韓藥作誌(Korean J. Medicinal Crop Sci.) 16(2) : 79 – 85 (2008)

# 흰민들레와 서양민들레 추출물의 세포독성 및 항산화 활성 비교

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# Cytotoxic and Antioxidant Effects of *Taraxacum coreanum* Nakai. and *T. officinale* WEB. Extracts

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**ABSTRACT : Some studies of dandelion have been carried out on bioactivities, however, no comparative analysis on antioxidant and cytotoxic activities in the Korean dandelion (*T. coreanum* NAKAI.; KD) and dandelion (*T. officinale* WEB.) have been reported. In this study, the extracts of KD and dandelion analyzed relative phenolic contents and free radical scavenging, antioxidant enzyme and cytotoxic activities. The extract of Korean dandelion leaves (KDL) exhibited a higher phenolics content (368 ± 11.5 ㎎/100 g) and a strong free radical scavenging activity ($RC_{50}$ value; 87.89 ug/㎖) than other parts and BHT (120.12 ug/㎖), synthetic antioxidant. At ascorbate peroxidase (APX) activity, the dandelion root (DR) had a greater (38.8 U/㎎ protein) antioxidant enzyme compare to the dandelion leaves (12.2 U/㎎ protein). The catalase (CAT) and superoxide dismutase (SOD) followed higher enzyme activity in Korean dandelion root (KDR) than other parts. In a cytotoxic activity against human cancer cell, the extracts of KDR was found to be active against Calu-6, HCT-116, and SNU-601 cell lines, with $IC_{50}$ values of 522.34, 532.74 and 614.85 ug/㎖, respectively. These results suggest that KD and dandelion would be an alternative antioxidant source, based on natural plant resources.**

***Key Words*** **:** *Taraxacum coreanum*, *Taraxacum. officinale,* DPPH, Antioxidnat Enzyme, Cytotoxic Activity.

## INTRODUCTION

Dandelion (*Taraxacum officinale* WEB.) is a common traditional plant in the Asia, Europe, and America, roadsides and ruderal sites. Young dandelion plant was consumed as a fresh salad, whereas roots are used as a coffee substitute. The root extract of dandelion include variety phytosterol, flavor and phenolic compound such as taraxasterol, stigmasterol, chicoric acid, caffeic acid, and scopoletin (Akashi *et al.*, 1994). Such medicine compounds have been used for diuretic, analgesic and hypoglycemia agents (Lee *et al.*, 2007). The water extract of dandelion has been reported to exhibit anti-carcinogenic activity and antitumor action (Koo *et al.*, 2004), moreover, their root was showed growth-stimulating activity of bifidobacteria (Trojanova *et al.*, 2004).

Korean dandelion *(Taraxacum coreanum* NAKAI.; KD), a traditional Korean medicine, has been used in the treatment of a women's disease and diuretic and anti-inflammatory medicine (Koo *et al.*, 2004). These functions of KD are related to its phytochemical compounds such as phenols and flavonoids, which are an important source of various natural antioxidants. These effects have led to an increasing demand of KD for use as a dietary supplement or as the agent in pharmaceutical products. However, KD is being disappeared rapidly by a strong propagation power of a dandelion. The content of the bioactive compounds as well as physiological and antioxidant activities in this plant have not been systematically researched. Also, biological activities of KD

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Received October 30, 2007 / Accepted April 3, 2008

have not been investigated to compare with a dandelion.

Therefore, in the present study, the cytotoxic and antioxidant activity of KD and the dandelion was evaluated by their abilities to inhibit the growth of various cancer cells, scavenge DPPH free radicals, and measure a total phenolic content. Furthermore, their SOD, APX and CAT activities were researched.

## MATERIAL AND METHODS

### 1. Plant materials and cell lines

KD and dandelion were collected from Naju and Hwasun of Jeonnam province, in March and April 2006. All the plants was fresh and frozen at −78℃ after harvesting.

Calu-6 (human pulmonary carcinoma cell line), HCT-116 (human colon carcinoma cell line), and SNU-601 (human gastric carcinoma cell line) cells were obtained from Korean cell line bank.

### 2. Preparation of plant extracts

Fresh plant parts were rinsed with water and freeze dried immediately. Dried plants were ground in a chilled mortar. The powder sample was extracted with ethanol (10ml of 95% ethanol/g of plant) at room temperature for 24 h. The extracts were filtered, followed by rotary evaporator under 40℃. The concentrated extracts were dissolved in a 95% ethanol and kept on the dark at −4℃.

### 3. Determination of total phenolic content

Total phenolic content of extracts was determined according to the Folin-Ciocalteu method (Kähkönen *et al.*, 1999). 700 ul of Folin-Ciocalteu reagent was added to 50 ul of extract and stirred vigorously by vortex and left to stand for 3 min. Then, 100 ul of 10% sodium carbonate solution was added and the solution was made up to 1 mℓ with distilled water, mixed thoroughly, and left to stand at room temperature for an hour. Absorbance was measured at 725 ㎚ and tannic acid was used as a standard in different concentrations (0, 25, 50, 100, 200 and 500 ㎎/100 g).

### 4. Assay of DPPH free radical scavenging activity

DPPH assay was carried out as described by Blois (1958). The extract was mixed 0.2 mM DPPH in ethanol solution. The mixture was stirred vigorously and then left at room temperature for 30 min. The UV absorbance was measured at 517 ㎚. The $RC_{50}$ value, which is the concentration of extract that reduces 50% of the free radical concentration, was calculated as ug/mℓ.

### 5. Assay of antioxidant enzyme activity

#### 1) Crude enzyme extraction

Each plant parts were homogenized in extraction buffer (50 mM phosphate buffer, pH 7.0; 1% triton X-100; 1% PVP-40). The homogenate was then centrifuged at 12,000 rpm for 20 min and the supernatant was used for enzyme activity assay. The protein content was determined by the Bradford *et al.*, (1976) using bovin serum albumin as a standard.

#### 2) Assay of SOD activity

SOD activity was detected by the method of Beauchamp and Fridovich (1971). The solution was mixed of 50 mM carbonic buffer (pH 10.2), 0.1 mM EDTA, 0.1 mM xanthine, 0.025 mM nitroblue tetrazolium, and crude enzyme extracts. Then, the mixture was reacted for 10 min at 25℃ and added xanthine oxidase ($3.3 \times 10^{-6}$ mM). The mixture was measured at 240 ㎚ at room temperature. The results defined as units per mg and one unit of SOD activity was defined as a mount of enzyme causing 50% inhibition of NBT.

#### 3) Assay of APX activity

APX activity was measured by the method of Nacano and Asada (1981). 1 mℓ of the substrate (50 mM potassium phosphate buffer pH 7.0, 0.5 mM ascorbate, 0.1 mM $H_2O_2$, 0.1 mM EDTA) was incubated with 100 ul of sample for 5 min at 37℃. The change in absorbance of the solution was read at 290 ㎚.

#### 4) Assay of CAT activity

CAT activity was assayed by the Aebi method using hydrogen peroxide ($H_2O_2$) (Aebi, 1984). The solution was consisted of 50 mM potassium phosphate buffer (pH 7.0) and 10 mM $H_2O_2$.. The decomposition of $H_2O_2$ was measured at 240 ㎚ at room temperature. CAT activity was calculated as units per mg protein. One unit is defined as the amount of the CAT enzyme required to consumption 1 uM of $H_2O_2$ per min.

### 6. Cytotoxicity assays

#### 1) Cell Culture

Calu-6, HCT-116, and SNU-601 cells were grown at 37℃ in an atmosphere of 95% air and 5% $CO_2$. Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovin serum (FBS) and 1% antibiotic antimycotic solution.

#### 2) MTT assay

The cytotoxic activity of extracts was evaluated using the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Lee *et al.*, 2001; Choi *et al.*, 2002). Cells ($3 \times 10^4$) were seeded in each well containing 90 ul of RPMI medium supplemented with 10% FBS in a 96-well microfilter plate and incubated overnight. After 24 h, 100 ul of new media and extracts was added, and the plates were incubated for 48 h. Cells were washed once before adding 50 ul of FBS-free medium containing 5 ㎎/㎖ MTT. After 4 h of incubation at 37℃, the medium was discarded and the formazan blue, which formed in the cells, was dissolved in 50 ul of DMSO. The optical density was measured at 540 ㎚. The concentration required to reduce absorbance by 50% ($IC_{50}$) in comparison to control cells was determined. The percentage of cell viability was calculated according to the following equation:

Percent of viable cells (%) = (optical density with cytotoxic drug/optical density without cytotoxic drug) × 100

### 7. Statistical analyses

All data are defined as mean ± standard deviation of triplicate experiments. Statistical differences were tested for significance using the student t-test (SPSS for Windows 12.0 package, SPSS Inc.).The values of p lower than 0.05 were considered significant (p is probability).

## RESULTS AND DISCUSSION

### 1. Total phenolic content

In this study we evaluated the total phenolic content of ethanolic extract of KDL, KDR, dandelion leaves (DL) and DR (Table 1). All the extracts contained relatively high total phenolics (130~368 ㎎/100 g). Especially KD (273~368 ㎎/100 g) were shown higher content than dandelion. The phenolic content of leaves in KD parts (368 ± 11.5 ㎎/100 g) was most high among the others

**Table 1.** Total phenolic contents in Korean dandelion *(T. coreanum)* and dandelion *(T. officinale)* extract.

| Sample | Total phenolic contents (㎎/100 g of plant) * |
|---|---|
| KDL | 368 ±11.5[a] |
| KDR | 273 ±15.1[b] |
| DL | 130 ±1.53 |
| DR | 143 ±5.69[a] |

KDL, Leaf of Korean dandelion; KDR, Root of Korean dandelion; DL, Leaf of dandelion; DR, Root of dandelion. *Means ($n = 3$) with different letters are significantly different ([a]$p < 0.001$, [b]$p < 0.05$, [c]$p < 0.01$).

which is 2.7 times high than the DR (130~143 ㎎/100 g).

Williams *et al.* (1996) isolated the phenolic constituents from dandelion such as Lu 7-glucoside, Lu 7-diglucoside, free luteolin, free chrysoeriol, and chicoric acid. Dandelion root from Poland contained 12.6 ㎎/100 g of total phenolics (Wojdylo *et al.*, 2007) and the aerial parts of dandelion were reported that contain 11.52 ㎎ GAE/g dw of total phenolics (Liu *et al.*, 2007). Plant phenolic compounds have a significant capacity to antioxidant activity (Hu and Kitts, 2003). Antioxidant activity has been ascribed on variety compounds, such as flavonoid and polyphenol isolated from various vegetables and fruits (Salah *et al.*, 1995). More than 30 polyphenol compounds in dandelion extract were identified by liquid chromatography coupled to mass spectrometry (Schütz *et al.*, 2005). However, total phenolics of KD have been not reported to date.

### 2. DPPH radical scavenging activity

In the DPPH assay, BHT were used as a standard and its $RC_{50}$ value determined to be 120.12 ug/㎖. Results showed that KDL and KDR extracts were most active in the DPPH assay with an $RC_{50}$ value of 87.89 ug/㎖ and 141.32 ug/㎖, respectively (Table 2, Fig. 1). The antioxidant activity of KDL contained high phenolics was shown stronger than BHT as a synthetic antioxidant. However, the extracts of DL and DR showed the lower values of 260.66 and 260.11 ug/㎖, respectively than KDL and KDR. These results indicated that the free radical scavenging activity is correlated with the total phenolics. This activity is generally accepted to be the means by which antioxidant compounds inhibit reactive oxygen species (ROS) and lipid peroxidation (Jadhav and Bhutani, 2002).

Chon *et al* (2004) reported that antioxidative effects of dandelion aerial part were the greatest among 13 species

**Table 2.** Free radical scavenging activity of the extracts of Korean dandelion *(T. coreanum)* and dandelion *(T. officinale)*.

| Sample | The DPPH assay (RC50 value in ug/mℓ) * |
|---|---|
| KDL | 87.89 ±6.2[b] |
| KDR | 141.32 ±9.5[c] |
| DL | 260.55 ±15.5[c] |
| DR | 262.04 ±8.2[c] |
| **Control group** | |
| Vit C.(ascorbic acid) | ≤ 25 |
| BHT | 123.04 ±7.91 |

KDL, Leaf of Korean dandelion; KDR, Root of Korean dandelion; DL, Leaf of dandelion; DR, Root of dandelion. *Means (n = 3) with different letters are significantly different ([a]$p < 0.001$, [b]$p < 0.05$, [c]$p < 0.01$).

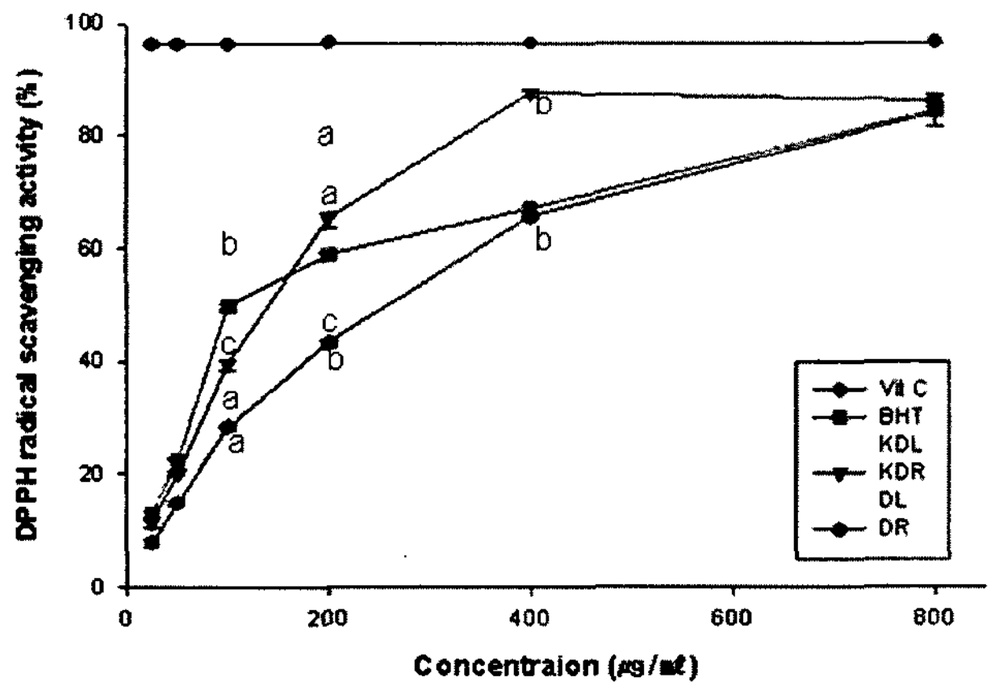


**Fig. 1.** DPPH radical scavenging activity of ethanol extracts from Korean dandelion *(T. coreanum)* and dandelion *(T. officinale)*. KDL, Leaf of Korean dandelion; KDR, Root of Korean dandelion; DL, Leaf of dandelion; DR, Root of dandelion. Each value is means ± S.D. of three separated experiments ([a]$p < 0.001$, [b]$p < 0.05$, [c]$p < 0.01$).

of Compositae plants in Rancimat and TBARS assay. Also, the aerial parts of dandelion showed 69.20% of DPPH scavenging activity according to report of Liu *et al.*, (2007).

### 3. Antioxidant enzyme (SOD, APX, CAT) activity

ROS and free radical can induce in a high number of damage (Park *et al.*, 2007). So, plants produce a lot of antioxidant enzymes to control the oxidative stress caused UV, free radical, and environmental stresses (Scartezzini and Speroni, 2000). Luteolin in dandelion flower scavenged ROS and prevented DNA from ROS induced damage (Katrin *et al.*, 2006)

In the KD and dandelion, we investigated the antioxidant enzymes SOD, APX, and CAT (Fig. 2). The total SOD

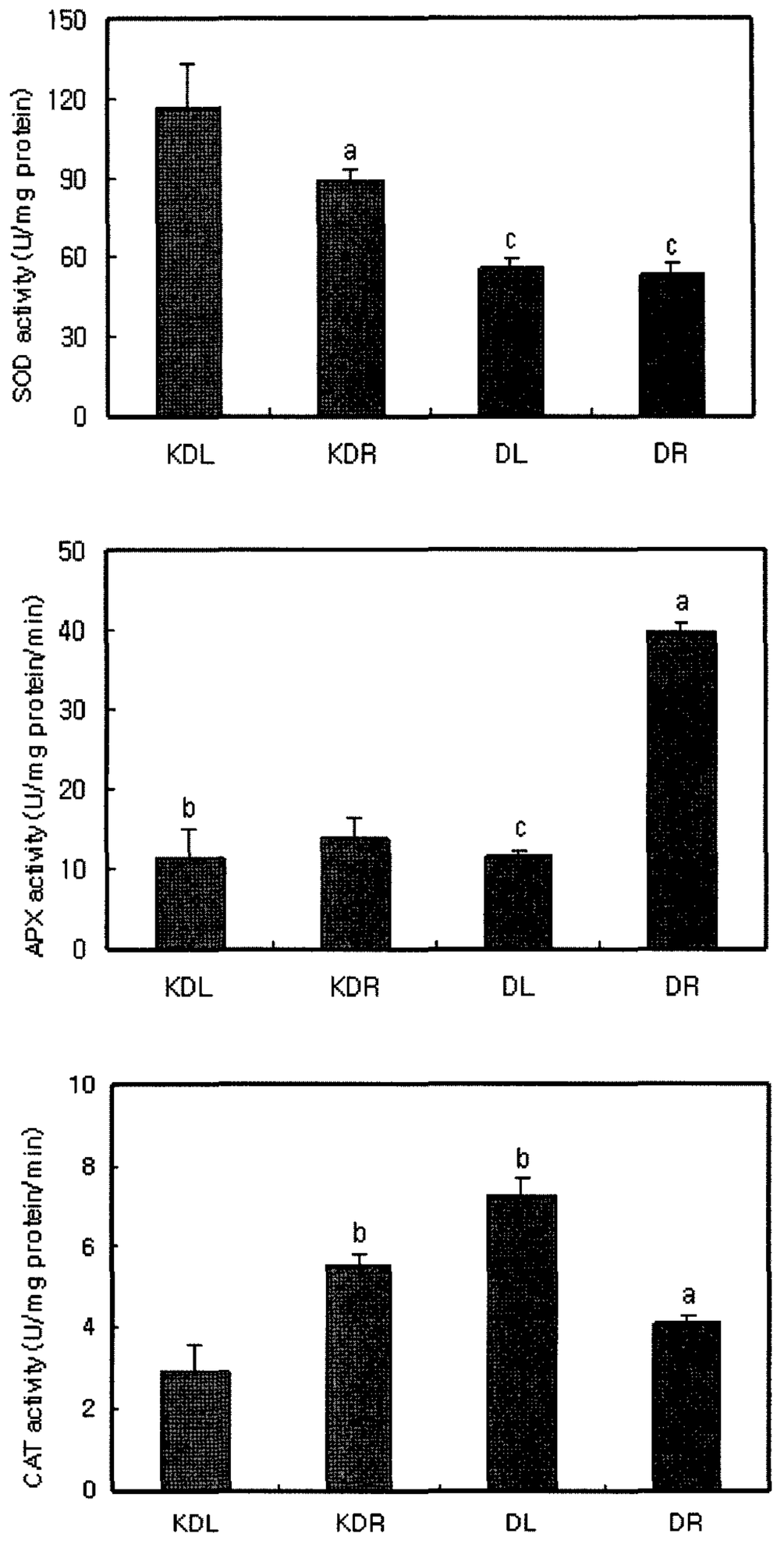


**Fig. 2.** Activities of SOD, APX, and CAT in extract from Korean dandelion *(T. coreanum)* and dandelion *(T. officinale)*. KDL, Leaf of Korean dandelion; KDR, Root of Korean dandelion; DL, Leaf of dandelion; DR, Root of dandelion. Each value is means ± S.D. of three separated experiments ([a]$p < 0.001$, [b]$p < 0.05$, [c]$p < 0.01$).

enzyme activity was found to be 119, 89, 56, and 52 U/mg protein in the KDL, KDR, DL, and DR, respectively. The SOD activity in KDL was shown higher than the other parts. At APX activities, the DR (38.8 U/mg protein) had greater antioxidant enzyme activity compare to the DL (12.2 U/mg protein). The KDL and KDR also exhibited similar APX activity to the DL at 11 and 13.9

U/mg protein, respectively. We also measured the CAT activity of KD and dandelion. The CAT activity in DL (7.2 U/mg protein) was most high and that of the others showed 2, 9, 5, 4, and 4.3 U/mg protein on the KDL, KDR, and DR, respectively. The CAT and SOD followed higher enzyme activity in KDR than in other samples.

Yoo *et al.* (2008) also reported that the phenolic concentrate of dandelion leaves enhanced SOD and CAT activities. SOD is present in all living cell, and its function is to provide a defense against the potentially damaging reactivates of superoxide radical (Chi *et al.*, 2005). APX of a major hydrogen peroxide detoxifying enzymes in plant has been shown to activity in defense to a number of environmental stress conditions, such as salt, drought, and cold (Shi *et al.*, 2001). SOD has been effective activity only when it is followed by the action of CAT and APX, because the dismutase activity of SOD induced $H_2O_2$ (Lee *et al.*, 2003). CAT catalyzed the composition of $H_2O_2$ to $H_2O$ and $O_2$. The results showed that the antioxidant enzymes activity of the SOD, APX, and CAT was different (→ three enzymes activity was no related reciprocally in KD and dandelion.). This is probable due to the different response of the antioxidant enzyme activity by various biological systems.

## 4. Cytotoxic activity against human cancer cells

The cytotoxic activity of the extracts on human pulmonary carcinoma cell (Calu-6), colon carcinoma cell (HCT-116) and gastric carcinoma (SNU-601) was measured by MTT assay (Fig. 3). The $IC_{50}$ value of KDR extract was found to be 522.34, 532.74 and 614.85 ug/mℓ on the Calu-6, HCT-116, and SNU-601 cell lines, respectively. The $IC_{50}$ value of the extracts from KDL, DR, and DL was found to be ≥ 800 ug/mℓ against all three cell lines.

Not many studies on cytotoxic activity of KD was previously reported against human cancer cell like as Calu-6, HCT-116, and SNU-601. Taraxinic acid from KD exhibited potent cytotoxic activity against various cancer cells like P-388, L-1210, and U-937 (Choi *et al.*, 2002).

Several earlier studies have showed that dandelion extract exhibits anti-tumor activities and induced cytotoxicity through TNF-α, IL-1α secretion in Hep G2 cells (Baba *et al.*, 1981; Koo *et al.*, 2004). Dandelion flower extract induced cytotoxicity in human colon adenocarcinoma cells and had the nitric oxide inhibitory activity (Chun and

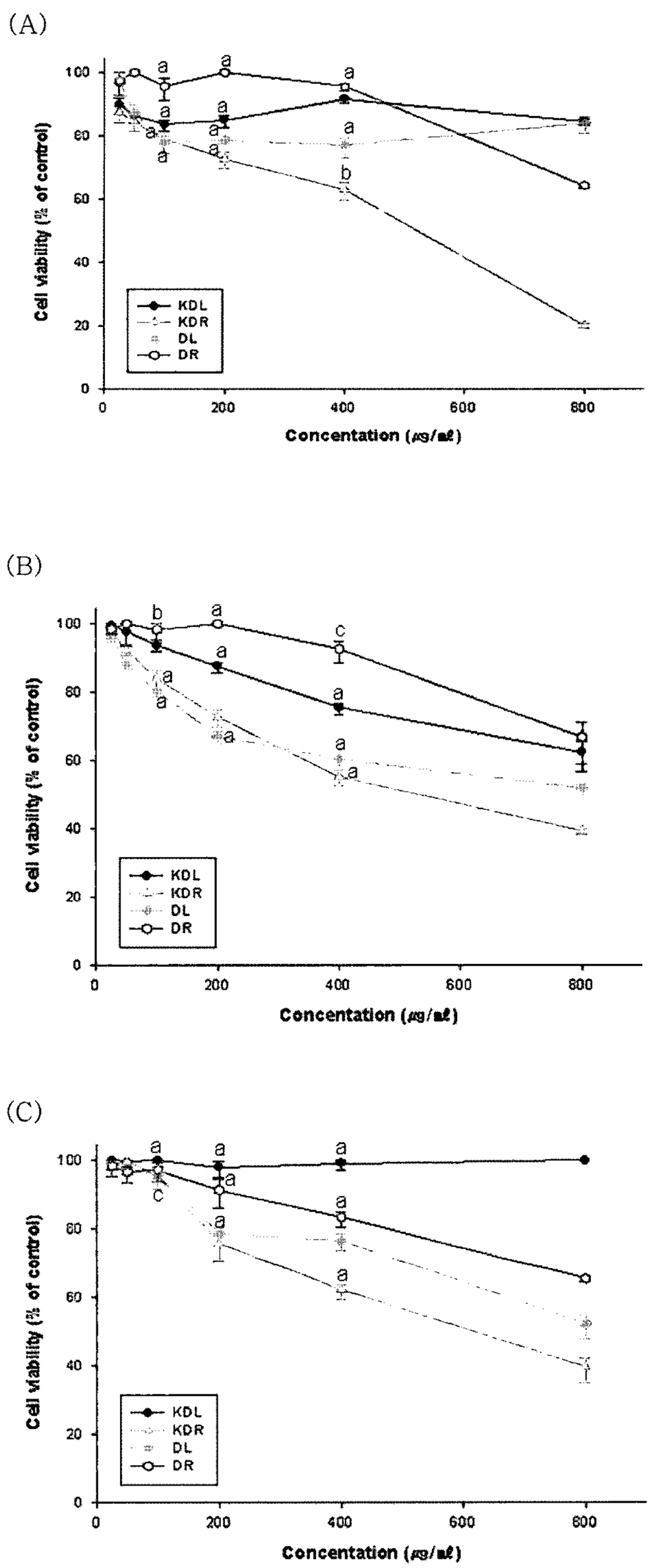


**Fig. 3.** The viability of cells was measured by MTT activities from Korean dandelion *(T. coreanum)* and dandelion *(T. officinale)* extract on human cancer cells Calu-6(A), HCT-116(B) and SNU-601(C). KDL, Leaf of Korean dandelion; KDR, Root of Korean dandelion; DL, Leaf of dandelion; DR, Root of dandelion. Each value is means ± S.D. of three separated experiments ($^{a}p < 0.001$, $^{b}p < 0.05$, $^{c}p < 0.01$).

Kitts, 2003; 2004). Antioxidant and cytotoxic activity of the plant extract maybe connected with various compounds like polyphenol, flavonoid, and pigment (Surh *et al.*, 2001; Scalbert *et al.*, 2002). These results indicate that contain compounds with a significant antioxidant and cytotoxic effect.

In conclusion, our results showed that Korean dandelion accumulated slightly higher activity of antioxidant and cytotoxic then the dandelion. Further studies to identify and characterize the cytotoxic mechanism from KD are in progress.

## Conclusion

In this study, the extracts of Korean dandelion (*T. coreanum*NAKAI.; KD)and dandelion (*T. officinale* WEB.) analyzed relative phenolic contents and free radical scavenging, antioxidant enzyme and cytotoxic activities. The extract of KD leaves exhibited a higher phenolics content (368 ± 11.5 ㎎/100 g) and a strong free radical scavenging activity (RC50 value; 87.89 ug/㎖) than other parts. The dandelion root showed a greater (38.8 U/㎎ protein) ascorbate peroxidase activity compare to the dandelion leaves (12.2 U/㎎ protein). The catalase and superoxide dismutase followed higher enzyme activity in KD root than other parts. In a cytotoxicity against human cancer cell, the extracts of KD root was found to be active against Calu-6, HCT-116, and SNU-601 cell lines, with IC50 values of 522.34, 532.74 and 614.85 ug/㎖, respectively. These results suggest that KD and dandelion would be an alternative antioxidant source, based on natural plant resources.

## Acknowledgement

This study was supported by research funds from Chosun University, 2006.

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