Korean J. Medicinal Crop Sci. 10(4) : 269-272(2002)

In vitro Antioxidant Activity and Anticancer Effects of The Extracts from *Eleutherococcus senticosus* Max

Myong Jo Kim*, Na Young Kim**, Won Hee Kang*, Won Cheol Choi*** and Chang Yeon Yu*†

* *Division of Applied Plant Science, Kangwon National University, Chunchon, 200-701, Korea*
** *Department of Food and Nutrition, Kyung Hee University, Seoul 130-701, Korea*
*** *Kwanghyewon Medical Cancer Center, Inchon, Korea*

ABSTRACT : **Ethanol and butanol fractionation of *E. senticosus* showed strong anti-oxidant activity, and methanol and water extracts also had high anti-oxidant activity. The antioxidant activities in ethanol and butanol fractionation were higher than or similar to those of α-tocopherol. The cytotoxic effect of root extract of *E. senticosus* was evaluated on seven different human cancer cell lines. The extracts of leaf and stem of *E. senticosus* also had strong antioxidant activity, but the antioxidant activity in root extract was higher than those in leaf and stem extracts. Methanol, hexane, and aqueous fraction layer had much higher inhibitory activities on lipid peroxidation in rat liver microsomes compared with α-tocopherol. The effect of root extract of *E. senticosus* was evaluated on six human cancer cell lines. The values of 50% growth inhibition (GI_{50}) for the extracts were mostly below 30μg/ml, and the extracts are considered as active inhibitory compounds on cancer cells.**

Key words : *Eleutherococcus senticosus*, antioxidant activity, anti-lipoperoxidant activity, cytotoxicity, anticancer activity

INTRODUCTION

Eleutherococcus senticosus (= *Acanthopanax senticosus* Harms) is a woody shrub found only in northeastern Asia including Korea, China, and Japan (Lee, 1979). These plants are distributed to the area of drainage basin of Woosuri in Russian, Hukryong river in China, Bukhaedo in Japan, and the mountains of Baekdu, Seolak, Dukyu, and Taegi in Korea (Han, 1983).

Eleutherococcus senticosus is an useful medicinal plant since its shoots and roots have been used as a remedy for robustness, anti-blood sugar diabets, neuralgia, antioxidant effect to several stress, palsy, sex- and learning-behaviour, anticancer, and so on (Brekhmam, 1960; Yook *et al.*, 1996; Hahn *et al.*, 1985). Wagner *et al.*(1982) analysed the characteristic lignan and phenylpropan derivatives by using TLC and HPLC of root extract from accessions of Korea, China, and Russia. The amount of eleutheroside of *Eleutherococcus senticosus* produced in Korea was much more than that in other countries. The biological activities of water, ethanol, and 50% ethanol extracts from the root bark of *Acanthopanax* were compared by the method of Kim et. al.(2000). Cell growth was inhibited by 94% in Hep3B (Liver cancer cell), and 90% in A549 (Lung cancer cell) by adding ethanol extracts of the root bark from *Eleutherococcus senticosus*. Also, cell growth and viability of Jurkat(T-cell) was more increased and activated by 275% by adding the 50% ethanol extracts from the root bark of *Eleutherococcus senticosus*. The biological activities of the extracts of *Eleutherococcus senticosus* were better than those of the extracts of *Eleutherococcus* spp. The objective of this

† Corresponding author (Phone) : 033-250-6411, E-mail : cyyu@kangwon.ac.kr
Received 10 September, 2002 / Accepted 30 October, 2002

study was to analyze the anti-oxidant, anti-peroxidative, and anti-cancer capacity of the extracts from *Eleutherococcus senticosus* on DPPH solution, rat liver microsomes, and human cancer cell lines, respectively.

MATERIALS AND METHODS

DPPH radical scavenging activity of *Eleutherococcus senticosus*

The 1,1-diphenyl-2-picryhydrazyl(DPPH) free radical scavenging activity was measured according to Xiong *et al.* One *ml* if 0.15mM DPPH in ethanol was added to sample solution containing 4*ml* of methanol and allowed to react for 30min at room temperature, then the optical density was measured at 517nm. For the blank, ethanol was used instead of DPPH solution, and for control, methanol was used instead of the sample represented the concentration of tested compound and the ordinate the average percent reduction of DPPH radical from three separate tests.

Anti-lipid peroxidation activity in rat liver microsomes

The inhibitory activity on lipid peroxidation in rat liver microsomes was measured by the levels of thiobarbituric acid (TBA, as index of lipid peroxidation) by the method (Kim *et al.*, 1996). Rat liver microsomes prepared according to the method of Ohkawa *et al.*,(1979) with some modification were suspended in 100mM Tris-HCl buffer (pH 7.4). Reaction was initiated by the addition of 100μM $FeSO_4 \cdot H_2O$. After 30min at 37℃ under reciprocal agitation, the reaction was stopped by the addition of 3M trichloroacetic acid in 2.5N HCl. Lipid peroxidation was assessed by measuring TBA reactive products. Percent inhibition was calculated as follows : (1-T-B)/(C-B))×100(%), in which T, C, and B are absorbance values at 530nm of the compound treatment, the control (peroxidation without compound) and the 0 time control (no peroxidation), respectively.

Cytotoxic sulforhodamine B (SRB) assay

Six human cancer cell lines were used and cultured with RPMI 1640 containing 10% fetal calf serum(FCS). For SRB assay, cells were cultured performed by previous method (Kim *et al.*, 1996). Cell suspension (3~40,000 cells/ml) was made in culture medium and inoculated to each well of 96-well microtiter plate. One day after plating, time zero control plate was made, compounds (1~4) were directly treated, and cells were incubated for further 48 hrs in a CO_2 incubator. Cells were fixed with 50μl of 50% trichloroacetic acid solution for 1h at 4℃ and plates were washed 5 times with tap water and air-dried. 100μl of SRB solution (0.4% in 1% acetic acid) was added and staining was done at room temperature for 30 min. Residual dye was washed out with 1% acetic acid and air-dried. To each well, Tris solution (10 mM, pH 10.5) was added. Optical density (OD) was measured with microtiter plate reader at 540nm. Growth inhibition was calculated according to the previous method. Briefly, OD of treated well was subtracted PD at time-zero(Tz) plate and divided by calculated value of untreated control. Growth inhibition of 50%(GI_{50}) was calculated by Probit method (Wu *et al.*, 1992).

RESULTS AND DISCUSSION

Antioxidant acitivities of the extracts from *E. senticosus*

A free radical, 1,1-diphenyl-2picrylhydrazyl (DPPH) was used to analyze the capacity of the extracts from root extracts of *E. senticosus* to restrain the oxidative process. Ethanol and butanol fractionation showed strong antioxidant activity, and methanol and water extracts of *E. senticosus* also have high antioxidant acititvies (Table 1). The antioxidant activity in ethanol and butanol fractionation was higher than or similar to those in α-tocopherol. The extract of leaf and stem in *E. senticosus* also showed strong antioxidant activity(data not showed), but the antioxidant activity in root extract was higher than that in leaf and stem extract. The effect of heaxane layer to restrain the oxidative process is eight times higher than the effect of α-tocopherol. Generally, the scavenging effects of root extracts of *E. senticosus* against DPPH free radical are almost same only in EtOAc layer, but the anti-oxidative effects of the extracts were greater than α-tocopherol. in all the other fractions.

Table 1. Scavenging effects of root extracts of *E. senticosus* against DPPH free radicals

Fraction	Antioxidant activity RC_{50} ($\mu g/ml$) DPPH removal activity
MeOH extract	20
Heaxane layer	〉100
EtOAc layer	10
n-BuOH layer	16
Aqueous layer	38
α-Tocopherol	12

Inhibitory activity on lipid peroxidation in rat liver microsomes

Ethanol fraction showed strong antilipid peroxidative activity and butanol fraction also had good inhibitory activity on lipid peroxidation in rat liver microsomes (Table 2). Methanol, hexane, and aqueous fraction layers resulted in much higher inhibitory activities on lipid peroxidation in rat liver microsomes, compared with α-tocopherol. More than 30-fold increase of inhibitory activity on lipid peroxidation in rat liver microsomes was detected from methanol, hexane, and aqueous fraction layers.

Table 2. Inhibitory activity on lipid peroxidation in rat liver microsomes of root extracts of *E. senticosus*

Fraction	Anti-lipid peroxidative activity RC_{50} ($\mu g/ml$)
MeOH extract	100
Heaxane layer	〉100
EtOAc layer	18
n-BuOH layer	56
Aqueous layer	〉100
α-Tocopherol	2.8

Cytotoxic effect of *E. senticosus* root extracts

The cytotoxic effect of root extract of *E. senticosus* was evaluated on six human cancer cell lines (Table 3). The values of 50% growth inhibition (GI_{50}) were mostly below 30μg/ml for crude extracts and the extracts can be considered as strong active compounds. The values of 50% growth inhibition in Hexane layer were much lower than those in other EtOAc layer, BuOH layer, aqueous layer and MeOH extraction. Some cell lines such as prostate(PC-3), Renal(ACHN), and Leukemia(MOLT-4F), showed relatively low GI_{50} values. Kim *et al.*,(2000) reported that the 50% ethanol extracts of *E. senticosus* inhibited the growth of Hep3B and A549 cells. In screening of immuno-modulating activities, the cell growth and viability of Jurkat(T-cell) were more increased and activated until 275% by adding ethanol extracts.

There were many reports that extract of *E. senticosus* was effective on gastric ulcer (Fujikawa *et al.*, 1996), immno-modulating (Li, 1991; Shen *et al.*, 1991; Xie, 1989), and serum-lipid (Shi *et al.*, 1990), and oxidation inhibition (Kim, 2000). Therefore, *E. senticosus* is one of the useful m[illegible]icinal plants for antioxidant medicine. immuno-stimulating activity on T

Table 3. Anticancer activity of root extracts of *E. senticosus*

Fraction	GI_{50}(μg/ml)						
	Prostate (PC-3)	Colon (HCT-15)	Renal (ACHN)	Colon (SW-620)	Lung (A549)	Leukemia (MOLT-4F)	GI_{50}
MeOH extraction	〉30	〉30	〉30	〉30	〉30	〉30	〉30
Hexane layer	20.51	〉30	20.22	24.79	〉30	14.29	23.30
EtoAC layer	〉30	〉30	〉30	〉30	〉30	〉30	〉30
BuOH layer	〉30	〉30	〉30	〉30	〉30	〉30	〉30
Aqueous layer	〉30	〉30	〉30	〉30	〉30	〉30	〉30
Adriamycin	0.13	0.16	0.13	〈 0.03	〈 0.03	0.05	0.09

cells and direct cytotoxicity activity of extract of *E. senticosus* might help inhibit tumor growth in a synergistic manner.

In the future research, it is necessary to investigate whether the effect of the extracts on anti-lipoperoxidant activity could partly explain the inhibition of lipid peroxidation, through decrease of reactive oxygen metabolites by inducing antioxidative genes, such as, glutathione peroxidase, ascorbate peroxidase, and superoxidase dismutase. Also there is possible involvement of mechanisms related to free radical scavenging effects by the extracts of *E. senticosus*. It also is necessary to measure the anti-peroxidative activity in addition to decrease in active oxygen formation by the extracts of *E. senticosus*. The mechanisms behind this anticarcinogenic effect from the extracts of *E. senticosus* are not known, although it has been shown that the extracts of *E. senticosus* are able to prevent the metabolic activation of carcinogens, inhibit tumor promotion and cell proliferation, and act as agents that induce antioxidant or oxidative-related enzymes. Further investigations will give us more clearer answers on the role of the extracts of *E. senticosus*.

LITERATURE CITED

Brekhman II (1960) A new medicinal plant of the family Araliceae the spiny *Eleutherococcus* Izv. Sibir. Otdel. Akad. Nauk. USSR 9 : 113-120

Fujikawa T, Yamaguchi A, Morita I, Takeda H, Nishibe S (1996) Protective effects of *Acanthopanax senticosus* Harms from Hokkai do and its components on gastriculcer in restrained cold water stressed rats. Biol Pharm Bull. 19(9) : 1227-1230.

Hahn DR, Kim CJ, and Kim JH (1985). A study on chemical constituents of *Acanthopanax koreanum* Nakai and its pharmaco-biological activities. Yakhakhoeji. 29(6) : 357-361

Jaccard A. Nouvelles recherches sur la distribution florale. Bulletin de la Societe Vaudise de Sciences Naturelles. 44 : 223-270

Kim CH (1997) Systematic of *Eleutherococcus* and related genera(*Araliaceae*). PH Dissertation. Chunbuk National Univ. pp. 1~82.

Kim HM, Han SB, Kim MS, Kang JS, Oh GT, Hong DH (1996). Efficient fixation procedure of human leukemia cells in sulforhodamine B cytotoxicity assay. J. Pharm. Tox. Method. 36 : 163-169.

Kim KY, Hun DY, Kim S, Park HK (1996) Studies on gathering Seeds of *Eleutherococcus Senticosus* Max. Korean J. Breed 28(2) : 120~121.

Kim SK, Kim YG, Lee MK, Han JS (2000) Comparison of biological activity according to extracting solvents of four *Acanthopanax* root bark. K. J. Med. Crop Sci. 8(1) : 21-28.

Kim WG, Kim JP, KimCJ, Lee KH, Yoo ID (1996b) Benzastatins A, B, C, and D : New free radical scavengers from Streptomyces nitrosporeus 30643. I. Taxonomy, fermentation, isolation, physico-chemical properties and biological activities. J. Antibiotics. 49 : 20-25.

Lee WT (1979) Distribution of *Acanthopanax* plants in Korea. Korean Journal of Pharmacology. 10 : 103-107.

Li XY (1991) Immunomodulating Chinese herbal medicines. Mem Inst Oswaldo Cruz. 86. Supplement. 2 : 159-164.

Nakai T (1927) *Araliaceae Flora* sylvatica Koreana. For. Exp. Sta. Govern., Seoul. 16 : 1~50.

Ohkawa, H., N. Ohishi and K. Ygi 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95 : 351-358.

Rohlf FJ (1989) Ntsys-PC Numerical taxonomy and multivalent analysis system, version 1 . 50. Exeter Pubi. New York.

Shen ML, Zhai SK, Chen HL, Luo YD, Tu GR, Ou DW (1991) Immunomo pharmacological effects of polysaccharides from *Acanthopanax senticosus* on experimental animals. Int. J. Immunopharmacol. 13(5) : 549-554.

Shi Z, Liu C, Li R (1990) Effect of a mixture of *Acanhopanax senticosus* and Elsholtzia splendens on serum-lipids in patients with hyper lipemia. Chung Hsi I Chieh Ho Tsa Chih. 10(3) : 155-156.

Wagner H, Heur YH, Obermeier A, Tittel G, Bladt S (1982) Die DC- and HPLC-analysis der Eleutherococcus Droge, Dc-and HPLC-analysis of Eleutherococcus. Planta Medica 44 : 193-198

Wu L, Smythe AM, Stinson SF, Mullendore LA, Monks DA, Paull KD, Koutsoukos AD, Rubinstein LV, Boyd M R, Shoemaker RH (1992) Multidrug-resistant phenotype of disease-oriented panels of human tumor cell lines used for anticancer drug screening. Cancer Res. 52 : 3029-3034.

Xie SS (1989) Immunoregulatory effect of polysaccharide of *Acanthopanax senticosus* (PAS). I. Immunological mechanism of PAS against cancer. Chung Hua Chung Liu Tsa Chih. 11(5) : 338-340.

Xiong Q, Kadota S, Tadata T, Namba T (1996) Antioxidative effects of phenylethanoids from *Cistanche deserticola*. Biol. Pharm. Bull. 19 : 1580-1585.

Yook C., Rho YS, Sed SH, Leem JY, Han SH (1996) Chemical components of *Acanthopanax divaricatus* and anticancer effect in leaves. Yakhakhoeji. 40(3) : 251-261.