ISSN (Print) : 0974-6846
ISSN (Online) : 0974-5645
Indian Journal of Science and Technology, *Vol 8(S1), 28–31, January 2015* DOI : 10.17485/ijst/2015/v8iS1/57442

Antioxidant Activities of Solvent-extracted Fractions from *Kummerowia striata* (Thunb.) Schindl

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Abstract

During the process of screening for antioxidant effects of natural plants by measuring the radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH), *Kummerowia striata* (Thunb.) Schindl (Leguminosae) was found to show potent antioxidant activity. It is very plentiful plant, used for treatment of inflammation and fever in Korea. Antioxidant activities of methanol extract and its fractions were determined by DPPH free radical scavenging effects, riboflavin-originated superoxide quenching activity and xanthine-originated superoxide quenching activity assays. Among them, ethyl acetate fraction showed the most significant free radical scavenging effects on DPPH, and the potent riboflavin and xanthine-originated superoxide quenching activities. These results suggest that *Kummerowia striata* could be used as a functional ingredient for anti-oxidation, anti-aging and anti-inflammation.

Keywords: DPPH, *Kummerowia striata*, Riboflavin-Originated Superoxide Quenching Activity, Xanthine-originated Superoxide Quenching Activity

1. Introduction

Kummerowia striata (Thunb.) Schindl (Leguminosae) is one year herbage and reproduced by seed in the field across all our nation. It is used to treat drainage, dysentery, infectious hepatitis and used for livestock feed and compost. It is also reported that it has an effect to suppress inflammation[1, 2].

Free radical such the reactive oxygen species (ROS; $\cdot O_2$-, $\cdot OH$, H_2O_2) are highly reactive molecules that are generated during normal metabolic process under aerobic conditions. Since free radicals can damage lipids, proteins, and DNA with oxidative stress, the body has developed several endogenous defense mechanisms, including Superoxide Dismutase (SOD), Glutathione Peroxidase (GSHpx), and Glutathione S-Transferase (GST). However, an imbalance of pro-oxidants and anti-oxidants in the organism can cause tissue damage and cell death. Therefore, antioxidants could be useful agents for diseases caused by ROS[3, 4]. So I carried this research in order to investigate its value for practical use because I came to know an antioxidant activity effect of *K. striata* which is grown common in all our nation

2. Materials and Methods

2.1 Plant Materials

K. striata which is used in this research is collected at an hillock near the Nambu university in Wolgye-dong, Gwangsan-gu, Gwangju, Korea and used in this research using in a powder after differentiation precisely.

2.2 Chemical and Reagent

All other chemicals and solvents were of analytical grade and used without further purification. 1-1-diphenyl-2-picryl-hydrazyl (DPPH), riboflavin, xanthine, xanthine oxidase, Vitamin C and BHA were obtained from Sigma

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Chemical Co. (St. Louis, MO, U.S.A.). Absorbance values of the resulting solution were measured using a microplate reader (Biotech, USA).

2.3 Extraction and Isolation

The shade dried plant material 200g was extracted three times with methanol at 50°C and filtered. The extracts were combined and evaporated *in vacuo* at 50°C. The resultant methanolic extract was successively partitioned as n-hexane, methylene chloride, ethyl acetate and n-butyl alcohol. The fractions were used in rotary vacuum evaporator (N-1000, EYELA, Tokyo, Japan) and freeze-dried.

2.4 DPPH Radical Scavenging Effect

Ethanol solutions of test samples at various concentrations (0.1–100 µg/mL) were added to a solution of 0.2mM DPPH in ethanol in 96 well plates. After storing these mixtures for 30 minutes at room temperature, the remaining amount of DPPH was determined by colorimetry at 520 nm using a microplate reader[5]. The radical scavenging activity of each compound was expressed by the ratio of the lowering of the DPPH solution in the absence of compounds. The mean values were obtained from triplicate experiments.

2.5 Superoxide Quenching Activity

The Superoxide Quenching Activities of test samples were photochemically measured using an assay system consisting of methionine, riboflavin, and Nitroblue Tetrazolium (NBT)[6, 7]. The reaction mixture was composed of 2.6 µM riboflavin, 13mM methionine, 75 µM NBT, 0.1 mM EDTA, PBS (pH 7.4) and various concentrations of test samples. The sample was randomly placed in a light storage box and replaced, randomly, every 5-min for 15-min. During the light illumination, the temperature within the light storage box was 20 ± 1°C. The light intensity at the sample level was 5,500 lux. During the light illumination, NBT was reduced to blue formazan formation that was measured by the absorbance at 560 nm. The inhibition of blue formazan formation was taken as a superoxide quenching activity.

2.6 Xanthine and Superoxide Scavenging Assay

Superoxide radicals were generated by xanthine/xanthine oxidase and measured by previously reported method[8]. In brief, test samples were mixed with 20 mM phosphate buffer (pH 7.8) containing 0.48 mM NBT and 1.6 mM xanthine. After 5-min, xanthine oxidase (0.05 U/mL) 100 µL was added. The absorbance of reaction mixture was read at 570 nm after 30-min incubation at 37°C. Superoxide radical scavenging activity was expressed by the degree of NBT reduction of a test group in comparison to that of control[9, 10].

3. Results

3.1 DPPH Radical Scavenging Effect

The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay is principally designed to evaluate the stable DPPH free radical scavenging activity by sample. So decrease of DPPH can realize scavenging effect of free radical progressing and predict decrease of reaction lipid peroxidation. DPPH appears maximum absorbance in 520nm and if it is reduced, absorption disappear. So reducing process of DPPH depend on reducing power[10]. The DPPH radical scavenging effects of methanol extract from *K. striata* are shown in Figure 1. *K. striata* methanol extract was showed a significant result as concentration-dependently. The DPPH radical scavenging effects of each solvent partitioned fraction from *K. striata* are shown in Figure 2. Vitamine C which was used as a positive control resulted (IC50 5.2 µg/ml) and ethyl acetate (IC50 24.1 µg/ml) showed higher activity than n-butanol (IC50 32.2 µg/ml) while methylene chloride and n-hexane showed lower activity.

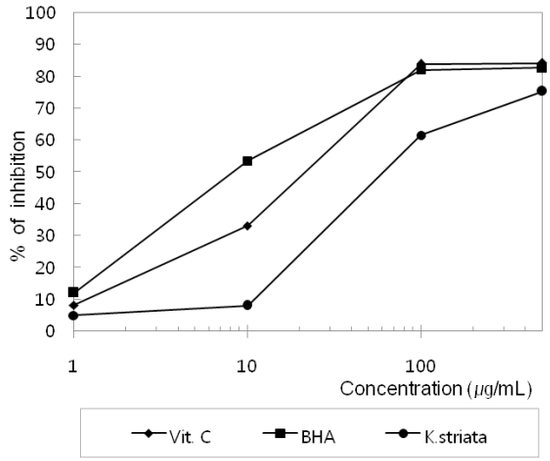

Figure 1. DPPH radical scavenging effects of *K. striata* methanol extract.

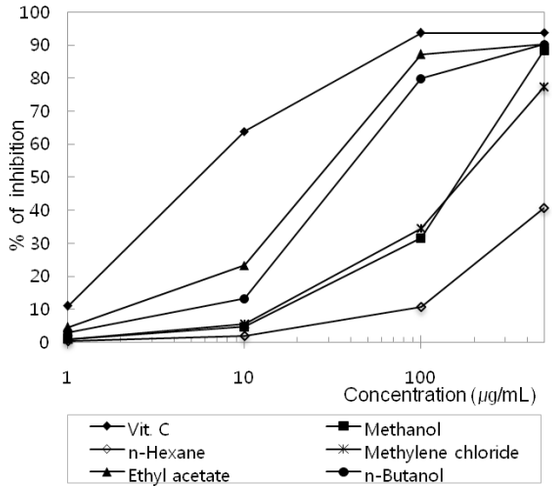

Figure 2. DPPH radical scavenging effects of fractions from *K. striata* methanol extract.

3.2 Riboflavin Originated Superoxide Quenching Activity

Reactive oxygen species that is called superoxide attacks unsaturated fatty acid that is component in cell membrane and causes oxidation response with lipide. And it stores lipoperoxide in body, reducing body function. Also, it causes many disease such as pigmentation, aging and adult disease[11–13]. SOD converts superoxide radical into hydrogen peroxide in body and is related to antioxidation and anti-aging. Riboflavin is degraded in the light of 5500lux and creates singlet oxygen and superoxide anion. It reduce NBT (Nitro Blue Tetrazolin) and produce blue formazan, material of reduction of NBT. Then, it has maximum absorbance in 560nm[14–16]. Superoxide quenching activity of each solvent partitioned fraction from *K. striata* are shown in Figure 3. Vitamine C which was used as a positive control resulted (IC50 4.6 μg/ml) and ethyl acetate (IC50 14.0 μg/ml) showed higher activity than n-butanol (IC50 27.1 μg/ml) while methylene chloride and n-hexane showed lower activity.

3.3 Xanthine Originated Superoxide Scavenging Assay

Xanthine oxidase affects as rate-limiting enzyme in terminal oxidation of purine and is a enzyme as a source of oxidizing agent like superoxide radical or hydrogen peroxid. superoxide radical[17,18]. The superoxide anion derived from oxidation of xanthin causes the oxidation of NBT to water-soluble formazan. The decrease of absorbance at 570 nm with antioxidants represents the quenching of the superoxide anionin reaction mixture. Xanthine and superoxide scavenging assay of each solvent partitioned fraction from *K. striata* are shown in Figure 4. Vitamine C which was used as a positive control resulted (IC50 1.3 μg/ml) and ethyl acetate (IC50 3.4 μg/ml) showed higher activity than n-butanol (IC50 5.2 μg/ml) while methylene chloride and n-hexane showed lower activity.

4. Discussion

The methanol extracts of *K. striata* were fractioned as n-hexane, methylene chloride, ethyl acetate and n-butyl alcohol. Each fraction was tested for the radical scavenging

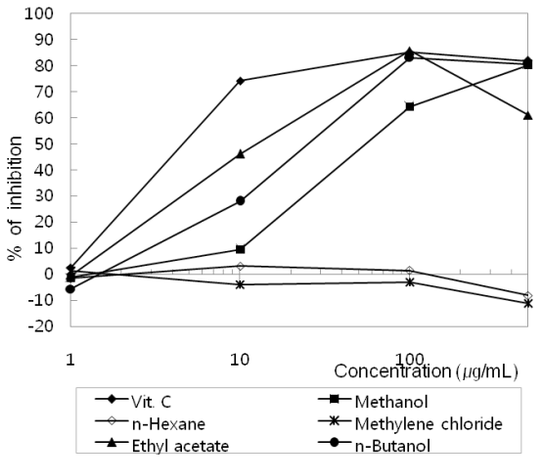

Figure 3. Riboflavin originated superoxide quenching activities of fractions from *K. striata* methanol extract.

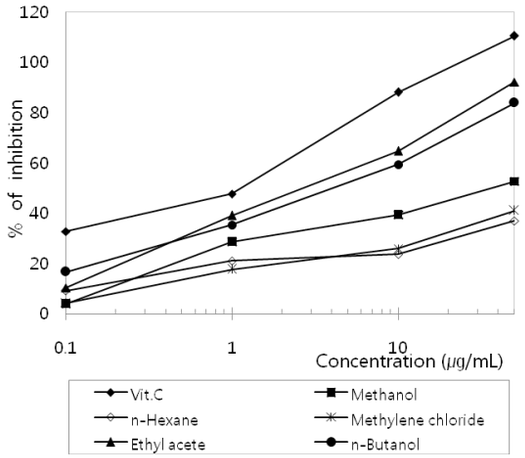

Figure 4. Xanthine originated superoxide quenching activities of fractions from *K. striata* methanol extract.

effect on DPPH radical scavenging effect, Superoxide quenching activity, Xanthine and superoxide scavenging assay. Among these fractions, the ethyl acetate soluble fraction showed the most significant free radical scavenging activity on DPPH, Superoxide quenching activity, Xanthine and superoxide scavenging assay. (Figure 2–4). As a result, ethyl acetate fraction is recognized as its value in a material of antioxidant and anti-aging, showing antioxidant which has a significance relation approaching control group.

5. References

1. Kang BH. The ecology of resource plant in Korea. Giobook. 2008; 1:0615–7.
2. Tao JY, Zhao L, Huang ZJ, Zhang XY, Zhang SL, Zhang QG. Anti-inflammatory effects of ethanol extract from *Kummerowia striata* (Thunb.) Schindl on LPS-stimulated RAW 264.7 cell. Inflammation. 2008; 31(3):154–66.
3. Cross CE, Halliwell B, Borish ET, Pryor WA, Ames BN, Saul RL. Oxygen radicals and human disease. Annals of Internal Medicine. 1987; 107(4):526–45.
4. Brand-Williams W, Cuvelier ME, Berset CL. Use of a free radical method to evaluate antioxidant activity. LWT–Food Sci Technol . 1995; 28(1):25–30.
5. Yoshida T, Mori K, Hatano T, Okumura T, Uehara I, Komagoe K, Fujita Y, Okuda T. Studies on inhibition mechanism of auto oxidation by tannins and flavonoids. Chem Pharm Bull. 1989; 37:1919–21.
6. Choi DS, Kim SJ, Jung MY. Inhibitory activity of berberine on DNA strand cleavage induced by hydrogen peroxide and cytochrome c. Biosci Biotechnol Biochem. 2001; 65:452–5.
7. Ginnopolitis CN, Ries SK. Superoxide dismutase. I. Occurrence in higher plants. Plant Physiol. 1977; 59:309–14.
8. Nishikimi M. Oxidation of ascorbic acid with superoxide anion generated by the xanthine-xanthine oxidase system. Biochem Biophys Res Comm. 1975; 63(2):463–8.
9. Blois MS. Antioxidant determination by the use of a stable free radical. Nature. 1958; 181:1199–2000.
10. Ukeda H, Maeda S, Ishii T, Sawamura M. Spectrophotometric assay for superoxide dismutase based on tetrazolium salt 3′-{1-[(phenylamino)-carbonyl]-3, 4-tetrazolium}-bis (4-methoxy-6-nitro) benzenesulfonic acid hydrate reduction by xanthine–xanthine oxidase. Anal Biochem. 1997; 251(2):206–9.
11. Kim DK. Antioxidative constituents from the whole plant of Actinostemma lobatum maxim. J Korean Soc Appl Bi. 2010; 53(6):746–51.
12. Marklund S, Marklund G. Involvement of superoxide amino radical in the oxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1975; 47:468–74.
13. Yu BP. Aging and oxidative stress: modulation by dietary restriction. Free Radix. Biol Med. 1996; 21:651–68.
14. Marx JL. Oxygen free radicals linked with many diseases. Sci 1987; 235:529–31.
15. Heo C, Chung JH, Jo BK, Kim HP, Heo MY. Antioxidative activities of 60 plant extracts. J Appl Pharmacol. 2003; 11:196–9.
16. Suk SH. Antioxidant effect of vitamin E on vascular endothelial cells damaged by reactive oxygen species. Kor J Ori Med Physiol Pathol. 2006; 20:685–9.
17. You HJ, Woo ER. The suppressive effect of medicinal herbs on the H_2O-induced hypoxanthine-guanine phosphoribosyl transferase (HPRT) mutation. Kor J Pharmacogn. 2004; 35:28–34.
18. Dziezak JD. Antioxidants. Food Technol 1986; 40:94–102.