

### Cytotoxic Activity of Medicinal Plant Extracts against Human Tumor Cell Lines

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The cytotoxic activities of the methanol extracts of 44 plant species in 31 families against five human solid A549 (lung), SK-OV-2 (ovarian), SK-MEL-2 (melanoma), XF-498 (central nervous system), and HCT-15 (colon) tumor cell lines were examined using the sulforhodamine B (SRB) assay. Responses varied with both cell line and plant species used. Potent cytotoxic activities (ED<sub>50</sub>, <40 μg/ml) against all model tumor cell lines were produced from the extracts of *Rhus chinensis* gall (Galla rhois), *Betula platyphylla* var. *japonica* bark, *Inula helenium* root, *Cinnamomum cassia* bark, *Cinnamomum sieboldii* root bark, *Lysimachia davurica* whole plant, and *Evodia rutaecarpa* fruit. These plants may be useful for developing new types of naturally occurring anti-tumor agents.

Key words: anti-tumor agent, cytotoxicicty, medicinal plant, sulforhodamine B assay.

Cancer therapy is primarily dependent upon repeated administrations of synthetic anti-cancer agents. Although effective, their continued or repeated use has led to widespread development of resistance to the agents in tumor cell lines<sup>1,3)</sup> and adverse effects on human health such as alopecia, leucopenia, sterility, and secondary malignancies in clinical trials.<sup>4)</sup> Decreasing efficacy and increasing concern over possible adverse effects of chemotherapeutic agents have highlighted the need for the development of selective alternatives.

Plants may be an alternative to currently used anti-cancer agents because they constitute a rich source of biologically active chemicals. Since many of them are largely free from adverse effects and have excellent pharmacological actions, they could lead to the development of new classes of possibly safer anti-cancer agents. Much efforts have, therefore, been focused on natural products for potentially useful products as commercial anti-cancer agents or as lead compounds.

In the laboratory study described herein, we assessed the cytotoxic activities of methanol extracts of 44 plant species against five human tumor cell lines to develop potentially new safer types of anti-tumor agents.

#### Materials and Methods

**Chemicals.** Sulforhodamine B (SRB) was purchased from Sigma (St. Louis, MO, USA). Fetal bovine serum and

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RPMI 1640 were supplied by Gibco (Gaithersburg, MD, USA). All other chemicals were of reagent grade.

Tumor cell lines and culture conditions. Five human tumor cell lines used in this study were A549 (lung), SK-MEL-2 (human melanoma), SK-OV-3 (ovarian), XF-498 (central nerve system), and HCT-15 (colon) tumor cell lines. They have been maintained in the laboratory as stocks in RPMI 1640 supplemented with 10% fetal bovine serum. Cell cultures were passaged once or twice weekly using trypsin-EDTA to detach the cells from their culture flasks.

Plants and sample preparation. A total of 44 plant species were selected (Table 1). With the exception of Chaenomeles sinensis and Cinnamomum camphora, the other plant materials were dried in an oven at 40 for 2 days and finely powdered using a blender. Each sample (50 g) was extracted twice with 300 ml methanol at room temperature for 2 days and filtered. Slices of the fresh Chaenomeles fruit (200 g) were ground by a blender, extracted twice with 900 ml methanol at room temperature for 1 day and filtered. The combined filtrate was concentrated to dryness by rotary vacuum evaporator at 40°C. The powder of C. camphora was purchased from Boeun Co. (Seoul, Korea).

**Bioassay.** SRB assay is applied for the measurement of cytotoxic activity of test plant materials against tumor cell lines used.<sup>5)</sup> The rapidly growing cells were harvested, counted, and inoculated at appropriate concentrations (1-2 10<sup>4</sup> cells/well) into 96 well microtiter plates. After incubation for 24 h, the extracts dissolved in culture medium were applied to the culture wells in triplicate, followed by incubation at 37°C for 48 h under 5% CO<sub>2</sub> atmosphere. The cultures fixed with cold trichloroacetic acid were stained

Table 1. Medicinal plants tested.

Plant species	Familiy	TS*	Yield (%) <sup>b</sup>	Plant species	Familiy	TSª	Yield (%) <sup>b</sup>
Rhus chinensis	Anacardiaceae	Ga	50.2	Cinnamomum sieboldii	Lauraceae	Rb	6.3
Acorus calamus var. angustatus	Araceae	Rh	10.1	Liriope platyphylla	Liliaceae	Tu	13.4
Acorus gramineus	Araceae	Rh	9.5	Illicium verum	Magnoliaeceae	Fr	26.1
Betula platyphylla var. japonica	Betulaceae	Ba	5.2	Sinomenium acutum	Menispermaceae	Rh	7.1
Artemisia princeps var. orientalis	Compositae	Wp	6.6	Eugenia caryophyllata	Myrtaceae	Fb	37.8
Inula helenium	Compositae	Ro	16.3	Paeonia suffruticosa	Paeoniaceae	Rb	18.6
Dioscorea batatas	Dioscoreaceae	Rh	2.4	Piper nigrum	Piperaceae	Fr	10.1
Eucommia ulmoides	Eucommiaceae	Le	12.1	Polygala tenuifolia	Polygalaceae	Ro	24.1
Astragalus membranaceus	Fabaceae	Ro	1.3	Rheum coreanum	Polygonaceae	Rh	41.6
Gleditsia horrida	Fabaceae	Fr	17.3	Lysimachia davurica	Primulaceae	Wp	9.0
Glycyrrhiza glabra	Fabaceae	Ro	21.9	Chaenomeles sinensis	Rosaceae	Fr	60.8
Pueraria thunbergiana	Fabaceae	Ro	12.6	Evodia rutaecarpa	Rutaceae	Fr	9.5
Corydalis turtschaninovii	Fumariaceae	Tu	1.3	Zanthoxylum piperitum	Rutaceae	Fr	20.7
Hierochloe odorata	Graminales	Wp	11.2	Zanthoxylum schinifolium	Rutaceae	Fr	16.2
Anemarrhena asphodeloides	Haemodoraceae	Rĥ	12.3	Santalum album	Santalaceae	Li	7.2
Juniperus rigida	Juniperaceae	Fr	5.4	Stemona japonica	Stemonaceae	Ro	15.2
Agastache rugosa	Labiatae	Wp	9.5	Aquillaria agallocha	Thymelaeaceae	Li	6.6
Schizonepeta tenuifolia	Labiatae	Wp	8.1	Angerica dahurica	Umbelliferae	Ro	17.7
Thymus mandschuricus	Labiatae	Wp	28.0	Cnidium officinale	Umbelliferae	Rh	10.0
Akebia quinata	Lardizabalaceae	St	17.1	Foenicum vulgare	Umbelliferae	Fr	4.9
Cinnamomum camphora <sup>c</sup>	Lauraceae			Nardostachys chinensis	Valerianaceae	Rh	12.9
Cinnamomum cassia	Lauraceae	Ba	5.1	Curcuma longa	Zingiberaceae	Rh	11.1

<sup>&</sup>quot;Tissue sampled: Ba, bark; Fb, flower bud; Fr, fruit; Ga, gall; Le, leaf; Li, lignin; Rh, rhizome; Rb, root bark; Ro, root; St, stem; Tu, tuber; and Wp, whole plant.

with 0.4% SRB dissolved in 1% acetic acid. After solubilizing the bound dye with 10 mM unbuffered Tris base by gyrotory shaker, the absorbance was measured at 520 nm with a microplate reader (Dynatech Model MR 700). All tests were triplicated. Fifty percent inhibitory dosage (ED $_{50}$ ) was defined as the dosage which reduced the absorbance of untreated wells, the control in the SRB assay, by 50%.

It has been generally acknowledged that plant extracts having cytotoxic effect at <40  $\mu$ g/ml (ED<sub>50</sub>) may be useful for developing anti-tumor agents. Therefore, the cytotoxic responses were classified as previously described: very strong response, ++++, ED<sub>50</sub> 10  $\mu$ g/ml; strong response, ++++, ED<sub>50</sub> 11-40  $\mu$ g/ml; moderate response, ++, ED<sub>50</sub> 41-100  $\mu$ g/ml; weak response, +, ED<sub>50</sub> 100-200  $\mu$ g/ml; and little or no response, -, ED<sub>50</sub> 200  $\mu$ g/ml.<sup>6)</sup>

#### Results and Discussion

The cytotoxic effects of test materials against five human solid tumor cell lines are given in Table 2. Potent cytotoxic activity (ED<sub>50</sub>, <40 μg/ml) against model tumor cell lines were produced from the extracts of *Rhus chinensis* gall (Galla rhois), *Betula platyphylla* var. *japonica* bark, *Inula helenium* root, *Cinnamomum cassia* bark, *Cinnamomum sieboldii* root bark, *Lysimachia davurica* whole plant, and *Evodia rutaecarpa* fruit. The extracts from the *Evodia* fruit in particular revealed very strong cytotoxic activities (ED<sub>50</sub>, <10 μg/ml) against all model tumor cell lines. Moderate

activities (ED<sub>50</sub>, 41-100 μg/ml) against all model tumor cell lines were obtained in the extracts of *Acorus gramineus* rhizome, *Corydalis turtschaninovii* tuber, *Juniperus rigida* fruit, *Agastache rugosa* whole plant, *Eugenia caryophyllata* flower bud, *Paeonia suffruticosa* root bark, *Zanthoxylum piperitum* fruit, and *Santalum album* lignin. The other plant materials exhibited weak or no cytotoxic effects.

It has been well acknowledged that many of the plant extracts and phytochemicals are potential alternatives to synthetic anti-cancer agents. Barclay and Perdue Barclay and Perdue Barclay and Perdue Suggested that the most promising botanical anti-tumor agents are in the families Annonaceae, Apocynaceae, Celestraceae, Cephalotaxaceae, Euphorbiaceae, Liliaece, Menispermaceae, Podocarpaceae, Rutaceae, Simanubaceae, Taxaceae, and Thymelaeaceae. In our study, potent cytotoxic activity was observed in plant species of the families Anacardiaceae, Betulaceae, Compositae, Lauraceae, Primulaceae, and Rutaceae.

The strong cytotoxic activities of the plants described confirm the superiority and usefulness of the plants as an anti-tumor agent, although it has been reported that methanol extract of Galla rhois has potent cytotoxic activity against the cancer cell lines of L1210, P388, and SNU-1. Additionally, some of the natural product-derived materials are found to be effective against cancer cells resistant to current chemotherapeutic agents. On the basis of our results and the earlier findings, some plants described may be useful for developing new types of anti-tumor agents.

<sup>&</sup>lt;sup>b</sup>Chaenomeles sinensis, (dry weight of methanol extract/fresh weight of C. sinensis fruit)×100; and the other plants, (dry weight of methanol extract/dry weight of test plant)×100.

<sup>&#</sup>x27;Powder was used.

Table 2. Cytotoxic activity of medicinal plant extracts against human tumor cell lines using SRB assay.

Plant species <sup>a</sup>	Tumor cell line							
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15			
R. chinensis	++++ <sup>h</sup>	++++	+++	++++	+++			
A. calamus angustatus	+	+	+	+	+			
A. gramineus	++	++	++	++	++			
B. platyphylla japonica	+++	+++	+++	+++	+++			
I. helenium	+++	+++	++++	+++	++++			
E. ulmoides	+	-	+	+	+			
G. glabra	++	+	+	++	++			
C. turtschaninovii	++	++	++	++	++			
H. odorata	+	+	+	+	+			
A. asphodeloides	+	+	+	+	+			
J. rigida	++	++	++	++	++			
A. rugosa	++	++	++	++	++			
C. cassia	+++	+++	+++	+++	+++			
C. sieboldii	+++	+++	+++	+++	+++			
I. verum	-	-	+	+	+			
S. acutum	+	+	+	+	+			
E. caryophyllata	++	++	++	++	++			
P. suffruticosa	++	++	++	++	++			
P. nigrum	+	+	+	+	+			
P. tenuifolia	+	-	+	-	+			
R. coreanum	+	+	+	+	++			
L. davurica	+++	+++	+++	++++	+++			
E. rutaecarpa	++++	++++	++++	++++	++++			
Z. piperitum	++	++	++	++	++			
Z. schinifolium	+	+	+	+	+			
S. album	++	++	++	++	++			
A. agallocha	++	_	+	+	++			
C. officinale	+	+	+	++	++			
N. chinensis	++	+	++	+	+			
C. longa	+	+	+	++	++			

<sup>&</sup>lt;sup>a</sup>Plants showing cytotoxic activity (ED<sub>50</sub> <200 μg/ml) are presented.

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#### References

- 1. Stark, G. R. and Calvert, H. (1986) Drug resistance. *Cancer Surveys*, **5**.
- 2. van der Bliek, A. M. and Borst, P. (1989) Multidrug resistance. Adv. Cancer Res. 52, 165-204.
- 3. Colegate, S. M. and Molyneux, J. J. (1993) In *Bioactive Natural Products: Detection, Isolation and Structure Determination*, CRC Press, Boca Raton.
- Malpas, J. S. (1991) Chemotheraphy. In *Introduction to the Cellular and Molecular Biology of Cancer*, Franks, L. M. and Teich, N. M. (2nd eds.) pp. 451-469, Oxford University Press, Oxford.
- Skehan, P., Storeng, R., Scudiero, D. A., Monks, A., McMahon, J. B., Visca, D. T., Warren, J. T., Bokesch, H., Kenney, S. and Boyd, M. R. (1990) New colorimetric cytotoxicity assay for anticancer drug screening. *J. Natl. Cancer Inst.* 82, 1107-1112.
- Lee, H. S., Lee, J. O., Lee, H. K., Oh, J. H. and Ahn, Y.
   J. (1998) Cytotoxic activity of leguminous seed extracts

- against human tumor cell lines. Agric. Chem. Biotechnol. 41, 246-250.
- Barclay, A. S. and Perdue, R. E. Jr. (1976) Distribution of anticancer activity in higher plants. *Cancer Treat. Rep.* 50, 1081-1113.
- 8. Cassady, J. M. and Douros, J. D. (1980) In Anticancer Agents Based on Natural Product Models, Academic Press, NY.
- Cragg, G. M., Boyd, M. R., Cardellina, J. H., Grever, M. R., Schepetz, S. A., Snader, K. M. and Suffness, M. (1993) Role of plants in the National Cancer Institute drug delivery and development program. In *Human Medicinal Agents from Plants*, Kinghorn, A. D. and Balandrin, M. F. (eds.) pp. 80-95, ACS Symp. Ser. No. 534, Am. Chem. Soc. Washington, DC.
- Perchellet, J. P., Gali, H. U., Perchellet, E. M., Laks, P. E., Botari, V., Hemingway, K. W. and Scalbert, A. (1994) In Food Phytochemicals for Cancer Prevention I, Fruits and Vegetables, Huang, M. T., Osawa, T. C., Ho, T. and Rosen, R. T. (eds.) pp. 303-327, ACS Symp. Ser. No. 546, Am. Chem. Soc. Washington, DC.
- Huang, Y. G., Kang, J. K., Liu, R. S., Oh, K. W., Nam, C. J. and Kim, H. S. (1997) Cytotoxic activities of various fractions extracted from some pharmaceutical insect relatives. *Arch. Pharm. Res.* 20, 110-114.

 $<sup>^{</sup>b}$ ++++,  $ED_{50}$  10  $\mu$ g/ml; +++,  $ED_{50}$  11-40  $\mu$ g/ml; ++,  $ED_{50}$  41-100  $\mu$ g/ml; +,  $ED_{50}$  101-200  $\mu$ g/ml; and -,  $ED_{50}$  200  $\mu$ g/ml.

#### pp. 1~6

The Quality Changes of Chungujang Prepared by Bacillus sp. CS-17 during Fermentation Time Dong-Hwa Son<sup>1</sup>, Oh-Jin Kwon<sup>2</sup>, Won-Dae Ji, Ung-Kyu Choi, O-Jun Kwon, Eun-Joung Lee, Young-Je Cho, Won-Seup Cha and Yung-Gun Chung\*(Dept. of Food Science and Technology, Yeungnam University, Kyongsan 712-749; <sup>1</sup>Dept. of Food Preparation, Taegu Polytechnic College, Taegu 706-020; <sup>2</sup>Dept. of Hotel Baking Technology, Taekyeung Broadcasting C&D College, Kyongsan 712-850; <sup>3</sup>Dept. of Food Engineering, Sangju National University, Sangju 742-711, Korea)

The quality changes of *chunggugjang* produced by *Bacillus* sp. CS-17 was investigated with fermentation time. The pH was gradually alkalized. L-value became low while b-value became high. Both strength and hardness extraordinarily decreased during fermentation. Total content of free amino acid was 8274.7~9301.4 mg% and phenylalanine, lysine, leucine and tyrosine were the most abundant components among the amino acids. The ratio of essential amino acid was 66.6~71.8%. As a result of sensory test, it was found that the *chunggugjang* fermented by *Bacillus* sp. CS-17 was suitable enough to be produced for commercial purpose.

Key words: chunggugjang, Bacillus sp. CS-17, free amino acid, commercial purpose

### pp. 7~11

### Fermentation Property of Chinese Cabbage Kimchi by Fermentation Temperature and Salt Concentration

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The effects of fermentation temperature(0~15°C) and salt concentration(1.5~4.0%) on the fermentation property of Chinese cabbage Kimchi were analyzed by response surface methodology. The pH decreased and acidity increased with increasing fermentation time. The reduction and increment velocities of pH and acidity were increased by increasing fermentation temperature and decreasing salt concentration. The optimum pH 4.2 was reached within 14~24 days at 5~15°C, while pHs of 24 days at 0~5°C were still lower value than 4.2. The effect of salt concentration more affected terminal fermentation period than initial fermentation period. The maximum edible acidity, 0.75%, was reached within 8 days at 15°C, while acidities of 24 days at 0°C were 0.35~0.43%. The effects of salt concentration at 0°C was higher than those at 15°C. The fermentation time, fermentation temperature and salt concentration were the first, second and third affecting factors on the pH and acidity of Kimchi. Based on the coefficients of determination, pH and acidity were highly fitted to the experimental data(r²>0.9276). For the suitable acidity range, 0.40~0.75%, the edible period of Kimchi at 15°C, 10°C and 5°C were 4 days, 10 days and 18 days at the 2.75% of salt concentration, respectively. The edible period increased from 14 days to 19 days with increased salt concentration from 1.50% to 4.00% at 5°C of fermentation temperature.

Key words: Chinese cabbage Kimchi, fermentation property, RSM, edible period

### pp. 12~17

#### Production of Ascorbic acid-2-Glucoside from Ascorbic acid with Rice a-Glucosidase

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For the enzymatic production of 2-O- $\alpha$ -D-glucopyranosyl-L-ascorbic acid (AA-2G) from ascorbic acid, rice seed was used as the source of  $\alpha$ -glucosidase having transglucosylation activity. Among six rice varieties, cultivated in Korea,  $\alpha$ -glucosidase activity of *Oryza savita* L. cv. Ilpumbyeo was the highest with 125.03 unit/ml and it had maximum specific activity with 8.52 unit/mg protein when rice seeds were grown for 3 days after germination. For the production of AA-2G using crude extract of *O. savita* L. cv. Ilpumbyeo, maltose was most effective glucose donor. The optimum concentration of maltose and ascorbic acid were 125 mM and 175 mM, respectively. The optimum

concentration of  $\alpha$ -glucosidase was 100 unit. The most effective buffer was 100 mM sodium citrate. The optimum pH and temperature were 5.0 and 60°C, respectively. Under the optimum condition, 108.43  $\mu$ M/unit of AA-2G was produced from ascorbic acid after 35 minutes of reaction, which corresponds to 6.2% of conversion ratio based on the amount of ascorbic acid used.

Key words: ascorbic acid, ascorbic acid-2-glucoside,  $\alpha$ -glucosidase, rice seed

# pp. 18-23 The Fine Structure of Amylopectin and Physicochemical Properties of Starch Granules from Endosperm Varieties in Glutinous Rice

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Fourteen varieties of glutinous rices were examined on amylopectin fine structure and physicochemical properties of starch granules. The amylopectin chain length distribution and short chain/long chain ratio were investigated by enzymatic treatments followed by high-performance size-exclusion chromatographic separation. Chain length distribution profiles of the isoamylase-debranched amylopectins showed distinct patterns according to varieties. Beongok showed the highest short chain/long chain ratio, while TP2579A1 showed the lowest one. Sharebyeo-152-1-B showed the highest hydrolysis rate to 15% H<sub>2</sub>SO<sub>4</sub>, while Sandong 47 showed the lowest one. Fourteen varieties of rice starch granules showed A-type pattern on X-ray diffractograms. Nongelitinized starch granules from Keochang 1 and Beongok had almost 100% hydrolysed by glucoamylase for 3 hrs at 370°C.

Key words: glutinous rice, chain length distribution, X-ray diffractogram, glucoamylase

### pp. 24-28 Clarification of Apple Vinegar by Ultrafiltration and Flux Charcateristics

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This study was conducted to evaluate the effect of ultrafiltration (UF) process variables on permeate flux and membrane resistance and to clarify apple vinegar for quality improvement. Apple vinegar was clarified in a laboratory ultrafiltration system with hollow fiber membrane made of polysulfone and MWCO 30,000 and 10,000. The permeate flux increased with the increase of flow rate and the optimum pressure was 1.5 kgf/cm² in this system. The turbidity of clarified apple vinegar treated UF largely decreased. pH and acidity of treated samples shpwed the same level as those of untreated apple vinegar. The permeate flux continuously declined while the fouling material accumulated on the membrane as the operation time increased. Resistance decreased with lower pressure, which could be explained by expansion of pore size at lower pressure and minor compaction of the polarized layer at lower pressure.

Key words: apple vinegar, ultrafiltration, clarification, membrane resistance

# pp. 29~33 Effect of Glutaminase on the Production of L-Glutamic Acid in Soybean Fermentation Products during Aging

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This study was carried out to investigate the effect of glutaminase added to Doenjang, Kochujang and Kanjang in manufacturing. The consequential changes of L-glutamic acid and amino nitrogen

contents were periodically analysed during aging. L-Glutamic acid contents in Kochujang, Doenjang and Kanjang aged for 45 days increased to 671.8%, 298.1% and 193.4% with glutaminase and also increased to 363.1%, 159.2% and 35.7% as compared with those without glutaminase. The 0.01% addition of glutaminase to Kochujang made L-glutamic acid content increased more than 3 times. The increase ratio of amino nitrogen was 216%, 120.8% and 84.5% in Kochujang, Kanjang and Doenjang with glutaminase which aged for 45 days, respectively. The effect of glutaminase added was the greatest in Kochujang. It increased to 35.7%, 8.4% and 40.3% as compared with those without glutaminase. The results of sensory evaluation showed that the products were favorably affected in taste, flavor and acceptability by glutaminase added.

Key words: glutaminase, L-glutamic acd, amino nitrogen, soybean sauce, paste

# pp. 34-38 Application Effects of Chitosan Fertilizer on the Growth of Cabbage and GABA Contents in the Cabbage

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To investigate the effects of chitosan on growth and quality improvement of vegetables, we utilized cabbage as a model plant system and SL-chitosan as a chitosan fertilizer. The chitosan fertilizer treatment increased the leaf lengths of cabbage seedlings compared with those of control groups. In addition, the content of  $\gamma$ -aminobutyric acid (GABA) in the fertilizer-treated cabbage seedlings was higher than that in the control group. Peripheral lengths and head weights of cabbages along with their GABA contents were also measured during the growth of cabbages in field. The fertilizer treatment, without changing the physico-chemical properties of main field soil after the cultivation of cabbage, significantly increased the peripheral length, average weight and GABA content compared with control treatment. These results may suggest that the quality and quantity of cabbage can be improved by chitosan treatments.

Key words: Chitosan, cabbage, GABA, growth

### pp. 39~45 Studies on the Stability of Multivitamin Solutions

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The stability of vitamin A,  $B_1$ ,  $B_2$ ,  $B_6$ , C in aqueous multivitamin solutions was carried out by means of estimation of reaction velocity and the results are described in this paper. The stability of vitamin A,  $B_1$  and C due to thermal degradation method in aqueous multivitamin solutions was evaluated at 40, 50, 60 and 70°C up to 40 days. The shelf-lives of vitamin A,  $B_1$  and C in this preparation, calculated using the Arrhenius equation, were 1493, 449 and 639 days at 25°C respectively. Examination was made on the effect of initial concentration of vitamin  $B_2$ ( $C_0$ ) on light fading of vitamin  $B_2$  in aqueous multivitamin solutions and it was found that the fading progressed according to the following formula:

$$-\frac{dc}{dt} = K_c \frac{C}{C_o}$$

where Kc is apparent light-fading rate constant relate to  $C_0$ . Photodecomposition of vitamin  $B_6$  in aqueous multivitamin solutions was apparently first order kinetics and was stable in polyethylene > brown color > glass container to sunlight. Photodecomposition of vitamin  $B_6$  in four seasons also investigated.

Key words: multivitamin solutions, stability, photodecomposition, heterogenous solution system

### pp. 46~51 Protective Enzymes of Paraquat-Resistant Conyza bonariensis

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The resistance of *Cornyza bonariensis* to herbicide paraquat was investigated by evaluating the activities of three enzymes concerning in scavenging paraquat-generated toxic oxygen species such as superoxide radical and hydrogen peroxide in resistant and susceptible biotypes. *Conyza bonariensis* inhabited in cultivated area was more tolerant to paraquat than that of uncultivated area. This is the first report that a biotype of *Cornyza bonariensis* has appeared in an area with repeated paraquat treatments of Korea. Superoxide dismutase activity of resistant biotype was 20% higher as 150 than that of susceptible biotype. Ascorbate peroxidase activity of resistant biotype was 44% higher than that of susceptible biotype. Glutathione reductase activity of resistant biotype was 64% higher than that of susceptible biotype. It can be concluded from above results that the resistance of *Conyza bonariensis* to paraquat depends partially on the toxic oxygen species-scavenging efficiency of protective multienzymatic system which is composed of three enzymes, superoxide dismutase, ascorbate peroxidase, and glutathione reductase.

Key words: Conyza bonariensis, paraquat, resistance, superoxide dismutase

### pp. 52~56

# N-phenyl Substitutent Effect on the Herbicidal Activity of 2-(4-(6-chloro-2-benzoxazolyloxy)phenoxy)-N-phenylpropionamide Derivatives against Rice Plant with Pre- and Post-emergence

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The influence of the 2-(4-(6-chloro-2-benzoxazolyloxy)phenoxy)-N-phenyl- propionamide derivatives on the herbicide activities against rice plant with pre-emer- gence and post-emergence in down land were examined and the structure activity relationship (SAR) were analyzed by Free-Wilson and Hansch method. In pre-emer- gence, the SAR approach is shown that the optimal,  $(p)_{opt} = 0.91$ , hydrophobicity with electron donating effect of the ortho substituted mono substituents and 2,3,4-substituted three substituents were found to be contribute the herbicidal activity. Whereas, in post-emergence, the optimal,  $(p)_{opt} = 0.50$ , hydrophobicity with electron withdrawing effect of meta substituted mono substituents and 2,3-substituted two substituents were found to be contribute the herbicide activity. The herbicide activities with post-emergence more increase than that of pre-emergence. It is assumed from the SAR equations that the 2-methyl-3-methoxy-4-cyano group substituent is selected as the most lowest herbicide activity against rice plant with post-emergence in green house. The hydroly-sis reaction was proceeded through nucleophilic addition-elimination  $(Ad._{Nu-E})$  with the orbital control between LUMO of substrate and HOMO of water molecule. And molecul-ar electrostatic potential (MEP) of none (H) substituent was discussed.

Key words: 2-(4-(6-chloro-2-benzoxazolyloxy)phenoxy)-N-phenylpropionamides, Rice plant, Herbicidal activity, QSAR. Frontier orbital interaction, Molecular electrostatic potential (MEP).

### pp. 57~62 Characteristics and purification of proteoglycan from Phellinus igniarius

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The proteoglycan, intracellular and extracellular, extracted from the liquid culture of *Phellinus igniarius* were purified and characterized. The mycelial productivity was proved to be better in shaking culture compared to standing culture. The productivity of intracellular proteoglycan of *Phellinus igniarius* appeared to be similar in two culturing methods. The standing culture of *Phellinus igniarius* produced 6 times as much extracellular proteoglycan compared to shaking culture. The proteoglycan were purified to a single peak by ion exchange chromatography(DEAE-cellulose) followed by gel filtration(Sepharose 2B). PIEPDG contained 79.0% total sugar and 7.2% protein. PIEPAG contained 56.7% total sugar and 40.8% protein. PIIPDG contained 64.8% total sugar and 17.4% protein. PIIPAG contained 56.9% total sugar and 41.5% protein. The molecular weights of all the fractions were estimated to be above 100,000, from 134KDa of PIEPDG to 560 KDa of PIEPAG. The results of sugar analysis by HPLC showed that PIEPDG contains glucose only. The sugar part of PIIPDG and PIIPAG were consisted of glucose and inositol. The PIEPAG contained three kinds of monosaccharides, glucose, fructose and inositol.

Key words: Phellinus igniarius, intracellular proteoglycan, extracellular proteoglycan

# pp. 63~66 Chemical Characteristics of the Leaves and the Seeds of Korean Dendropanax (Dendropanax morbifera Lev.)

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Proximate analyses of free sugars, free amino acids, fatty acids, total vitamin C and the soluble tannin content of the leaf and seed of *Dendropanax morbifera* Lev. were determined. Moisture content was 70.2% in leaf and 72.6% in seed, and crude protein contents were 1.2% in leaf and 6.2% in seed, and ash contents were 1.7% in leaf and 0.9% in seed. Total vitamin C and soluble tannin in leaf were 56.9% and 10.7% which were five times and eleven times higher in seed, respectively. Free sugar content in leaf was higher than that in seed, with the major free sugars consisting of sucrose, glucose and fructose. Turanose and xylose were not detected in leaf, but were detected in seed in small amounts. Unsaturated fatty acids were predominant in both of leaf and seed, but major fatty acids were quite different from each other. Low levels of free amino acids were found to consist mainly of arginine, aspartic acid and glutamic acid. The highest content of mineral elements in leaf and seed were calcium and potassium, respectively.

Key words: Dendropanax morbifera Lev., Korean dendropanax, chemical characteristics

### pp. 67~71 Termiticidal Activities of Chamaecyparis obtusa Endl. Heartwood.

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Termiticidal activities of *Chamaecyparis obtusa* were quantitatively evaluated, and the activity differences between supporting materials such as woodmeal and filter paper or between species were defined based on the termiticidal activity value(TAV). It was found that TAV was high in the following order; *C. obtusa* > *Litsea coreana* > *C. obtusa var. formosensis* > *Ternstroemia gymnanthera*. In particular, termiticidal activity of *C. obtusa* heartwood was stronger by 5 to 9 times than that of other three species. Median survival dosage(MSD) of *C. obtusa* was 108.8 mg. In case of woodmeal or filter paper tests with *C. obtusa*, termiticidal activities were inversely proportional to logarithms values of an added sample weight and median survival time(MST). The difference of termiticidal activities between woodmeal and filter paper in the methanol extracts was small, but that in the neutral fraction was enormous as 3.21 times. However, termiticidal activity of neutral fraction was corresponded to 17 to 53% of original woodmeal, and 47 to 83% of termiticidal activity was considered as a loss in test process.

Key words: termite, Chamaecyparis obtusa, termiticidal activity value, median survival dosage, supporting material

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### Isolation of Anticonvulsant Compounds from the Fruits of Schizandra chinensis Ball.

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The repeated silica gel colum chromatographies of EtOAc fraction, showing anticonvulsant activity, obtained from MeOH extracts of *Schizandra chinensis* B. fruits led to isolation of a sesquiterpenoid, four lignans and a sterol glycoside. Their chemical structures were determined to be chamigrenal, gomisin A, gomisin H, gomisin N, schizandrin and daucosterol. Among them, schizandrin and daucosterol inhibited GABA degrative enzymes, succinic semialdehyde dehydrogenase and succinic semialdehyde reductase, respectively. It is postulated that the schizandrin and daucosterol are able to elevate the neurotransmitter GABA levels in central nervous system by inhibitory action on GABA degrative enzymes and act as anticonvulsant drugs.

Key words: Schizandra chinensis, anticonvulsant, succinic semialdehyde dehydrogenase, succinic semialdehyde reductase, lignan, chamigrenal, daucosterol

### pp. 78~80

Hyperin, Antioxidant Compounds Isolated from the Branch of Uncaria rhynchophylla Miq. Jae-Taek Han, Seoung-Jun Oh, Hae-Yeong Kim, Young-Doo Park and Nam-In Baek\*(Kyung Hee University, Department of Life Sciences & Institute of Life Sciences, Suwon 449-701, Korea)

Key words: Uncaria rhynchophylla Miq., antioxidation, DPPH radical scavenging activity, hyperin, ursolic acid