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Antioxidant and Anticancer Activities of Extracts from *Picrasma quassioides* (D. Don) Benn.

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The antioxidant and anticancer activities of *Picrasma quassioides* (D. Don) Benn. were investigated. The superoxide radical and hydroxyl radical scavenging activities were assayed by the electron spin resonance (ESR) spin-trapping technique. Anticancer activity of *Picrasma quassioides* (D. Don) Benn. extracts was tested by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay on gastric cancer cell line NCI-N87. Aqueous extracts of *Picrasma quassioides* (D. Don) Benn. had higher superoxide radical (IC_{50} = 121.4 μg/mL) and hydroxyl radical (IC_{50} = 1.13 mg/mL) scavenging activity than methanol and ethanol extracts. The cytotoxicity of each extracts showed strong anticancer activity, especially ethanol (56.3 %) and methanol (53.6 %) extracts. Present results show that *Picrasma quassioides* (D. Don) Benn. extracts has antioxidant and anticancer activities.

Key Words: Apoptosis, Electron spin resonance, MTT assay, NCI-N87 cells, *Picrasma quassioides* (D. Don) Benn.

INTRODUCTION

Biological free radical reactions are involved in the reduction of molecular oxygen to yield reactive oxygen species (ROS) such as the superoxide anion and hydroxyl radical[1]. Various diseases such as cancer, liver disease and diabetes may reduce the number of these radicals and reactive oxygen species may be essential for cellular functions such as ingestion of bacteria and redox regulation of signal transduction[2]. These reactive oxygen species cause destructive and damage to the components of a cell, such as lipids, proteins and DNA[3]. Moreover, lipid peroxides promote the formation of additional free radical in a type of chain reaction[4].

Plants have many phytochemicals with various bioactivities including antioxidant, anticancer and antiinflammatory activities. Many plants have been examined to identify new and effective antioxidant and anticancer compounds, as well as to elucidate the mechanisms of cancer prevention and apoptosis[5-7]. In particular, oriental medicinal plants are considered to be one of the most promising sources due to their variety of species and applications.

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Picrasma quassioides (D. Don) Benn. is distributed in China, Korea, India and Japan. Its bark and stem contains a number of medicinal compounds and has been shown to be anthelmintic, antiamoebal, antiviral, bitter and hypotensive. It increased the flow of gastric juices, in Korea it is used in the treatment of digestive problems, especially chronic dyspepsia, in East Asia used as herbal medicine[8].

In a previous study, it was reported that the water and methanol extract of *Picrasma quassioides* (D. Don) Benn. exhibited DPPH radical scavenging activity[9]. BuOH fraction of MeOH crude extract from *Picrasma quassioides* (D. Don) Benn. strong inhibitory activity against human cancer cell (HT-29) growth[10]. In this study, the superoxide radical and hydroxyl radical scavenging activities were assayed by the electron spin resonance (ESR) spin-trapping technique. In addition, the anticancer activity of the *Picrasma quassioides* (D. Don) Benn. extracts was also examined.

EXPERIMENTAL

The gastric cancer cell lines were purchased from Korean cell line bank. RPMI 1640, fetal bovine serum and trypsin-EDTA were acquired from Gibco BRL (Grand Island, NY, USA) and the 48-well plates were obtained from Nunclon Brand Products. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from the Sigma-Aldrich Company (St. Louis, MO, USA).

Sample preparation: Bark of *Picrasma quassioides* (D. Don) Benn. was obtained from Chuncheon, Korea. The samples were washed thoroughly in tap water, shade-dried and powdered. One hundred gram of *Picrasma quassioides* (D. Don) Benn. were extracted with water, 70 % ethanol, 100 % ethanol, 70 % methanol and 100 % methanol for 3 days, respectively, in to 2 L solvent volume.

Scavenging activity of superoxide anion radicals by ESR spectrometry: An ESR spectrometer was used to measure superoxide anion radicals ($^{\bullet}O_2^-$) in the form of spin adducts of $^{\bullet}O_2^-$ (DMPO-OOH)[11]. The relative intensity of the signal of the DMPO-OOH spin adduct was measured as a ratio of the intensity of the Mn^{2+} signal. Mixtures contained 180 µL of 0.1 M Kpi buffer (pH 7.4), 4 µL of 50 mM hypoxanthine, 2 µL of 5 mM diethylenetriaminepentaacetic acid (DETAPAC), 5 µL extract, 2 µL of 100 µM catalase, 4 µL of 9 mM DMPO (5,5-dimethyl-1-pyrroline-oxide) and 3 µL containing 25 U xanthine oxidase (XOD). Two minutes after the addition of XOD, the spin adduct DMPO-OOH was determined by ESR spectrometry (JEOL-JES-TM200, JEOL, Tokyo, Japan). ESR spectra were recorded at 37 ºC with a field setting of 335.4 ± 5.0 mT for superoxide radicals, modulation frequency was 100 KHz, modulation amplitude was 0.79 × 0.1 mT, response time was 0.1 s, sweep time was 0.5 min, microwave power was 8.0 mM (9.423 GHz) and receiver mode was 1st.

Scavenging activity of hydroxyl radicals by ESR spectrometry: The hydroxyl radical was generated by Fenton reaction[12]. The total 200 µL mixture included the extract, 90 mM DMPO, 0.2 mM EDTA and 0.2 mM ferrous sulfate. After mixing, the solution was transferred to an ESR quartz cell and placed at the cavity of the

ESR spectrometer and hydrogen peroxide was added to a final concentration of 5 mM. Deionized water was used instead of sample solution for blank experiments. After 40 s, the relative intensity of the signal of DMPO-OH spin adduct was measured. The spin adduct DMPO-OH was determined by ESR spectrometry. The conditions of ESR spectra were similar with scavenging activity of superoxide anion radicals.

MTT assay: MTT assay was performed according to Mosmann procedure with slight modification[13]. A gastric cancer cell suspension (2×10^5 cells/well) in 96-well plates was incubated for 24 h at 37 °C. Then, the cells were rinsed and grown in a RPMI 1640 containing each extracts (1 mg/mL). After incubation for 72 h at 37 °C, the RPMI 1640 was removed. And the cells were again incubated again with 0.25 mL of RPMI 1640 and 0.05 mL of a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) solution (0.5 µg/mL) for 4 h. 0.7 mL of the lysing buffer (20 % sodium dodecyl sulfate in 50 % N,N-dimethylformamide, pH 4.6) was added to each wells to dissolve the purple formazan produced by the MTT and the cells were incubated for further 2 h. The plate was read using a spectrophotometer (Beckman model DU-64) at a wavelength of 540 nm. The cytotoxicity was obtained by comparing the absorbance between the samples and the control.

Annexin V and propidium iodide assay: In order to evaluate the apoptosis/necrosis cell death ratio, phosphatidylserine (PS) exposure was analyzed using annexin V and propidium iodide (PI), as described previously[14]. Annexin V-FITC Apoptosis Detection Kit (MBL, Nagoya, Japan) was used in accordance with the manufacturer's protocol. The cells were washed once with PBS and resuspended in 500 µL of binding buffer. The mixture that containing 5 µL Annexin V-FITC and PI was added to each sample and then incubation at room temperature for 5 min in the shadowy place, 400 µL of binding buffer was added and analyzed by a FACS can flow cytometer (Becton-Dickinson, Franklin Lakes, NJ, USA).

Statistical analysis: All experimental data are presented as means ± standard errors (SE), Duncan's test was used to evaluate the significant difference between groups and $p < 0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

Superoxide radical scavenging activity: The superoxide radical scavenging activities of different extracts are shown in Table-1. The order of superoxide radical scavenging activity was: water > ethanol > 70 % methanol > methanol > 70 % ethanol > hot water. The superoxide radical scavenging activity of the extracts was obtained from the generated signal intensities. IC_{50} of the aqueous extract of *Picrasma quassioides* (D. Don) Benn. was 121.4 µg/mL (Table-1). Measurement of the ESR signals showed that the *Picrasma quassioides* (D. Don) Benn. extract was reduced the DMPO-OOH signal in a dose-dependent manner (Fig. 1). Comparison of the ESR signal intensities at different concentrations of aqueous of *Picrasma quassioides* (D. Don) Benn. extract with the manganese oxide (Mn^{2+}) signal as internal standard revealed potent inhibition at 100 µg/mL.

TABLE-1
ASSAY OF SUPEROXIDE RADICAL SCAVENGING ACTIVITY

Sample	Concentration (μg/mL)	Inhibition (%)	SOD-like activity (μg/mL)	IC_{50} (μg/mL)
Water	100	53.20	0.54	121.40
Hot water	100	32.50	0.16	247.10
EtOH	100	50.50	0.49	134.20
70 % EtOH	100	40.10	0.30	176.40
MeOH	100	47.90	0.44	159.40
70 % MeOH	100	50.90	0.50	134.40

Calibration curve: y = 55.2X+223.495, where y = inhibition rate (%), X = SOD (μg/mL).

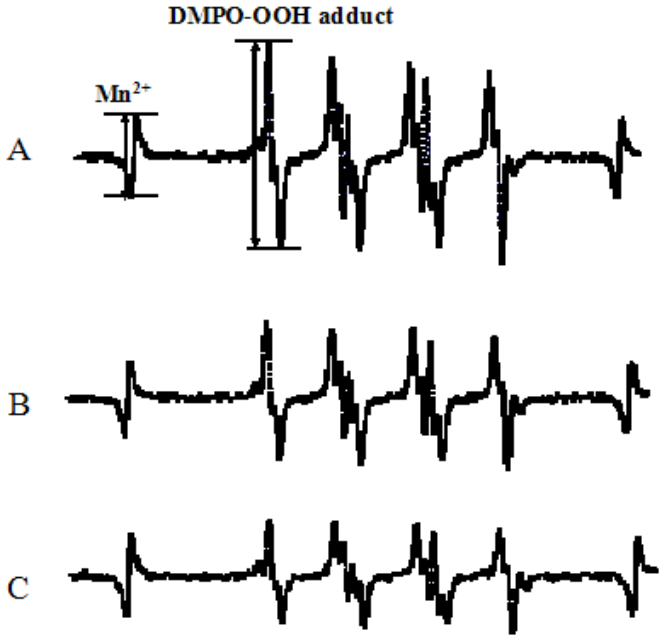

Fig. 1. ESR signals of the superoxide radical (DMPO-OOH) measured by ESR spectrometry. Mn^{2+}, manganese oxide signal as a standard. The effect of different concentrations of aqueous extract of *Picrasma quassioides* (D. Don) Benn. (A) Control; (B) 50 μg/mL; and (C) 100 μg/mL

Scavenging activity of hydroxyl radicals by ESR spectrometry: The hydroxyl radical scavenging activities of different extracts are shown in Fig. 2. The order of hydroxyl radical scavenging activity was: water > hot water > 70 % methanol > methanol > ethanol >70 % ethanol. The hydroxyl radical scavenging activity of the extracts was obtained from the signal intensities generated (Fig. 2). The changes of the intensity of DMPO-OH spin signal in ESR spectrometry were used to evaluate the scavenging activity of hydroxyl radicals of *Picrasma quassioides* showed higher and dose-dependently scavenging activities against hydroxyl radicals, the 2 mg/mL

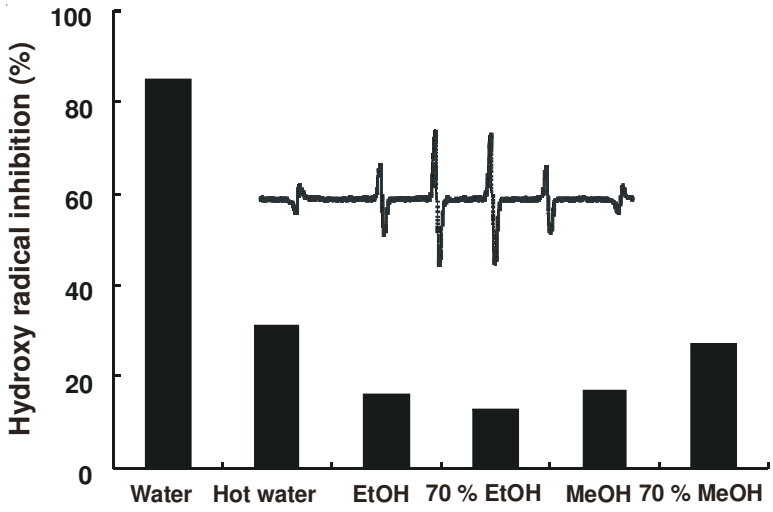

Fig. 2. Protective effect of different extracts of *Picrasma quassioides* (D. Don) Benn. on hydroxyl radical formation. Inset shows the ESR spectrum of the hydroxyl radical

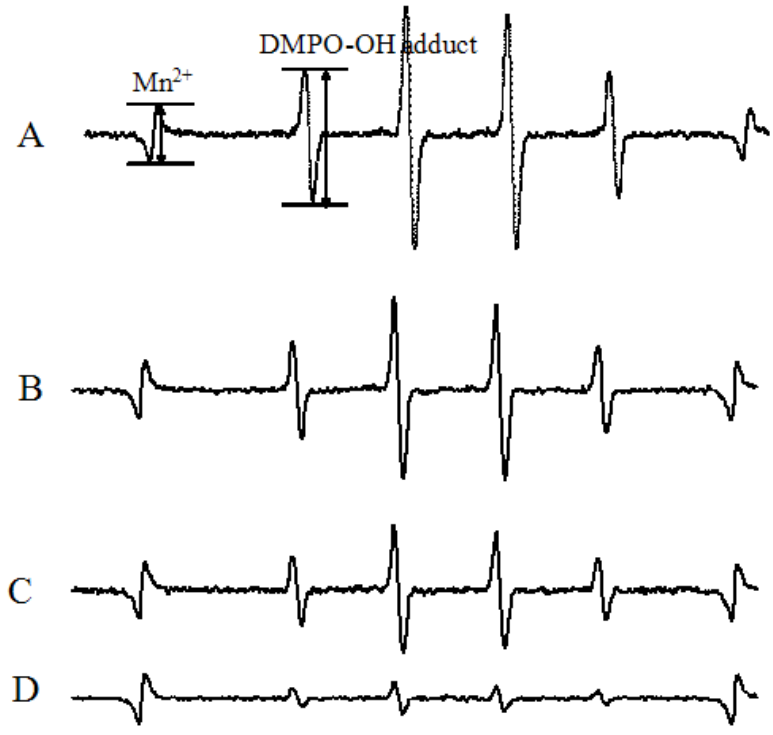

Fig. 3. ESR signals of the hydroxyl radical (DMPO-OH) measured by ESR spectrometry. Mn^{2+}, manganese oxide signal as a standard. The effect of different concentrations of aqueous extract of *Picrasma quassioides* (D. Don) Benn. (A) Control; (B) 500 μg/mL; (C) 1000 μg/mL; (D) 2000 μg/mL

aqueous *Picrasma quassioides* (D. Don) Benn. extract showed 85 % scavenging activity against hydroxyl radicals (Fig. 3). Clearly, this natural product contains a free radical scavenging activity that can inhibit tumor cell proliferation-related activites[15].

Cytotoxicity activity on cancer cells: The anticancer activities of the *Picrasma quassioides* (D. Don) Benn. extracts were investigated using a MTT assay on gastric cancer cell lines NCI-N87. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is proportional directly to the number of viable cells[13]. Fig. 4 shows that the extract inhibit significantly cancer cell growth at a concentration of 1 mg/mL ($P < 0.01$) and EtOH and MeOH extracts exhibited the highest cytotoxicity (> 50 %) in NCI-N87 cell lines. Meanwhile, other extracts not only have high cytotoxicity on NCI-N87 cells, but also have high cytotoxicity on human normal kidney cells[16].

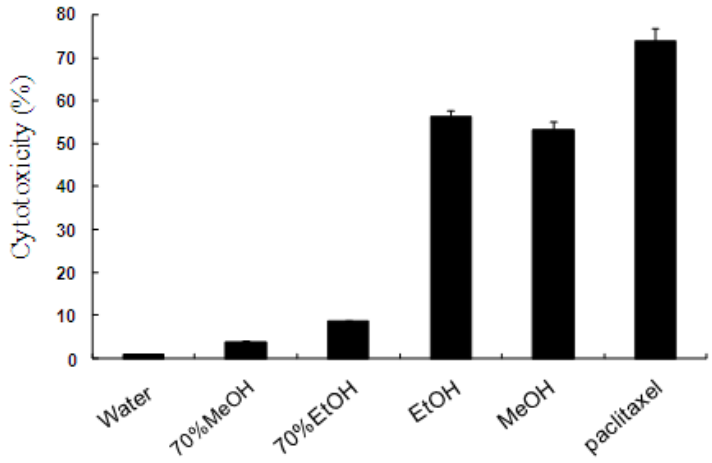

Fig. 4. Cytotoxicity test of *Picrasma quassioides* (D. Don) Benn. extract on the NCI-N87cell line after 72 h incubation, measured by MTT assay (mean ± SD; n = 6). The concentrations of each extract are 1 mg/mL and paclitaxel 0.1 mg/mL

***Picrasma quassioides* (D. Don) Benn. EtOH extract induces apoptosis in NCI-N87 cells:** Cell death includes necrosis and apoptosis. The necrosis is the death of cells or tissues through injury or disease, especially in a localized area of the body, while apoptosis is programmed cell death. Most compounds can kill cancer cells by necrosis, but they can not be used as anticancer drugs. Apoptosis is a standard to test a compound can be used as an anticancer drug or not[17]. Occurrence of apoptosis was confirmed using annexin-V/PI double staining. NCI-N87 cells were treated with concentration of *P. quassioides* (D.Don) Benn. EtOH extract as 1 mg/mL and 10 mg/mL for 48 h (Fig. 5). Shifts from control (down-left quadrant) to early (down-right quadrant) and late apoptosis (up-right quadrants) were clearly evident in dot

plots of Fig. 5. The percentage of apoptotic cells expressed as annexin V positive cells. The corresponding quantities of necrosis and apoptosis were 3.44 and 10.4 %, 11.2 and 13.8 %, 11.5 and 25.0 %, respectively (Fig. 5). These results suggested that the EtOH extract can induce apoptosis on NCI-N87 cells[18,19].

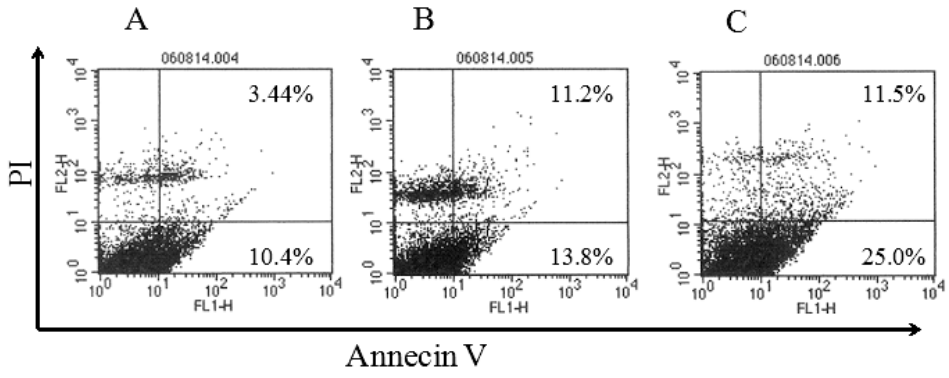

Fig. 5. Assessment of apoptosis by annexin V on the NCI-N87 Cells. The cells were pretreated with or without 48 h. (A) control NCI-N87 cells; (B and C) NCI-N87 cells treatment with *Picrasma quassioides* (D. Don) Benn. EtOH extract at 1 mg/mL and 10 mg/mL for 48 h. The percentage of apoptosis and necrosis HepG2 cells were determined by Annexin V-FITC/PI staining method

Conclusion

In this study, the extract of *Picrasma quassioides* (D.Don) Benn. was confirmed to exhibit antioxidant and anticancer activities. The superoxide radical and hydroxyl radical scavenging activities were assayed by the electron spin resonance (ESR) spin-trapping technique. Aqueous extracts of *Picrasma quassioides* (D. Don) Benn. had higher superoxide radical (IC_{50} = 121.4 μg/mL) and hydroxyl radical (IC_{50} = 1.13 mg/mL) scavenging activity when compared with the methanol ethanol extracts. MTT assay also revealed that *Picrasma quassioides* (D.Don) Benn. contains a strong anticancer compound, which exhibited cytotoxicity on the NCI-N87 cancer cell lines. However, further studies needed to identify the exact molecular structures of both the antioxidant and anticancer compounds.

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