

Effect of *Euphorbia supina* Extract on Antioxidant Activity

Hyang Mi Choi and Sun Young Lim*

Division of Marine Environment & Bioscience, Korea Maritime and Ocean University, Republic of Korea

Abstract: We investigated the effect of *Euphorbia supina* extract on the production of cellular reactive oxygen species (ROS) in order to evaluate its antioxidative activity. The H₂O₂-induced ROS generation was measured with dichlorofluorescein-diacetate (DCFH-DA) assay. The acetone+methylene chloride (A+M) and methanol (MeOH) extracts dose-dependently decreased ROS production induced by H₂O₂ compared to the extract-free control during the 120 min assay. The MeOH extract showed a higher inhibitory effect on the A+M at all concentrations tested. All tested fractions showed a decrease in ROS production with increase in concentration. Treatments with *n*-hexane and 85% aqueous methanol (MeOH) fractions (0.5 mg/mL concentrations) showed 82% and 97% inhibition rate, respectively, suggesting that the 85% aqueous MeOH fraction had ROS scavenging effects. These results indicate that the 85% aqueous MeOH fraction inhibited cellular oxidation and may contain valuable active compounds.

Keywords: Antioxidant, cellular oxidation, *Euphorbia supina*, reactive oxygen species.

INTRODUCTION

Euphorbia supina is an annual summer broadleaf weed and spotted spurge belonging to the Euphorbiaceae family. This broadleaf weed is often found in disturbed sites, such as poorly maintained lawns, waste areas, cultivated ground, and glades, and along sidewalks, railroads, and roadsides. The stems and foliage exude a milky sap when injured, and the stems are pinkish in color and densely hairy [1]. In Korea, *E. supina* has traditionally been known to be effective in treating bronchitis, jaundice, and hemorrhage [2, 3]. *Euphorbia* species are known to possess biologically active compounds [4]. For example, jatropane diterpenoids from *E. pubescens* [5], *E. fischeriana* [6], *E. dendroides* [7], and *E. turczaninowii* [8] have been shown to act as regulators of multidrug resistance. Some studies identified several key polyphenol components from *E. supina*, which were suggested to be highly associated with the antioxidant activity of this species [9, 10]. The findings of previous study showed that *E. supina* contained linolenic acid (18:3n-3) and suggested that this fatty acid might be related to the various biological activities of this plant [11]. Thus, we investigated the potential biological benefits of *E. supina* extracts, including its antioxidant properties, cytotoxicity against human cancer cell lines, and modulation of immune processes.

Reactive oxygen species (ROS) include both radical and non-radical substances. Radical ROS include hydroxyl, superoxide, peroxy, and alkoxy radicals, where non-radical ROS include hydrogen peroxide and single oxygen. Cellular ROS are known to play an important role in affecting human

health by causing several chronic diseases, such as cancer, inflammation, aging, asthma, hypertension and cardiovascular diseases [12]. These ROS are generated during normal cell metabolism [13]. To scavenge cellular ROS, intake of antioxidants from foods or supplements is recommended. Some studies suggest that diets rich in medical plants and marine organisms protect against ROS-induced oxidative stress since they contain several types of bioactive compounds with antioxidant activity [14, 15]. Thus, in the present study, we investigated the antioxidant activity of *E. supina*, which grows wild in Korea, in order to determine its potential as a natural antioxidant supplement.

MATERIALS AND METHODS

Materials and Cell Culture

Dulbecco's modified Eagle's medium (DMEM), fetal serum albumin (FBS), phosphate buffered saline (PBS), dimethylsulfoxide (DMSO), penicilline-streptomycin, 2'-7' dichlorofluorescein-diacetate (DCFH-DA) were obtained from Sigma-Aldrich (St. Louis, MO). Human fibroblast cell line HT-1080 was obtained from the Korea Cell Line Bank. The cells were maintained at 37°C under 5% CO₂ in DMEM containing 10% FBS and 100 units/mL penicillin-streptomycin.

Extraction and Fractionation

E. supina samples were collected from lawns and sidewalks in Korea Maritime and Ocean University, Busan city, Korea on July-August, 2013. The sample was authenticated by Professor Moo Ryong Huh who is a plant taxonomist in Gyeongsang National University. *E. supina* samples were dried in dark area, finely cut and used for the plant material. Dried *E. supina* samples (520 g) were extracted twice with acetone+methylene chloride (A+M) and

*Address correspondence to this author at the Division of Marine Environment & Bioscience, Korea Maritime and Ocean University, Republic of Korea; Tel: +82-51-410-4757; Fax: +82-51-404-475; E-mail: sylim@kmou.ac.kr

methanol (MeOH). The combined crude extracts were fractionated with *n*-hexane and 85% aqueous MeOH, and the aqueous layer was also further fractionated with *n*-butanol (*n*-BuOH) and water, resulting in the *n*-hexane (5.6 g), 85% aqueous MeOH (2.7 g), *n*-BuOH (6.7 g) and water (1.9 g) fractions. The crude extracts and four types of fractions with different polarities were concentrated to dryness and the residues were kept at 4°C.

Intracellular ROS Measurement

Cellular oxidative stress owing to ROS generation from H₂O₂ was measured by DCFH-DA method [16]. DCFH-DA is diffused through the cell membrane and is enzymatically hydrolyzed by intracellular esterases to nonfluorescent DCFH, which is rapidly oxidized to the highly fluorescent DCF in the presence of ROS. HT-1080 cells were first cultured in 96-well plates (5 × 10⁵/well) for 24 h. After washing with PBS, cells were treated with 20 μM DCFH-DA and pre-incubated for 20 min. Then samples were treated and incubated for 1 h. After DCFH-DA was removed and washed with PBS, 500 μM H₂O₂ was added and incubated for 120 min. DCF fluorescence intensity was measured with

an excitation wavelength at 485 nm and emission wavelength at 535 nm, using a fluorometric plate reader (VICTRO3, Perkin Elmer, Wellesley, MA).

Statistical Analysis

Data were presented as mean ± standard error of the mean (SEM). ANOVA was used to determine the significance of the treatments using the SIGMASTAT Statistical program package (Jandel Co., Erkrath, Germany): Comparisons of the treatment mean were done with the use of Tukey's post-hoc test at 5% level of significance.

RESULTS AND DISCUSSION

Effect of Extracts and Fractions of *E. supina* on the Production of Cellular ROS

The inhibitory effects of extracts of *E. supina* on ROS production induced by H₂O₂ in HT-1080 cells are presented in Fig. (1). The A+M and MeOH extracts dose-dependently decreased ROS induced by H₂O₂ compared to the levels seen in the control without the extracts during 120 min. Treatments with A+M and MeOH extracts (0.5 mg/mL concentrations) reduced ROS generation by 98% (Table 1).

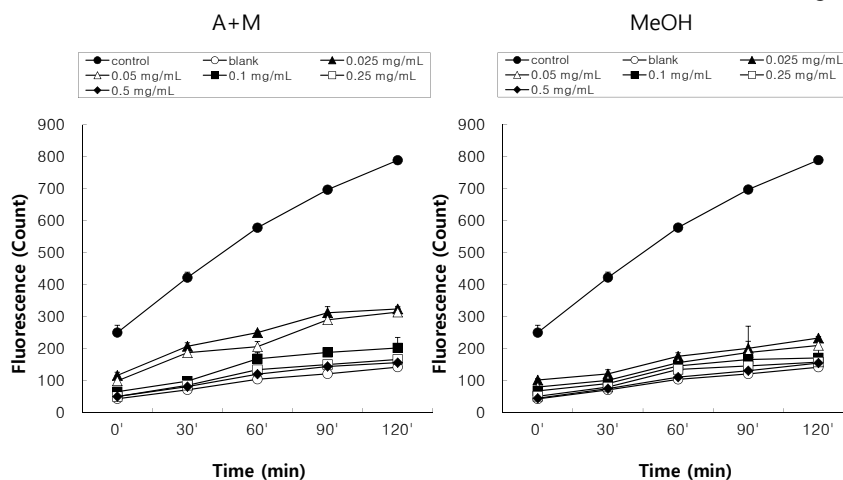


Fig. (1). Effect of acetone+methylene chloride (A+M) and methanol (MeOH) extracts from *E. supina* on the levels of reactive oxygen species in HT-1080 cells. Control, sample was treated with 500 μM H₂O₂ and phosphate buffered saline; Blank, sample was treated with phosphate buffered saline without H₂O₂.

Table 1. Effect of extracts and fractions from *E. supina* on the production inhibition (%) of reactive oxygen species in HT-1080 cells.

Extracts/Fractions	Treatment Concentration (mg/mL)*				
	0.025	0.05	0.1	0.25	0.5
A+M	71.9 ± 2.5 ^{a,1}	73.4 ± 3.5 ^{a,1}	90.7 ± 7.2 ^{a,2}	96.3 ± 1.5 ^{a,2}	97.8 ± 2.0 ^{a,2}
MeOH	85.9 ± 2.2 ^{a,1}	89.5 ± 2.5 ^{a,1}	95.5 ± 2.4 ^{a,2}	97.5 ± 3.1 ^{a,2}	98.0 ± 3.5 ^{a,2}
Hexane	65.4 ± 1.1 ^{b,1}	72.7 ± 3.1 ^{a,1}	80.8 ± 4.3 ^{a,2}	89.0 ± 1.4 ^{a,2}	91.0 ± 2.0 ^{a,2}
85% aq. MeOH	78.0 ± 0.7 ^{a,1}	81.9 ± 0.8 ^{a,1}	86.4 ± 0.2 ^{a,1}	94.3 ± 0.7 ^{a,2}	97.4 ± 0.5 ^{a,2}
BuOH	57.3 ± 1.0 ^{b,1}	61.9 ± 1.8 ^{b,1}	68.3 ± 0.9 ^{b,1}	77.5 ± 1.0 ^{b,2}	81.7 ± 1.6 ^{b,2}
Water	39.4 ± 2.5 ^{c,1}	44.5 ± 1.8 ^{c,1}	54.4 ± 4.6 ^{b,2}	59.7 ± 2.8 ^{c,2}	61.0 ± 1.8 ^{c,2}

*Values are expressed as mean ± standard deviation of inhibition rate. Values in the same column with different letters and row with different Arabic numerals are significantly different at $P < 0.05$ using Turkey's test. A+M, acetone with methylene chloride extract; MeOH, methanol extract; Hexane, *n*-hexane fraction; 85% aq. MeOH, 85% aqueous methanol fraction; BuOH, *n*-butanol fraction; Water, water fraction.

In particular, the MeOH extract showed a higher inhibitory effect all tested concentrations compared to the A+M extract. Figs. (2-5) show the inhibitory effect of solvent fractions (*n*-hexane, 85% aqueous MeOH, *n*-BuOH and water) on ROS levels. All tested fractions decreased of ROS production in a concentration-dependent manner. Treatments with *n*-hexane, 85% aqueous MeOH and *n*-BuOH fractions (0.5 mg/mL concentrations) resulted in 91%, 97%, and 82% ROS inhibition, suggesting that the 85% aqueous MeOH fraction had the strongest effect in reducing ROS production.

Nugroho *et al.* [17] investigated the peroxynitrite-scavenging activity of extracts and fractions of *E. supina* and found that the ethylacetate fraction exhibited the strongest ONOO⁻ scavenging activity and contained the highest content of polyphenols. They also suggested that the potent activity of this fraction was related to high content of

galloylated flavonoid glycosides and ellagic acid, which are believed to strong ONOO⁻ scavengers and are therefore beneficial for the treatment or prevention of gastrointestinal diseases. Hong *et al.* [9] also found the highest radical scavenging activity in the ethylacetate fraction of *E. supina* and isolated eight flavonoids from this fraction. Heo *et al.* [18] evaluated the antioxidant activity of an extract of *E. humifusa*, and found that the methanol extract showed DPPH radical scavenging activity. Another study on *E. humifusa* indicated that its fractions show antioxidant effects in biological systems, particularly in skin exposed to ultraviolet radiation, by scavenging ¹O₂ and other ROS, and inhibit damage to cellular membranes against ROS [19]. Farhan *et al.* [20] suggested that ethanol and water extracts from *E. macroclada schyzoceras* are effective antioxidants; some aromatic compounds and fatty acids were detected

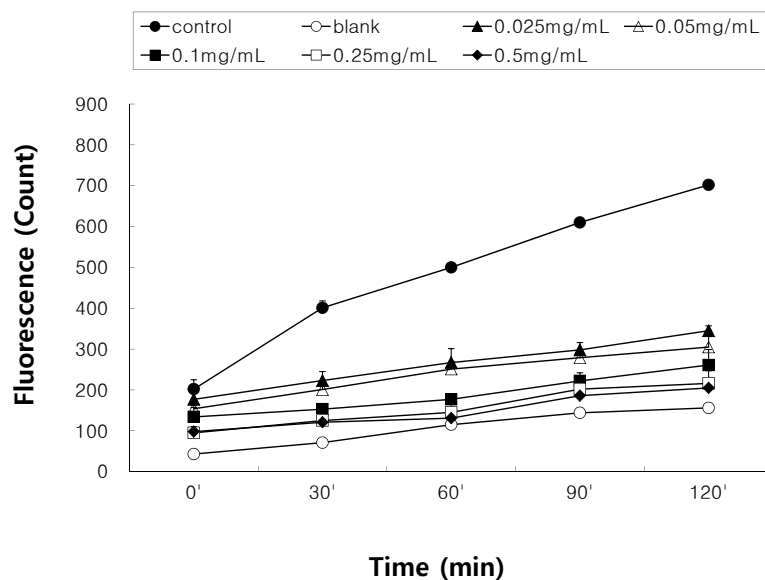


Fig. (2). Effect of *n*-hexane fraction from *E. supina* on the levels of reactive oxygen species in HT-1080 cells. Control, sample was treated with 500 μ M H₂O₂ and phosphate buffered saline; Blank, sample was treated with phosphate buffered saline without H₂O₂.

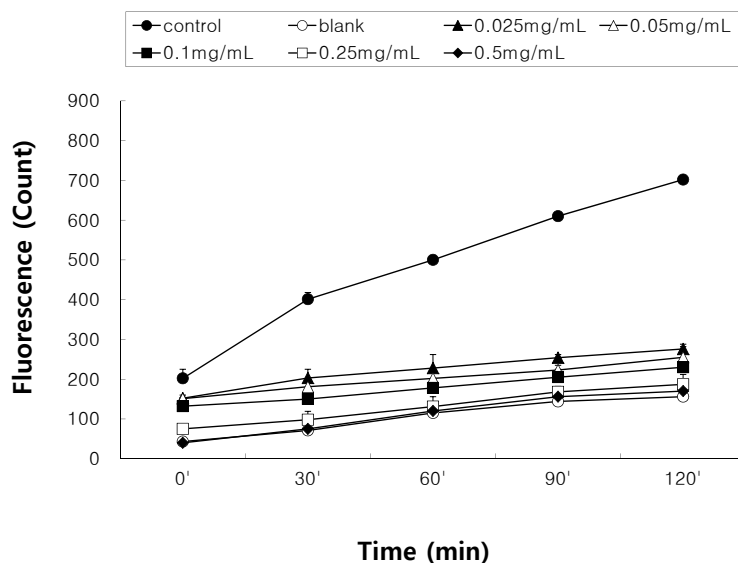


Fig. (3). Effect of 85% aqueous methanol fraction from *E. supina* on the levels of reactive oxygen species in HT-1080 cells. Control, sample was treated with 500 μ M H₂O₂ and phosphate buffered saline; Blank, sample was treated with phosphate buffered saline without H₂O₂.

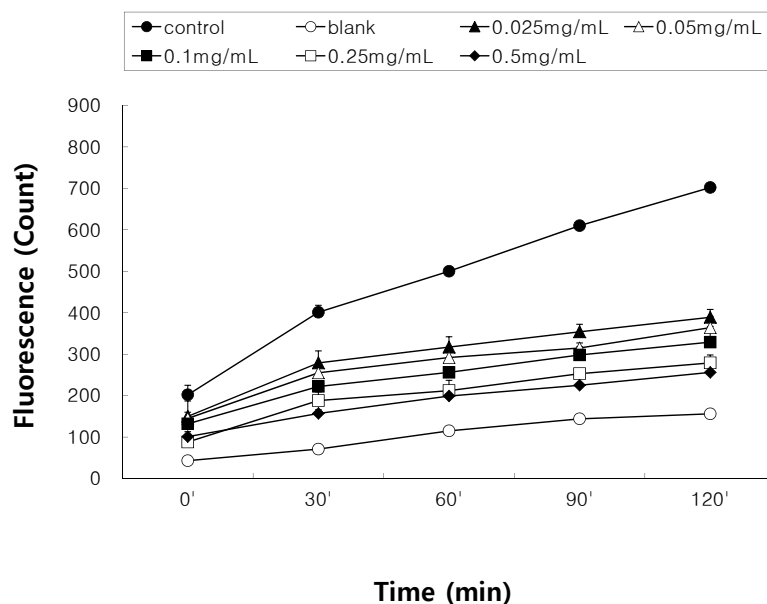


Fig. (4). Effect of *n*-butanol fraction from *E. supina* on the levels of reactive oxygen species in HT-1080 cells. Control, sample was treated with 500 μ M H₂O₂ and phosphate buffered saline; Blank, sample was treated with phosphate buffered saline without H₂O₂.

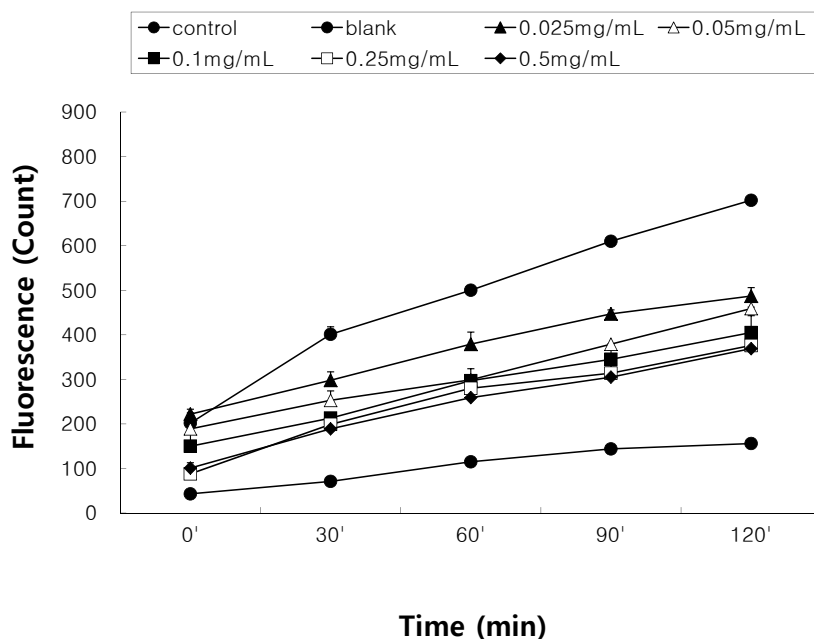


Fig. (5). Effect of water fraction from *E. supina* on the levels of reactive oxygen species in HT-1080 cells. Control, sample was treated with 500 μ M H₂O₂ and phosphate buffered saline; Blank, sample was treated with phosphate buffered saline without H₂O₂.

among the chemical compounds in these extracts. Taken together, these results show that plants of the genus *Euphorbia* can be used for preventing a number of diseases related to oxidative stress. Our previous study on the fatty acid composition of *E. supina* demonstrated that among the fractions tested, the 85% aqueous MeOH fraction contained the highest percentage of 18:3n-3 and showed a potent inhibitory effect on the proliferation of human cancer cells [11]. In conclusion, the 85% aqueous MeOH fraction

inhibited cellular oxidation and may therefore contain valuable active compounds. Therefore, analysis of the primary functional components of *E. supina* demonstrated the antioxidant effects of this plant, indicating its potential to modulate oxidative stress.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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