The two provenances were air-dried at room temperature for two weeks. The air-dried sample was pulverized using an electric mill.

In-vtro Antibacterial Activity:

Test Bacteria: The tested bacteria included two Gram-positive bacteria (*Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 13301) and two Gram-negative bacteria (*Pseudomonas fluorescens* KCCM 41443 and *Salmonella typhimurium* KCCM 11862).

Preparation of Extract: The air-dried and powdered plant materials (100 g of each) were soaked in 1,000 mL ethanol at room temperature for 24 h and then filtered through Whatman No.2 paper. The crude ethanol extract was partitioned with 500 mL of hexane and then the top layer was concentrated (comprising the hexane fraction). The remaining layer was successively fractionated with 500 mL of diethyl ether and then ethyl acetate (forming the ether and ethyl acetate fractions). The remaining residue was the water fraction. Each fraction was concentrated with a rotary evaporator (30 °C) to 30 mL and stored at 5 °C until tested.

Determination of Antibacterial Activity: Each bacterial strain was grown in a nutrient broth at 30 °C for 18-24 h prior to testing and subcultured three times for another 18-24 h. The turbidity of bacterial cell suspensions was brought to 0.3 optimal density at 660 nm by adding sterile broth and was then used for the tests. We uniformly poured 0.1 mL of the bacterial cell suspensions on nutrient broth agar plates.

The paper disks containing the extracts were carefully placed on the seeded Petri dishes. The diameters of the resulting inhibition zones were measured in mm after the cultures were incubated at 38 °C for 24 h. At the end of the incubation period, the antibacterial activity was evaluated by measurement of the inhibition zone. The minimal inhibition concentration (MIC) was determined as the lowest concentration that caused an inhibition zone. MIC was measured for the ether and ethyl acetate fraction.

In-vtro Antioxidant Activity:

Preparation of Extract: 100 g of the powdered cortex and sprout was macerated with 1,000 mL of ethanol and hot water for 6 h, respectively. The percolates were then filtered (ADVANTEC No.2). The extracts were then concentrated *in-vacuo* to 100 ml at 30°C and tested for the DPPH and ABTS radical scavenging activities.

DPPH Radical Scavenging Activity: The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of the extracts was evaluated using a modified version of the method described by Blois [8]. Briefly, 160 µL of each extract 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 (EL800, Bioteck, Vinooski, VT, USA).

Each extract's DPPH radical scavenging activity was expressed in terms of IC_{50} values (the concentration required to inhibit DPPH radical formation by 50%). L-Ascorbic acid was used as a positive control.

ABTS Radical Scavenging Activity: The experiment was carried out using a modified 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) decolouration assay [9]. ABTS radical cation was produced by reacting ABTS stock solution (7 mM in water) and 2.45 mM potassium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing the mixture to react at room temperature in the dark for 12 h. And the solution was diluted with ethanol to absorbance of 0.70 ± (0.02) at 734 nm. 2.0 mL of the diluted ABTS was added to 50 µL of each extract and then the absorbance was measured on a microplate spectrophotometer reader (EL800, Bioteck, Vinooski, VT, USA) at 734 nm [10].

Each extract's ABTS radical scavenging activity was expressed in terms of IC_{50} values (the concentration required to inhibit ABTS radical formation by 50%). L-Ascorbic acid was used as a positive control.

Determination of Total Phenolic Content: The total polyphenol content was determined using a modified version of the Folin-Denis method[11].Each 25 µL sample extract (1mg/mL) was mixed with 500 µL of Folin-Denis' reagent (diluted 10-fold with distilled water) was added. The mixtures were allowed to stand at room temperature for 5 min and then centrifuged at 1200 rpm for 10 min, and the supernatant was collected.

The 0.1 mL of clear supernatant of the samples were mixed with 0.75 mL of Folin-Denis' reagent was added. After 5 min, 500 µLof sodium bicarbonate (7.5 % in distilled water) was added and the solution was allowed to stand at 30 °C in darkness. The absorbance was then measured at 765 nm using a microplate spectrophotometer reader (EL800, Bioteck, Vinooski, VT, USA). A standard curve prepared from gallic acid (100-1000 µg/mL) was used for quantification and the total polyphenol content was expressed as mg of gallic acid/g dry weight.

Determination of Total Flavonoid Content: The total flavonoid content was determined according to Moreno *et al.*, [12] with slight modification. 10 µL of each sample fraction (1mg/mL) was diluted with 80% aqueous ethanol (90 µL). An aliquot of 0.5 mL was added to the test tube containing 2 µL of 10% aluminum nitrate, 2 µL of 1 M aqueous potassium acetate and 86 µL of 80% ethanol. The solution was allowed to stand at room temperature for 40 min.

The absorbance was then measured at 415 nm using a microplate spectrophotometer reader (EL800, Bioteck, Vinooski, VT, USA). A standard curve prepared from quercetin (100-1000 µg/mL) was used for quantification and the total flavonoid content was expressed as mg quercetin/g dry weight.

Statistical Analysis: Data were expressed as mean± standard deviation values (n = 3). Statistical analysis was performed using SPSS software (version 24.0; SPSS Inc., Chicago, IL, USA).The significance of differences between means was evaluated using Duncan's test.

RESULTS AND DISCUSSION:

Antibacterial Activity: Antibacterial activity of the ether and ethyl acetate fraction was evaluated according to their MIC values against 2 Gram-positive and 2 Gram-negative bacteria **Table 1**. The results revealed that the tested fractions have weak antibacterial activity against the tested bacteria, having the MIC values 0.5~5.0 mg/mL, moreover, *S. typhimurium* was resistance to the tested fractions. *Bacillus cereus* was the most sensitive bacterium to the tested fractions. This result was similar to other studies. And the results indicated that Gram-negative bacteria are more resistant to the tested fractions than Gram-positive bacteria, which was similar to some results [13-15].

The tested bacteria used in this study were more susceptible to the cortex fractions than sprout fractions and the ether fractions were shown more potent than the ethyl acetate fractions. Neglo *et al*. showed that effective extraction and assessment of antimicrobial agents and antioxidants from plants are crucial in promoting their application as pharmaceuticals [16].

TABLE 1: MIC OF FRACTIONS OF ETHANOL EXTRACTS OF CORTEX AND SPROUT FROM *ARALIA CORDATA* AND *KALOPANAX SEPTEMLOBUS* AGAIST 4 BACTERIA

Fractions			Minimum Inhibition Concentration (MIC, mg/mL)			
			B. cereus	***S. aureus***	***P. fluorescens***	***S. typhimurium***
Aralia cordata	Cortex	Ether	0.5	2.0	1.5	-
		Ethyl acetate	5.0	5.0	5.0	-
	Sprout	Ether	0.5	1.0	0.5	-
		Ethyl acetate	2.5	2.5	-	-
Kalopanax septemlobus	Cortex	Ether	0.5	2.0	1.5	-
		Ethyl acetate	0.5	0.5	0.5	5.0
	Sprout	Ether	1.0	2.5	2.0	-
		Ethyl acetate	2.5	-	-	-

No clear zone was formed.

Antioxidant Activity: The beneficial effects derived from the phenolic compounds have been attributed to their antioxidant activity. Numerous researches have been focused on the obtaining natural antioxidants [17].

In the present study, the results are expressed as IC_{50}, which represents the antioxidant concentration necessary to decrease the initial DPPH and ABTS radical concentration by 50%. The determination of DPPH radical scavenging is a widely used and common method for the relatively rapid evaluation of antioxidant activity [18]. The DPPH free radical scavenging activity of the two species is shown in **Table 2**. The lower values indicate greater free radical scavenging activities of the extracts. The IC_{50} values of ethanol extracts of *Aralia cordata* and *Kalopanax septemlobus* cortex were 0.10 and 0.05 mg/g, respectively. In contrast, those of hot water extracts of *Aralia cordata* and *Kalopanax septemlobus* cortex were 0.63 and 0.67 mg/g. The DPPH free radical scavenging activities of ethanol extracts were greater than those of hot water extracts, regardless of species and samples, moreover, the ethanol extracts were more potent than ascorbic acid, a well-known antioxidant compound.

Table 3 shows ABTS free radical scavenging activities of *Aralia cordata* and *Kalopanax septemlobus* extracts. The IC_{50} values of ethanol extracts of the two species were lower than those of hot water extracts. The ABTS free radical scavenging activities of cortex extracts were greater than those of sprout extracts, regardless of species and solvents. These results are similar to the DPPH free radical scavenging activities.

TABLE 2: DPPH FREE RADICAL SCAVENGING ACTIVITY OF SOLVENT EXTRACTS OF CORTEX AND SPROUT FROM *ARALIA CORDATA* AND *KALOPANAX SEPTEMLOBUS*

Extracts			DPPH Free Radical Scavenging Activity (IC_{50}, mg/g)
Aralia cordata	Cortex	Ethanol	0.10 ±0.01
		Hot water	0.63±0.06
	Sprout	Ethanol	0.22±0.05
		Hot water	1.72±0.13
Kalopanax septemlobus	Cortex	Ethanol	0.05±0.01
		Hot water	0.67±0.08
	Sprout	Ethanol	0.28±0.07
		Hot water	1.46±0.11
	Ascorbic acid		0.27±0.01

Values are expressed as Mean±SD (n=3).

TABLE 3: ABTS FREE RADICAL SCAVENGING ACTIVITY OF SOLVENT EXTRACTS OF CORTEX AND SPROUT FROM *ARALIA CORDATA* AND *KALOPANAX SEPTEMLOBUS*

Extracts			ABTS Free Radical Scavenging Activity (IC_{50}, mg/g)
Aralia cordata	Cortex	Ethanol	0.36±0.06
		Hot water	0.65±0.03
	Sprout	Ethanol	1.19±0.04
		Hot water	1.80 ±0.23
Kalopanax septemlobus	Cortex	Ethanol	0.25±0.01
		Hot water	0.72±0.02

	Sprout	Ethanol	0.99±0.08
		Hot water	1.45±0.02
Ascorbic acid			0.25±0.01

Values are expressed as Mean±SD (n=3).

Plant is said to have antimicrobial and antioxidant activity due to polyphenols and flavonoids [19]. The amount of total polyphenol varied in the plant and solvent and ranged from 1091.2 to 1603.6 milligrams gallic acid equivalents per gram of sample of dry material (mg Gallic acid/g). The highest total polyphenol content was detected in the ethanol extract of *Kalopanax septemlobus* cortex, whereas the lowest value is shown in the hot water extract of *Aralia cordata* sprout **Table 4**. The results are related to its DPPH and ABTS radical scavenging activities. Previous studies have shown that the amount of phenol compounds in plant depend on antioxidant activity [20, 21].

The total flavonoid content of the extracts is presented in **Table 5**. Among the studied extracts, the highest total flavonoids content was found in the ethanol extract of *Kalopanax septemlobus* sprout (823.7 mg quercetin/g). The results are shown that the cortex extracts of the two tested species contained mainly non-flavonoid phenolic compounds, whereas the sprout extracts were composed of flavonoids. Phenolic compounds and flavonoids are considered powerful antioxidants, and plant extracts' antioxidant activities strongly correlate with their total polyphenol content and total flavonoid content [22].

TABLE 4: TOTAL POLYPHENOL CONTENT OF SOLVENT EXTRACTS OF CORTEX AND SPROUT FROM *ARALIA CORDATA* AND *KALOPANAX SEPTEMLOBUS*

Extracts			Total Polyphenol Content (mg Gallate/g)
Aralia cordata	Cortex	Ethanol	1,092.3±8.2
		Hot water	1,564.7±14.8
	Sprout	Ethanol	1,117.1±12.6
		Hot water	1,091.2±18.2
Kalopanax septemlobus	Cortex	Ethanol	1,603.6±30.9
		Hot water	1,282.2±3.8
	Sprout	Ethanol	1,319.2±9.7
		Hot water	1,570.0±76.1

Values are expressed as Mean±SD (n=3).Gallic acid equivalent (y=0.0034x+0.7427, R^2=0.9936).

TABLE 5: TOTAL FLAVONOID CONTENT OF SOLVENT EXTRACTS OF CORTEX AND SPROUT FROM *ARALIA CORDATA* AND *KALOPANAX SEPTEMLOBUS*

Extracts			Total flavonoid content (mg Quercetin/g)
Aralia cordata	Cortex	Ethanol	65.3±15.3
		Hot water	68.3±5.8
	Sprout	Ethanol	362.0±0.0
		Hot water	352.0±15.3
Kalopanax septemlobus	Cortex	Ethanol	112.3±5.8
		Hot water	125.3±15.3
	Sprout	Ethanol	823.7±5.8
		Hot water	858.7±26.5

Values are expressed as Mean±SD (n=3).Quercetin equivalent (y=0.0002x+0.0458, R^2=0.9942).

CONCLUSION: This is the first comparison study on the antibacterial and antioxidant activities of *Aralia cordata* and *Kalopanax septemlobus* extracts. The ether fraction of ethanol extract from the two species was more potent antibacterial activities than the ethyl acetate fraction. Our results also showed that ethanol extracts of the two species have remarkable antioxidant activities determined by DPPH and ABTS radical scavenging assay. The total phenolic and flavonoid contents are shown the association with antioxidant activity, it was not consistent exactly. The obtained results are useful to further research such as identifying specific compounds responsible for the antibacterial or antioxidant activities.

ACKNOWLEDGEMENT: This work was supported by a Research promotion program of SCNU.

CONFLICTS OF INTEREST: The authors have no conflicts of interest in this work.

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How to cite this article:
Seo K and Yun KW: *In-vitro* antibacterial and antioxidant activities of *Aralia cordata* and *Kalopanax septemlobus*. Int J Pharm Sci & Res 2023; 14(12): 5613-18. doi: 10.13040/IJPSR.0975-8232.14(12).5613-18.