ejpmr, 2020,7(12), 23-28

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DPPH AND ABTS RADICAL SCAVENGING ACTIVITY IN KALOPANAX SEPTEMLOBUS

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Article Received on 24/09/2020 **Article Revised on 14/10/2020** **Article Accepted 04/11/2020**

ABSTRACT

The aim of this study was to investigate the antioxidant activity of several solvent extracts including water from leaves, stems, and spines of *Kalopanax septemlobus*. The antioxidative activities of the extracts were determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging methods. DPPH scavenging activity of water extract for leaves evaluated at 1.0 mg/ml was 65.5% and that of butanol was only 51.0% at same concentration. The antioxidant properties of leaf extract were high. Stem and spine effects were not great levels. There was no significant difference among three tissues (p>0.05). DPPH and ABTS scavenging ability at two concentrations (0.5 mg/ml and 1.0 mg/ml) showed significant differences in all tissues (p<0.01-0.001). The Trolox (positive control as ABTS) exhibited inhibitory activity against the ABTS cation of 44–91% at concentration ranges of 1.0 µg/mL. According to antiradical efficiency parameter, the results of DPPH and ABTS showed that the leaf, stem, and spine of *K. septemlobus* were low antiradical activity as an effectivenatural source of antioxidant.

KEYWORDS: ABTS, DPPH, *Kalopanax septemlobus*.

INTRODUCTION

Antioxidants are the defense system of the body against the damage of reactive oxygen species, which is normally produced during the various physiological processes in the body. Natural antioxidants are mostly derived from plant species. In recent decades, alternate of synthetic food antioxidants by natural ones has fostered interest on vegetable sources and the screening of inexpensive raw materials.

DPPH is a stable free radical that is able to accept one electron or hydrogen atom to form a stable diamagnetic molecule.[1] When a solution of DPPH is mixed with a substance that can donate a hydrogen atom, the reduced form of the radical is generated, accompanied by loss of color. This delocalization is also responsible for the deep violet color, characterized by an absorption band in ethanol solution at about 517 nm.

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) is a chemical compound used to observe the reaction kinetics of specific enzymes. Based on the special chemical properties of formed free radicals, ABTS assay has been used to determine the antioxidant capacity of food products.

ABTS+, a stable free radical, is frequently used for estimating the total antioxidant capacity (TAC) of natural products, including crude extracts,[2-6] polyphenols,[7] phenolic acids,[8] flavonoids,[9] and others.[10-11] The original ABTS+ radical-scavenging assay was measured according to the activation of metmyoglobin with hydrogen peroxide in the presence of ABTS to produce the radical cation, in the presence or absence of antioxidants.[12]

Kalopanax septemlobus (Thunb.) Koidz., or *Kalopanax pictus* (Thunb.) Nakai, commonly called castor aralia or tree aralia, is the only large tree found in the aralia family. We use cookies to enhance your experience on our website.By continuing to use our website, you are agreeing to our use of cookies. You can change your cookie settings at any time. *K. septemlobus* grows in East Asian countries (Japan, China, Korea and eastern coastal Russia.[13] Despite its rather exotic appearance, the tree aralia, *K. septemlobus*, is a hardy, deciduous and highly ornamental tree. The leaves of *K. septemlobusare* highly variable in terms of how lobed they are – some plants will have leaves that are very deeply lobed, whereas others will not. The Kalopanax septemlobusleaf has been used as a traditional medicine herb for the treatment of various human diseases for hundreds of years. We use cookies to enhance your experience on our website. By continuing to use our website, you are agreeing to our use of cookies. You can change your cookie settings at any time. The stem bark of K. septemlobushas traditionally been utilized to treat diabetes mellitus, rheumatoid arthritis and neuropatic pain.[14-15] The bark

contains a range of bio-active constituents, including saponins, flavonoids and lignans. It has antifungal and liver protecting properties. It is used in Korea in the treatment of contusions, beri-beri, lumbago, neuralgia and pleurisy. An infusion of the leaves is used to make a stomachic tea. The root is expectorant. A decoction of the wood is used for skin diseases.

The purpose of the present study is to evaluate *K. septemlobus* as sources of antioxidants for DPPH and ABTS radical scavenging activity. In this study, the DPPH and ABTS scavenging capacities of *K. septemlobus* and several classic solvents including water were compared.

MATERIALS AND METHODS

Sample extract

In this study, a mixture of plant leaves, stems, and spines of *K. septemlobus* was used. The plant materials were ground using a Retsch GM 200 mill (Fisher Bioblock, France). Tissues were extracted with methanol, ethanol, hexane, ethyl acetate, butanol, and hot water. To prepare the each solvent extract, the same process was performed and 500 mL of solvent was added to 100 g tissues. The mixture was stirred for two days on a magnetic stirrer under a refluxcondenser. To prepare water, tissues (200g) were added to 1,000 ml of water that had just reached the boil in a stainless steel pan and cooked for 3 hours. It was conducted 5 times a day. In addition, ultrasound processing was performed with physical treatment to allow more active ingredients to be released. The ultrasound extraction was carried out for 120 min at 60°C using an ultrasonic bath (5510, Branson, USA). The mixture was shaken vigorously for a day at room temperature. After filtration, the water was removed in a rotary vacuum evaporator (N-1001S-W, Eyela, Tokyo, Japan) at 65-70°C. To get dry powder, samples placed in a low temperature vacuum chamber. These powders were then used to determine antioxidant activities. All analyses were realized as much as possible in an area protected against light.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) Assay

The hydrogen atoms or electrons donation ability of the corresponding extracts and some pure compounds were measured by bleaching the purple colored methanolic solution of DPPH. DPPH is a dark, purple and stable free radical and decreases in antioxidants and turns colorless. Free radical scavenging activities of solutions of the plant extracts and synthetic antioxidant substances used in the studyprepared in methanol at concentrations of 0.25, 0.5 and 1.0 mg/mL were determined in accordance with the Brand-Williams et al.[16] method, which is based on the principle of scavengingthe DPPH radical. 0.3 ml of each sample was put into 2.7 ml of 0.1 mM DPPH solution diluted with ethanol, being reacted at room temperature for 30 minutes. Afterwards, its absorption was measured at 517 nm. Corresponding blank sample was prepared and L-Ascorbic acid (0.25, 0.5, and 1.0 mg/ml) was used as reference standard (positive control).

ABTS free radical scavenging assay

The free radical scavenging activity as a measure of hydrogen donating capacity was determined using the 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) cation decolourization, or trolox equivalent antioxidant capacity (TEAC), method described by Re et al.[12] with some modifications. 1 ml of 0.1 mM ABTS solution in ethanol was mixed with 1 ml of the previous extracts of various concentrations (0.25, 0.5, and 1.0 mg/ml). ABTS was added to the solutions prepared with extracts and standard antioxidant substances and stirred. ABTS radical solution at a concentration of 7 mm was prepared bydissolving 13.2 ug of ABTS in 10 ml of 50% methanolicsolution and 76.8 ug of potassium persulphate in 10 ml distilled water. The two solutions were mixed together andmade up to 200 ml with 50% methanol, and kept in the dark at roomtemperature for 12 h. A stock solution of the compounds was prepared at 1 mg/ml in DMSO. The stock solution was diluted to varying concentrations in 96-well microplates. Then, 5 µl of ethanol ABTS solution (final concentration 300 µM) was added to each well. The plate was shaken to ensure thorough mixing before being wrapped with aluminum foil and placed into the dark. The radical scavenging reaction was carried out at 37℃ in dark for 30 min. The optical density (OD) of the solution was read using the Microplate Reader at the wavelength 515 nm. Corresponding blank sample was prepared and Trolox (6-Hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), a water-soluble vitamin E analog, serves as a positive control inhibiting the formation of the radical cation in a dose dependent manner. Relative inhibitor rate of raw materials and other samples for Trolox was calculated.

Statistical analysis

All experiments were performed thrice and the results averaged data were expressed as mean±SD. Data was conducted using Microsoft Excel and SPSS 21.0 for Windows (Chicago, IL, USA). A one-way and a two-way analysis of variance (ANOVA) followed by the Tukey post hoc test were used to analyze statistical significance. Significance and confidence level were estimated at $p < 0.05$.

The percent inhibition was calculated as the decolourization percentage of the test sample using the following formula: Inhibition (%) = (IA-As)/IA×100

Where IA is the absorbance of the 100% initial and As is the absorbance of the sample. IA and As were the values which were subtracted the average absorbance of the blank wells.

IC_{50} which is an inhibitory concentration of each extract required to reduce 50% of the nitric oxide formation was determined. Regression analysis by a dose response curve was plotted to determine the IC_{50} values. The time needed to reach the steady state to IC_{50} concentration

(T_{IC50}) was calculated graphically.[17] Lower IC_{50} value proves the higher antioxidant ability of studied substrate time.

RESULTS

DPPH radical scavenging assay

The DPPH radical scavenging ability of *K. septemlobus* on several concentrations was shown Table 1. A concentration dependent inhibition against DPPH was observed in this assay. The DPPH scavenging activity of the water extract was the most highest among solvents. DPPH scavenging activity of water extract for leaves evaluated at 1.0 mg/ml was 65.5% and that of butanol was only 51.0% at same concentration. The antioxidant properties of leaf extract were high. Stem and spine effects were not great levels. There was no significant difference among three tissues (p>0.05). However, DPPH scavenging ability at two concentrations (0.5 mg/ml and 1.0 mg/ml) showed significant differences in all tissues (p<0.01-0.001). In particular, as the concentration increased, there was significant in DPPH scavenging ability.

Figure 1 was shown the rate of DPPH inhibitory of L-ascorbic acid (positive control) and relative inhibitory rate for *K. septemlobus* on 1.0 M. The values for leaf, stem, and spine of water extract were 86.6%, 75.7%, and 69.6%, respectively. The those values for butanol extract were 66.3%, 53.9%, and 46.3%, respectively. An IC_{50} value is the concentration of the sample required to scavenge 50% of the free radicals present in the system. IC_{50} value was inversely related to the antioxidant activity of crude extracts. The values of IC_{50} for metanol, hexane, ethanol, butanol, and warer extracts of leaves were 325.6, 327.8, 311.2, 351.4, and 317.3 μg/ml, respectively (Table 3). Those of IC_{50} for stem and spine were similar to those of leaf. According to antiradical efficiency parameter, the results showed that the leaf, stem, and spine of *K. septemlobus* were low antiradical activity as an effectivenatural source of antioxidant.

ABTS free radical scavenging assay

The scavenging activity of *K. septemlobus* leaf extracts tested at the concentration range of 0.25 to 1.0 μg/mL was assessed using the ABTS cation assay. The ethyl acatate and hot water extracts were the least active extracts with an inhibitory activity ≥ 66% at the highest concentration tested, while the butanol extract showed inhibitory percentage activity ≤ 49% (Table 2). The antioxidant properties of leaf extract were high. However, stem and spine effects were not great levels. There was no significant difference among three tissues (p>0.05). However, ABTS scavenging ability at two concentrations (0.5 mg/ml and 1.0 mg/ml) showed significant differences in leaf anf spine tissues (p<0.01-0.001). In particular, as the concentration increased, the difference between leaves and spine was significant in ABTS scavenging ability. However, there was no significant difference on stem 1.0 μg/mL for five extrat solvens (p>0.05).

The butanol extract for leaf exhibited an IC_{50} of 361.2 μg/mL, followed by the hexane and metanol extracts, which had 342.2 and 341.1 μg/mL, respectively. Trolox served as a positive control and was tested at 1/10 lower concentrations to that of the extracts. The Trolox exhibited inhibitory activity against the ABTS cation of 44–91% at concentration ranges of 1.0 μg/mL (Fig. 2). The ABTS cation scavenging activity results of the extracts and the positive control were not significant as they had large IC_{50} variations.

Table 1. DPPH radical scavenging activity (%) of *Kalopanax septemlobus* at different concentrations.

Tissues	Concentration (㎛)	Solvent					*t*-test
		Methanol	Hexane	Ethyl acetate	Butanol	Water	
Leaves	0.25	30.57±0.80	27.43±2.72	24.46±6.60	18.90±3.68	35.91±0.87	0.804
	0.5	45.96±4.23	46.01±3.13	48.93±9.56	39.52±2.28	49.65±2.38	39.184**
	1.0	61.34±1.18	59.02±0.91	64.06±2.81	50.96±5.23	66.51±1.69	89.439***
Stem	0.25	32.44±1.69	18.42±1.94	19.36±0.59	15.64±5.86	36.45±1.64	1.092
	0.5	42.92±3.69	30.57±1.71	33.21±5.45	32.09±3.86	47.13±3.57	42.023**
	1.0	55.17±5.08	41.43±3.92	47.73±2.23	41.38±1.03	58.17±4.53	36.256**
Spine	0.25	27.52±3.23	6.84±2.70	16.58±5.76	7.41±0.26	30.22±3.09	2.569
	0.5	39.04±0.90	26.84±1.25	29.07±6.45	25.26±2.97	43.51±4.53	37.211**
	1.0	50.01±1.41	38.53±1.61	39.98±2.94	35.59±3.60	53.42±0.53	53.980***
t-test		0.017	0.003	0.007	0.009	0.017	

**, p<0.01; ***, p<0.001.

Table 2. ABTS radical scavenging activity (%) of *K. septemlobus* at different concentrations.

Tissues	Concentration (㎛)	Solvent					*t*-test
		Methanol	Hexane	Ethyl acetate	Butanol	Water	
Leaves	0.25	27.01 ±3.17	23.75±0.40	20.66±9.23	14.79±6.45	34.17±3.21	0.983
	0.5	43.39±5.88	39.08±1.51	48.18±4.85	36.84±3.78	54.49±3.76	49.825**
	1.0	59.78±1.92	51.65±1.87	66.72±1.93	48.49±6.96	68.89±0.88	74.834***
Stem	0.25	14.91±0.20	11.99±0.32	16.70±4.97	11.20±9.03	28.38±2.16	1.219
	0.5	34.76±1.62	32.74±2.89	34.56±6.32	28.17±6.88	36.23±5.32	27.264**
	1.0	48.15±1.66	44.57±6.24	47.02±3.75	39.99±4.51	45.40±0.91	0.761
Spine	0.25	9.88±1.91	4.95±1.83	13.45±4.55	7.65±3.65	24.87±3.28	0.040
	0.5	31.57±1.22	24.15±2.26	31.71±5.92	19.47±8.88	33.60±3.25	18.372*
	1.0	41.67±6.18	33.60±0.80	39.58±3.22	29.34±3.51	41.44±2.47	48.296**
t-test		0.001	0.006	0.020	0.041	0.020	

*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

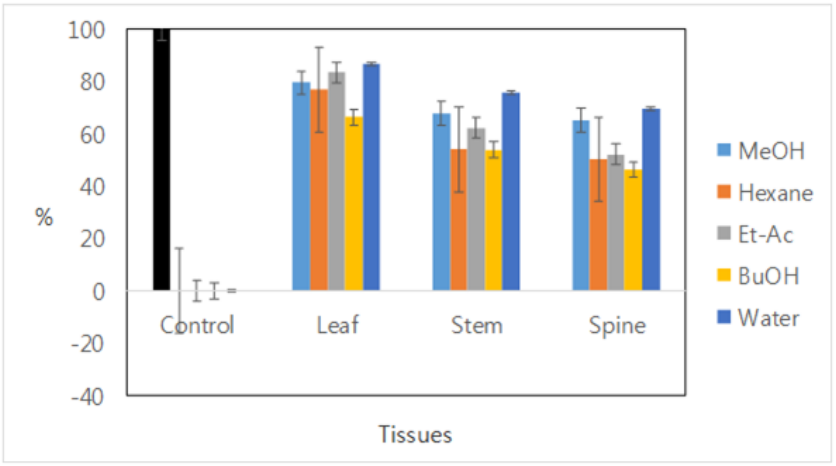

Figure: 1. The rate of DPPH inhibitory of L-ascorbic acid (positive control) and relative inhibitory rate for *Kalopanax septemlobus* on 1.0 mg/ml. MeOH: Methyl alcohol (methanol), BuOH: Butyl alcohol (butanol), Et-Ac: ethyl acetate.

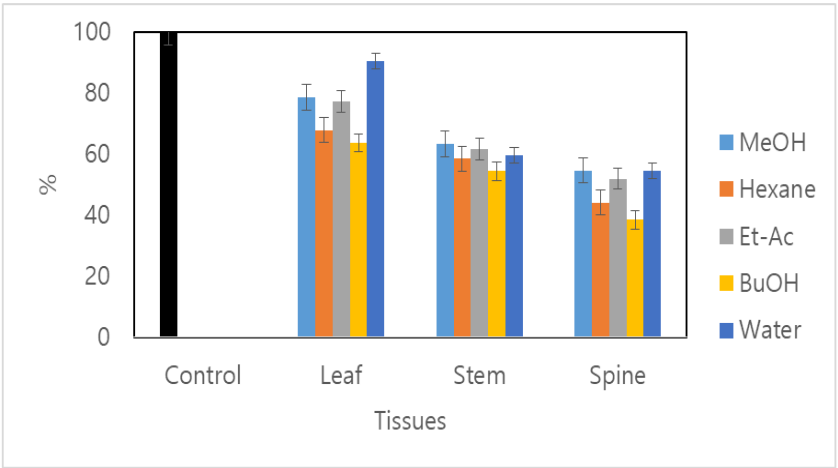

Figure: 2. The rate of ABTS inhibitory of Trolox (positive control) and relative inhibitory rate for *Kalopanax septemlobus* on 1.0 mg/ml.

Table 3. The 50% inhibition (IC_{50}) of DPPH for *K. septemlobus.*

Sample	Anti-oxidant	IC_{50}	T_{IC50}	AE (10^{-4})	Antiradical efficiency classification
Leaf	Methanol	325.6	8.0	3.84	Low antiradical activity
	Hexane	327.8	8.1	3.77	Low antiradical activity
	Ethanol	311.2	7.8	4.12	Low antiradical activity
	Butanol	351.4	9.5	3.00	Low antiradical activity
	Water	317.3	7.5	4.20	Low antiradical activity
Stem	Methanol	342.5	9.2	3.17	Low antiradical activity
	Hexane	337.1	9.4	2.90	Low antiradical activity
	Ethanol	342.2	8.5	3.44	Low antiradical activity
	Butanol	366.6	9.4	2.90	Low antiradical activity
	Water	322.1	8.8	3.53	Low antiradical activity
Spine	Methanol	352.2	9.5	2.99	Low antiradical activity
	Hexane	399.6	10.5	2.38	Low antiradical activity
	Ethanol	396.5	10.5	2.40	Low antiradical activity
	Butanol	411.2	10.6	2.29	Low antiradical activity
	Water	336.5	9.2	3.23	Low antiradical activity

Table 4. The 50% inhibition (IC_{50}) of ABTS for *K. septemlobus.*

Sample	Anti-oxidant	IC_{50}	T_{IC50}	AE (10^{-3})	Antiradical efficiency classification
Leaf	Methanol	341.1	8.2	3.58	Low antiradical activity
	Hexane	342.3	8.5	3.44	Low antiradical activity
	Ethanol	315.6	7.6	4.17	Low antiradical activity
	Butanol	361.2	9.4	2.95	Low antiradical activity
	Water	315.6	7.5	4.22	Low antiradical activity
Stem	Methanol	396.6	8.4	3.06	Low antiradical activity
	Hexane	342.7	8.5	3.43	Low antiradical activity
	Ethanol	365.4	9.2	2.97	Low antiradical activity
	Butanol	367.9	9.3	2.92	Low antiradical activity
	Water	377.8	8.1	3.27	Low antiradical activity
Spine	Methanol	366.7	9.3	2.93	Low antiradical activity
	Hexane	389.1	8.5	3.02	Low antiradical activity
	Ethanol	377.4	9.6	2.76	Low antiradical activity
	Butanol	399.6	9.4	2.66	Low antiradical activity
	Water	351.3	9.0	3.16	Low antiradical activity

DISCUSSION

In the result of DPPH(1,1- diphenyl-2-picryl -hydrazyl) scavenging radical activity, 70% EtOH extracts of *K. septemlobus* leaf showed 93.1% and it was similar to BHA (butylated hydroxyanisole) effect at 1,000ppm concentration.[18] Xanthine oxidase inhibition of hot water extracts and 70% EtOH extracts of *K. septemlobus* leaf were 46.6% and 60.4% at 1,000ppm, respectively. In these results, *K. septemlobus* leaf has a great potential as a cosmeceutical ingredient with a natural anti-oxidant source.[18]

The tree stems and spines of *K. septemlobus* were hard and were treated for a long time with extractive solvents. The solvent was volatile, so the concentration of the solvent was supplemented several times by heating it and stirring it with a stirrer. In the case of water-soluble extraction, the boiling time was usually 1 to 3 hours, but in this study, it was conducted 5 times a day. In addition, ultrasound processing was performed with physical treatment to allow more active ingredients to be released. Thus, hot water extracts could show slightly higher antioxidant function than other solvents (Tables 1 and 2). Generally, total phenolic compound was highest on the polar solvents with hot water recording the highest and ethyl acetate recording the least.[19] Notably, boiled water extracts had higher TPC than 50% methanol.[19] Efficiency of the extraction is strongly affected by the extraction method, temperature, extraction time, the composition of phytochemicals, and the solvent used.[20-

21]

K. septemlobus (synonyms: *K. pictus*) is used in traditional Korean and Chinese medicine to treat rheumatoidal arthritis and neurotic pain.[22] The young shoots are sold in local food markets in Korea.[23]. Young leaves and young shoots – cooked. Young leaves are used for food, but long leaves are not eaten because they are tough. Such old leaves can be used as antioxidants according to this study.

CONCLUSIONS

In the present investigation, leaf extract of *K. septemlobus* exhibited somewhat scavenging effects on DPPH and ABTS. Thus, *K. septemlobus* had the potential of using water extract, which showed potential health benefits (antioxidant activity).

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