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Biological Properties of Different Types and Parts of the Dandelions: Comparisons of Anti-Oxidative, Immune Cell Proliferative and Tumor Cell Growth Inhibitory Activities

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Abstract

Dandelions have been reported to have medicinal properties and bioactive components that impact human health. However, the precise biological properties of dandelions and the parts of the plants possessing bioactive components remain uncertain. In this study, we evaluated 3 different types of dandelions based on their cultivation origin (Songpa, Uiryung, and native Uiryung types) as well as their 4 different plant parts (leaf, flower, root, skin). Each sample was extracted with 80% methanol and then compared for the biological activities (anti-oxidative, immune cell proliferative and tumor cell growth inhibitory activities). All 3 types of dandelions possessed a degree of biological functions including the hydroxyl radical scavenger activity, immune cell proliferative activity and tumor cell growth inhibitory activity. However, there was no significant difference in these activities between the 3 dandelion types. Leaves of all three dandelion types showed the highest levels of all biological activities. To a lesser degree, the flower and root parts displayed biological activities. In the skin parts, anti-oxidative activity was also detected only at higher doses of dandelion extracts. Heating the dandelion leaf extract did not affect the biological activity, suggesting a heat-stable nature of the biological compounds. Taken together, these collective data suggest that dandelions, in particular their leaves, possess a high concentration of heat-resistant biological compounds, which are responsible for anti-oxidative, immune cell proliferative and tumor cell growth-inhibitory activities.

Key words: dandelion, anti-oxidation, immune cell proliferation, tumor cell growth inhibition

INTRODUCTION

Dandelions are widely distributed perennial herbs that grow in most places throughout the year. In oriental medicine, dandelions have been well known for their effects on making one's stomach strong, for treating an abscess, a tubercle, jaundice and women's diseases, as well as for alleviating a fever (1,2). In Europe, dandelions have been known to be effective for treating constipation, rheumatoid arthritis, neurosis and night blindness. As physiological roles of dandelions have been recently evaluated, there have been more focused approaches to utilizing dandelions as a component of either functional foods or medicinal herbs. Recently, dandelion extracts have been reported to possess antibiotic activities (3,4), anti-oxidative properties (5,6) and anti-tumor substances (7,8).

There are more than 2000 dandelion species in the world. In particular, the most typical and edible domestic dandelions include native dandelions (*Taraxacum mongolicum*, *T. platycarpum*), overseas dandelions (*T. officinale*), Cheju dandelions (*T. hallaisanense*), and mountain dandelions (*T. ohwianum*) (9). In the composition of dandelions, the major part of terpenoid and sterol compounds include taraxacin and taraxacerin, which are distributed equally in the roots, leaves and flowers (10). Other terpene/sterol compounds include beta-amyrin, taraxasterol, taraxerol, sitosterin, stigmasterin, and phytosterin. Since ancient times, the roots and sprouts of dandelions have been used as vegetables or stew components, and sometimes food substitutes. In Europe and North America, the leaves, roots and flowers have been used in salads and as coffee substitutes or wine constituents. However, differences in physiological functions between dandelion types and parts have not been reported.

In this study, we chose 3 different types of dandelions based on their geographical origin (Songpa, Uiryung, and native Uiryung types) and then obtained 4 different tissue parts of the dandelions (leaf, flower, root, skin). These separate samples were subsequently tested for their bio-

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logical properties including anti-oxidative, immune cell proliferative and tumor cell growth inhibitory activities.

MATERIALS AND METHODS

Collection and extraction of dandelions

Different types of dandelions (Songpa, Uiryung, native Uiryung types) were chosen for this study. We harvested the dandelions in the agricultural fields of Seoul and Uiryung, Kyungnam, followed by washing several times with water. Each part of the dandelion (leaf, flower, root, skin) was separately collected and then frozen for lyophilization. In particular, the skin part was mostly derived from the Songpa type as Uiryung and native Uiryung types gave little amount of skin parts. The lyophilized samples were broken using Electric Mixer (Cupiter Gold, Angel, Korea) and then extracted with 80% methanol in a ratio of 1 g samples per 20 mL 80% methanol by shaking for 24 h. The extracts were filtered using Whatmann filter paper and then filtrates were collected, followed by enrichment using Rotavapor (Büchi, Switzerland). Enriched samples were subsequently lyophilized for obtaining a powder using Lyophilizer (Freezone plus 6, Labconco, USA). The powder was quantitated and then stored in a -4°C freezer.

Anti-oxidation TBA test

Hydroxyl radical scavenging activity was tested as follows: 0.2 mL of 0.1 mM $FeSO_4$/EDTA solution was added to a test tube, followed by addition with 0.2 mL of 10 mM 2-deoxyribose, and then with 1.2 mL of 0.1 M phosphate buffer (pH 7.4) containing an increasing concentration of extract samples (0.1~100 μg/1.2 mL). Finally, 0.2 mL of 10 mM H_2O_2 was added. This mixture was reacted for 4 h at 37°C. This was followed by addition with 1 mL of 2.8% TCA (trichloroacetic acid) and then with 1 mL of 1.0% TBA (thiobarbituric acid)/50 mM NaOH solution. This was heated at 100°C for 10 min for color development and then cooled on ice. Subsequently, optical density (OD) values of each sample were observed at 532 nm. As a control, no dandelion extracts were added. The hydroxyl radical scavenging activity (%) was calculated as follows:

$$\text{OH-radical scavenging activity (\%)} = \left(1 - \frac{As - Ao}{Ac - Ao}\right) \times 100$$

where Ao, OD of blank; Ac, OD of control; As, OD of sample.

Immune cell proliferation MTT assay

Spleen was obtained from mice (Balb/c) and single cells were prepared as previously described (11). The cells were resuspended in 3 mL of ACK buffer (4.15 g NH_4Cl, 0.5 g $KHCO_3$, 0.019 g Na_2EDTA in 500 mL H_2O) for 1 min to remove red blood cells, and then washed 3 times in cRPMI (RPMI supplemented with 10% fetal bovine serum albumin, 1% L-glutamine and 1% penicillin/streptomycin). One hundred μL of cRPMI containing 1, 10, 50 and 100 μg dandelion samples/mL in a final concentration was added to each well of a 96 well plate. This was followed by addition of immune cells (3×10^5/well/100 μL). Cells were then incubated for 3 days at 37°C in 5% CO_2. Finally, 15 μL of MTT (2 mg/mL) was added to each well and the cells were incubated for 4 h. The cell culture supernatants were then carefully replaced with 150 μL of dimethylsulfoxide to dissolve formazan precipitate. Subsequently, OD values were read at 490 nm using ELISA reader (EL×800, Bio-Tek, USA).

Tumor cell growth inhibition MTT assay

For determining the effects of dandelion extracts on tumor cell growth inhibition, tumor cells were treated with each extract sample for MTT assay as previously described (12). Briefly, tumor cells were incubated with a final concentration of 500 and 1,000 μg/mL of dandelion extracts. After 3 days incubation, 15 μL of MTT (5 mg/mL) was added to cells, followed by incubation for 4 h at 37°C in 5% CO_2. Cell supernatants were carefully replaced with 150 μL of dimethylsulfoxide. Subsequently, OD values were read at 490 nm using an ELISA reader (EL×800, Bio-Tek, USA). The tumor cells (TC-1) (a kind gift from T.-C. Wu, Johns Hopkins Medical Institutions) were grown in cRPMI supplemented with 400 μg per mL of G418. TC-1 cells are of murine epithelial cell origin and express human papillomavirus 16 E6 and E7 oncogenes, as well as activated *ras* genes.

Statistical analysis

Statistical analysis was done using the Student's *t*-tests (two sample *t*-test) using the SPSS10.1 software program. Values between 2 different groups were compared and considered statistically significant when p values were <0.05.

RESULTS AND DISCUSSION

Hydroxyl radical scavenging activity of dandelions

We determined whether different types and parts of dandelions might have different levels of anti-oxidative activities. Extracts were prepared from three different types of dandelions (Songpa, Uiryung, native Uiryung types) and 4 different tissue parts of dandelions (leaf, flower, root, skin), and then tested at different concentrations using TBA methods. As shown in Fig. 1, the leaf part of dandelions in all 3 dandelion types showed

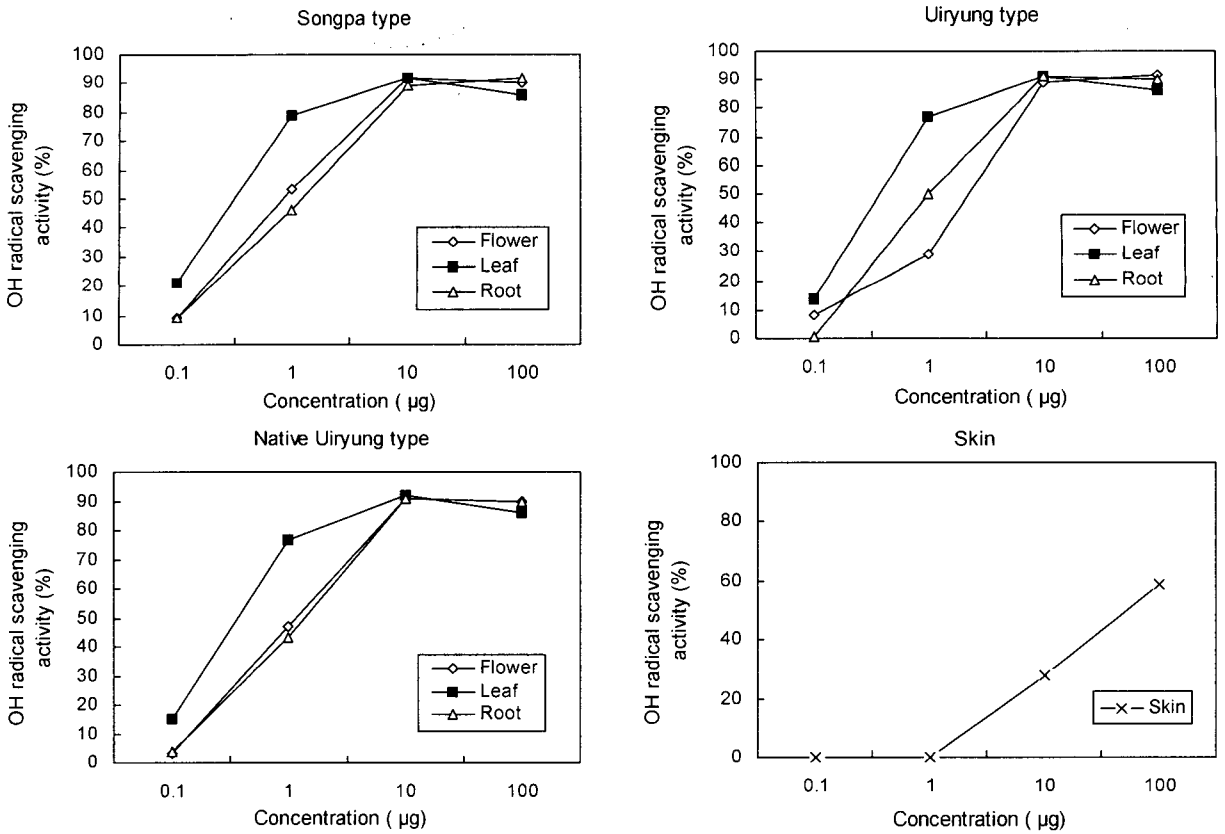

Fig. 1. Hydroxyl radical scavenging activity (%) of different dandelion types and parts. Four different parts (leaf, flower, root, skin) of 3 dandelion types were extracted with 80% methanol. The extracted samples of dandelions (Songpa, Uiryung, and native Uiryung types) and the skin part were added to 0.1 M phosphate buffer (pH 7.4) at a final concentration of 0.1~100 μg per 1.2 mL. In particular, the skin parts are most from the Songpa type. This was followed by TBA assay as described in the Methods and Material section. This was repeated 2 times with similar results.

the highest degree of anti-oxidative activity at the concentrations of 0.1 and 1 μg per 1.2 mL. In contrast, the flower and root parts exhibited lower anti-oxidative activity than the leaf part at these concentrations. However, the anti-oxidative activity of the leaf, flower and root parts was similar at the tested concentrations of 10 and 100 μg per 1.2 mL, suggesting that more than 10 μg of samples per 1.2 mL might be within saturation dose ranges. In the case of the skin part of dandelions, no anti-oxidative activity was detected at the 1 μg dose per 1.2 mL while anti-oxidative activity was detected at the doses of 10 and 100 μg. In all 3 different types of dandelions, however, no big difference in anti-oxidative activity was noted. Overall, leaf, flower/root and skin parts at a dose of 1 μg showed approximately 80%, 40~50%, and 0% of hydroxyl radical scavenging activities, respectively. Thus, this suggests that the leaf part of dandelions possesses the highest amount of anti-oxidative substance(s). Our observation is compatible with the previous finding that dandelions have anti-oxidative activities (5,6). Previous reports have also shown that there are some differences in the compositions of dandelions (13,14). For instance, a whole part of dandelions mainly contain taraxasterol, taraxarol, and taraxerol. However, the leaf parts contain higher concentrations of lutein, violaxanthin, and plastoquinone, while the flower parts contain arnidiol, lutein and flavoxanthin. This suggests that the differences in anti-oxidative activity between the dandelion parts might be due to chemical compounds differently distributed between dandelion parts. However, we observed no dramatic difference in anti-oxidative activities between 3 different dandelion types tested here.

Immune cell proliferative activity of dandelions

We next tested the immune cell proliferative activity of extracts of the 3 different dandelion types (Songpa, Uiryung, native Uiryung types). Each sample was tested in splenocytes for MTT assay. In this study, however, we expected that the leaf part might contain the highest level of immune cell proliferative activity, as compared to the root and flower parts. This is due to our observation that the leaf part possessed the highest level of anti-oxidative activities, as compared to the other parts. As shown in Fig. 2, the leaf part of all 3 dandelion types alone showed an immune cell proliferative activity in a dose-dependent manner. However, the flower, root

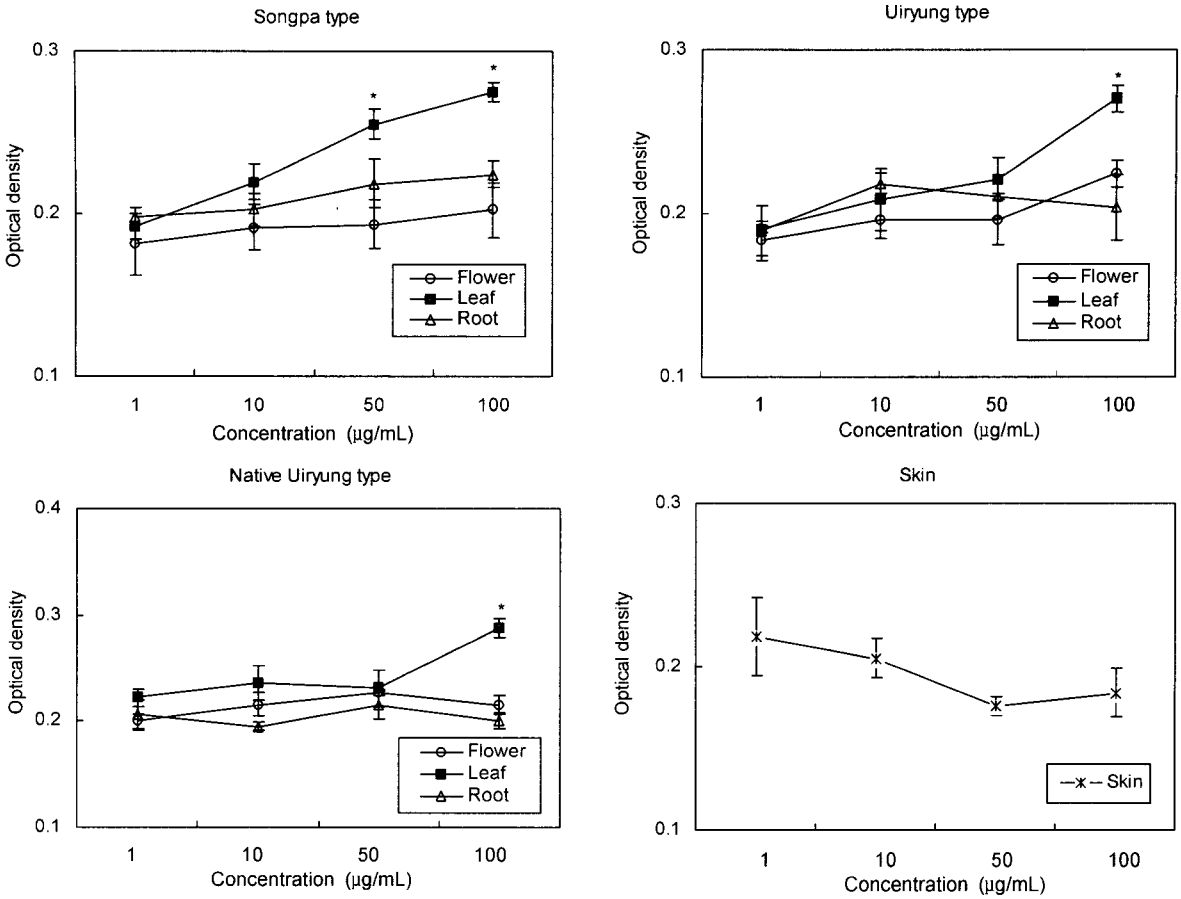

Fig. 2. Immune cell proliferative activity of different dandelion types and parts. Spleen cells were obtained from mice (Balb/c). Spleen immune cells were stimulated *in vitro* with 1~100 μg/mL extracts of different parts (leaf, flower, root) of 3 dandelion types (Songpa, Uiryung, and native Uiryung types) as well as the skin part of the 3 dandelions. After three days stimulation, cells were added with MTT for the cell proliferation assay. OD values were read at 490 nm. This was performed in triplicate. As a positive control. Con A treatment (1 μg/mL) resulted in OD values of 0.5~0.6 in all tested immune cells.
*Statistically significant at $p<0.05$ using the Student's *t*-test compared to the flower or root parts.

and skin parts failed to show such activity. In particular, there was a significant increase in immune cell proliferation at the dose of 100 μg/mL leaf extracts in the case of Uiryung and native Uiryung types of dandelions, as compared to flower or root extracts ($p<0.05$). In contrast, the Songpa type displayed immune cell proliferative activity at both doses of 50 and 100 μg/mL leaf extracts significantly higher than flower or root extracts ($p<0.05$). This suggests that the leaf part of dandelions contains immunostimulatory molecules which are responsible for immune cell proliferation. Our finding is consistent with previous reports showing a big difference in the composition of chemical compounds in dandelion parts (13,14). Furthermore, it would be interesting to determine which subsets of immune cells are involved in this dandelion-mediated immune cell activation.

Tumor cell growth inhibitory activity of dandelions

We next evaluated the tumor cell growth inhibitory activity of extracts of 3 dandelion types (Songpa, Uiryung, native Uiryung types). Each sample was incubated with tumor cells for 3 days for MTT assay. As shown in Fig. 3, the leaf part of dandelions had the highest level of tumor cell growth inhibitory activity, as compared to the other parts (flower and root). Furthermore, the flowers and to a lesser degree the roots also exhibited tumor cell growth inhibitory activity. Overall, all 3 parts of dandelions showed tumor cell growth inhibitory activity significantly higher than positive control (no sample added) ($p<0.05$). In contrast, the skin part showed no tumor cell growth inhibitory activity. However, there was also no dramatic difference in the tumor cell growth inhibitory activity between these 3 different dandelion types (Songpa, Uiryung, and native Uiryung types). These collective data indicate that the leaves of dandelions have the highest amount of tumor cell growth inhibitory substances. A component of dandelion roots, taraxasterol, is known to exhibit anti-carcinogenic properties (7,15). However, our observation showed the highest degree of tumor cell growth inhibitory activity in the leaf part. This

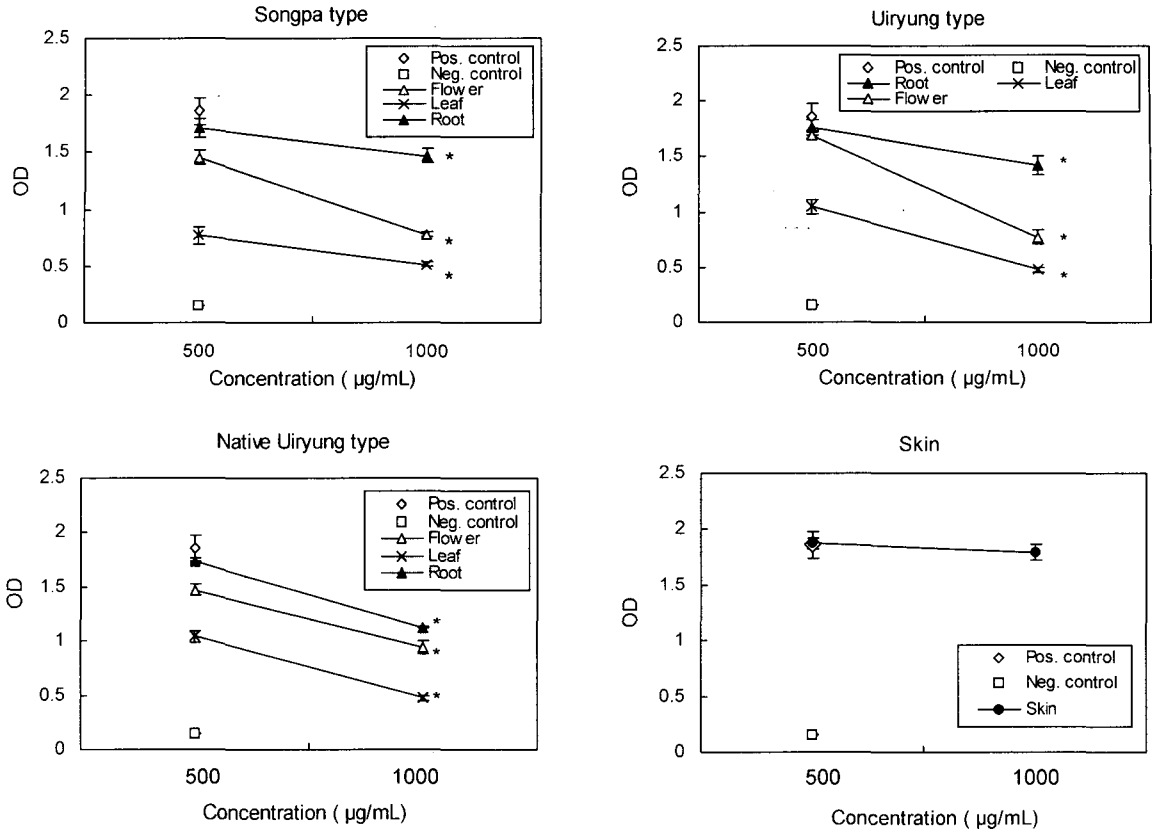

Fig. 3. Tumor cell growth inhibitory activity of different dandelion types and parts. TC-1 tumor cells (3×10^4/well) were incubated *in vitro* with extracts of different parts (leaf, flower, root) of 3 dandelion types (Songpa, Uiryung, native Uiryung types) as well as the skin part of the 3 dandelions at a final concentration of 500 and 1,000 μg/mL. After 3 days incubation, cells were added with MTT for cell growth assay. OD values were read at 490 nm. This was performed in triplicate.
*Statistically significant at $p < 0.05$ using the Student's *t*-test compared to positive controls.

suggests that taraxasterol might be a major compound of the leaf part. Alternatively, some other anti-tumor cell substances besides taraxasterol might exist in the leaf part. However, this needs to be further confirmed. Furthermore, anti-tumor cell effects of many natural compounds have been previously reported by others including us (12,16-19).

Heat resistance of biologically active substances

We were next interested in testing the heat stability of the biological functions of the dandelion leaf extracts. We chose the leaf extracts of the Songpa dandelion types, since they showed a little higher biological activity, as compared to other parts. The sample was boiled for 15 min for heat treatment. Fig. 4A showed that there was no difference in hydroxyl radical scavenging activity at the tested doses of 0.1, 1 and 10 μg per 1.2 mL between heat-treated group and non-treated group. This suggests that the anti-oxidative substance(s) of dandelions are not heat-labile. Fig. 4B shows the immune cell proliferative activities of heat-treated leaf extracts of the Songpa dandelion types. When immune cells were treated with heat-treated dandelion extracts, immune cells were stimulated in a manner similar to non-treated controls, demonstrating that dandelions have a heat-resistant substance responsible for immune cell proliferation either directly or indirectly. Fig. 4C shows tumor cell growth inhibitory activity of the dandelion extracts after heat treatment. Heat-treatment also had no effect on tumor cell growth inhibition, confirming that the substance(s) are heat-resistant. Previously, we reported that fungal extracts of *Lentinus edodes* possess tumor cell growth inhibitory activity and that this activity is destroyed by heat treatment (12). In this case, however, dandelion extracts displayed heat-resistance for anti-tumor, anti-oxidative and immune cell proliferative activities. These data indicate that dandelions contain heat-resistant substances that are responsible for their biological activities.

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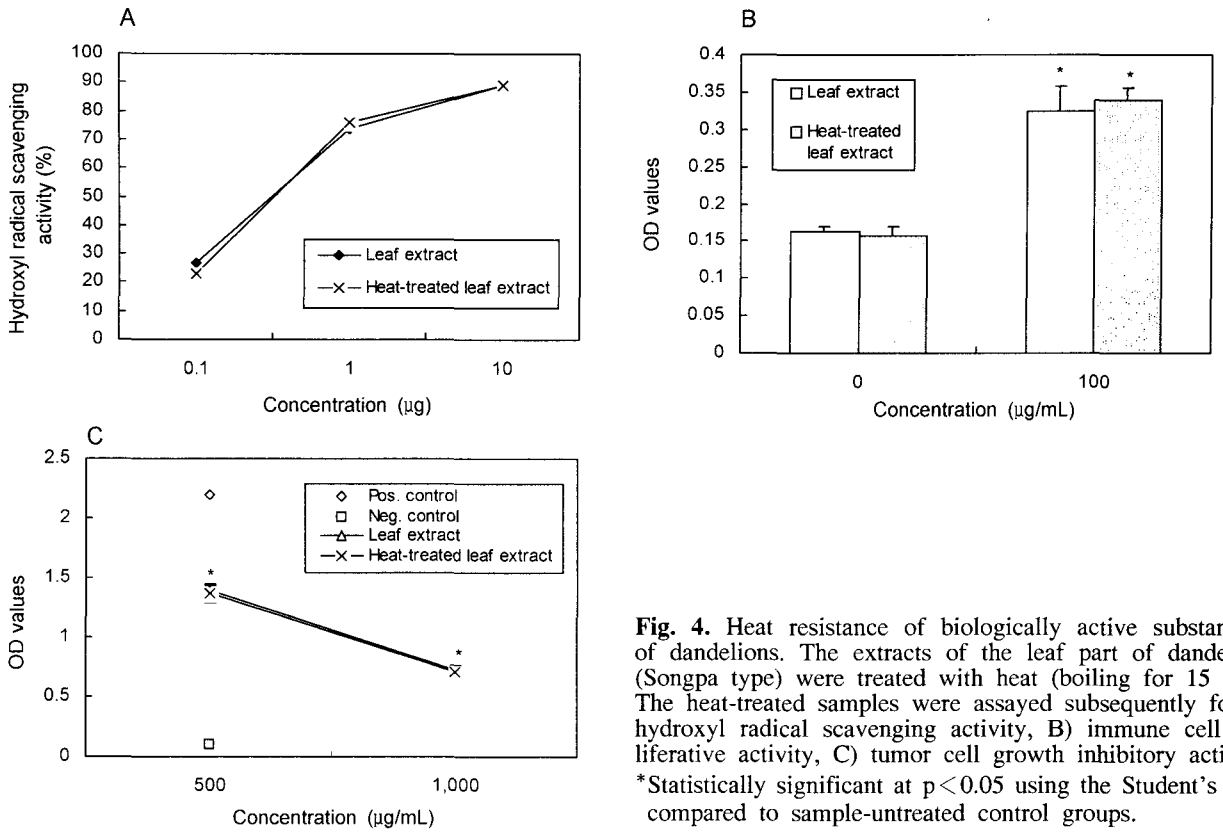

Fig. 4. Heat resistance of biologically active substance(s) of dandelions. The extracts of the leaf part of dandelions (Songpa type) were treated with heat (boiling for 15 min). The heat-treated samples were assayed subsequently for A) hydroxyl radical scavenging activity, B) immune cell proliferative activity, C) tumor cell growth inhibitory activity. *Statistically significant at $p<0.05$ using the Student's *t*-test compared to sample-untreated control groups.

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