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Cytotoxcity Effects of Triphlorethol-A on Various Cancer Cells

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ABSTRACT : *Ecklonia cava* is a brown alga (Laminariaceae) that is abundant in the subtidal regions of Jeju island in Korea. Phlorotannins were identified to be responsible for the biological activities in Ecklonia species. In the present study, triphlorethol-A, a phlorotannin, was isolated from *Ecklonia cava* and its anticancer properties were investigated. Triphlorethol-A was investigated whether it may show cytotoxicity effects against U937, HeLa, NCI-H460 and MCF-7 cancer cells by MTT test. As a result, triphlorethol-A did not show cytotoxic effects against tested four cell lines.

Key words : **Triphlorethol-A, *Ecklonia cava*, Anticancer effect**

Introduction

Cancer is the leading cause of death in Korea. Among the possible causes of cancer, damage to DNA and other cellular molecules by reactive oxygen species (ROS) ranks high as a major factor in the onset and development of cancer (Gutteridge, 1993; Borek, 1991; Borek, 1997). Oxidative stress induces gene mutation and promotes carcinogenesis, thereby leading to cancer. (Shi *et al.*, 1998; Sporn and Suh, 2000). Experimental studies support that dietary antioxidants (eg, vitamin E, vitamin C, β-carotene, and other phytochemicals) as well as endogenous antioxidants (eg, glutathione) that neutralize or trap ROS act as cancer preventive agents (Borek, 1997; Borek *et al.*, 1986).

Ecklonia cava is a brown alga (Laminariaceae) that is abundant in the subtidal regions of Jeju island in Korea. Recently, it has been reported that Ecklonia species exhibits radical scavenging activity (Kang *et al.*, 2003; Kang *et al.*, 2004), anti-plasmin inhibiting activity (Fukuyama *et al.*, 1989a, 1989b, 1990), HIV-1 reverse transcriptase and protease inhibiting activity (Ahn *et al.*, 2004) and tyrosinase inhibitory activity (Park *et al.*, 2000). Phlorotannins such as eckol (a phloroglucinol trimer, a closed-chain trimer), 6,6-bieckol (a hexamer), dieckol (a hexamer) and phlorofucofuroeckol (a pentamer) were identified to be responsible for the biological activities in Ecklonia species.

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In the present study, triphlorethol-A, a phlorotannin, was isolated from *Ecklonia cava* and its anticancer properties were investigated.

Materials and Methods

Preparation of triphlorethol-A

The dried *Ecklonia cava* (4 kg), collected from Jeju island in Korea, was immersed in 80% methanol at room temperature for 2 days. The aqueous methanol was removed in vacuo to give a brown extract (1 kg), which was partitioned between ethyl acetate and water. The ethyl acetate fraction (230 g) was mixed with celite. The mixed celite was dried and packed into a glass column, and eluted in the order of hexane, methylene chloride, diethyl ether and methanol. The obtained diethyl ether fraction (14 g) was subjected to Sephadex LH-20 chromatography using $CHCl_3$: MeOH gradient solvent (2/1 → 0/1). The triphlorethol-A (220 mg) was obtained from these fractions and was identified according to the previously reported method (Fig. 1) (Fukuyama *et al.*, 1989). The purity of triphlorethol-A assessed by HPLC was 90%. Triphlorethol-A was freshly dissolved in dimethylsulfoxide; the final concentration of which did not exceed 0.1%.

Reagents

MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] bromide) were purchased from Sigma Chemical Company (St. Louis, MO, USA).

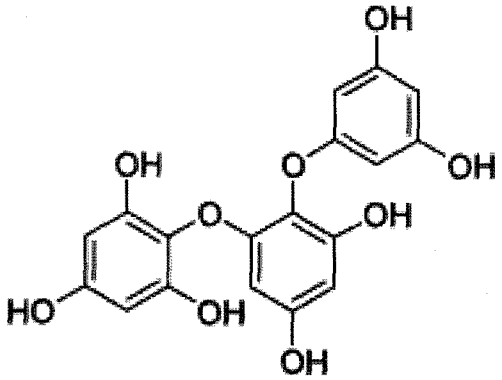

Fig. 1. Chemical structure of triphlorethol-A.

Cell culture

To study the cytotoxic effect of triphlorethol-A against cancer cells, NCI-H460 (human non-small cell lung), HeLa (human cervix cancer cell), U937 (human monocytic leukemia cell) and MCF-7 (human breast cancer cell) from the American Type Culture Collection were used. These cancer cells were maintained at 37°C in an incubator with a humidified atmosphere of 5% CO_2 and were cultured in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum, streptomycin (100 μg/ml) and penicillin (100 units/ml).

Cell viability

The cell viability of triphlorethol-A against various cancer cells are determined using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] bromide (MTT) assay, which is based on the reduction of a tetrazolium salt by mitochondrial dehydrogenase in the viable cells (Carmichael *et al.*, 1987). Triphlorethol-A were treated and incubated for 24 h. The MTT solution was added and incubated for 4 h. The formazan crystals in each well were dissolved in 150 ul dimethylsulfoxide and the absorbance was measured at 540 nm.

Statistical analysis

All the measurements were made in triplicate and all values were represented as means ± standard error. The results were subjected to an analysis of the variance (ANOVA) using the Tukey test to analyze the difference. $p < 0.05$ were considered significantly.

Results and Discussion

To find the anticancer compounds from from *Ecklonia cava*, triphlorethol-A, a phlorotannin, was isolated. It was detected using MTT assay whether triphlorethol-A have cytotoxic effects against various cancer cells. As

Table 1. Cytotoxic effect of triphlorethol-A against NCI-H460 cells

Triphlorethol-A (μg/ml)	NCI-H460
0	100.0 ± 7.0%
30	70.2 ± 1.8%*
60	75.8 ± 3.2%*
90	65.1 ± 0.9%*

Cytotoxic effect of triphlorethol-A against NCI-H460 cells at 30, 60, 90 μg/ml was determined by MTT assay. The measurements were made in triplicate and values are expressed as means ± standard error. *significantly different from control ($p < 0.05$).

Table 2. Cytotoxic effect of triphlorethol-A against HeLa cells

Triphlorethol-A (μg/ml)	HeLa
0	100.0 ± 1.6 %
30	89.8 ± 0.6 %*
60	98.0 ± 0.5 %*
90	93.9 ± 1.7 %*

Cytotoxic effect of triphlorethol-A against HeLa cells at 30, 60, 90 μg/ml was determined by MTT assay. The measurements were made in triplicate and values are expressed as means ± standard error. *significantly different from control ($p < 0.05$).

Table 3. Cytotoxic effect of triphlorethol-A against U937cells

Triphlorethol-A (μg/ml)	U937
0	100.0 ± 3.3%
30	83.9 ± 6.4%
60	79.9 ± 4.2%*
90	75.2 ± 1.5%*

Cytotoxic effect of triphlorethol-A against U937cells at 30, 60, 90 μg/ml was determined by MTT assay. The measurements were made in triplicate and values are expressed as means ± standard error. *significantly different from control ($p < 0.05$).

Table 4. Cytotoxic effect of triphlorethol-A against MCF-7 cells

Triphlorethol-A (μg/ml)	MCF-7
0	100.0 ± 2.5%
30	70.1 ± 2.1%*
60	79.0 ± 2.6%*
90	65.5 ± 1.2%*

Cytotoxic effect of triphlorethol-A against MCF-7 cells at 30, 60, 90 μg/ml was determined by MTT assay. The measurements were made in triplicate and values are expressed as means ± standard error. *significantly different from control ($p < 0.05$).

shown in Table 1, triphlorethol-A at 30, 60, 90 μg/ml showed the cell viability of 70%, 75% and 65% against NCI-H460 cells, respectively. In HeLa cells, it showed the cell viability of 90%, 98% and 94%, respectively (Table 2). And triphlorethol-A at 30, 60, 90 μg/ml showed the cell viability of 84%, 80% and 75% against U937 cells, respectively (Table 3). In MCF-7 cells, showed the cell viability of 70%, 79% and 66%, respectively (Table 4). Phlorotannins, which are marine algal polyphenols and are also known as brown algae, are polymers of phloroglucinol (Shibata *et al.*, 2002). Although some reports suggest that phlorotannins from algae exhibit the antioxidant effect on free radicals (Kang *et al.*, 2003; Kim *et al.*, 2004; Nakamura *et al.*, 1996), there are no reports on the anticancer activity of triphlorethol-A, isolated from *Ecklonia cava*. However, triphlorethol-A did not show cytotoxic effects against tested four cell lines.

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