

Anti-oxidant and Anti-inflammatory Effects of *Acanthopanax Cortex* Hot Aqueous Extract on Lipopolysaccharide(LPS) Simulated Macrophages

Na Young Jo and Jeong Du Roh*

Department of Acupuncture & Moxibustion Medicine, Je-Cheon Hospital of Traditional Korean Medicine, Semyung University



[Abstract]

Objectives : This study is to investigate the effects of *Acanthopanax Cortex* hot aqueous extract on nitric oxide(NO), prostaglandin E2(PGE₂) production and DPPH(1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity in macrophages.

Methods : *Acanthopanax Cortex*(200 g) was heated at 100 °C with distilled water(2 L) for 4hrs. The extract was filtered and concentrated to 100 ml using a rotary evaporator and was frozen at -80 °C, then was freeze-dried. The RAW 264.7 macrophages were subcultured. In order to evaluate cytotoxicity, MTT assay was performed. Experimental groups were divided into five(control, AC 25, 50, 100 and 200 µg/ml) and we measured cytotoxicity. The concentrations of NO were preprocessed by Griess assay. The RAW 264.7 macrophages was pretreated by 10 µg/ml LPS and experimental groups were divided into five and we measured NO production. The concentrations of PGE₂ were measured by enzyme immunoassay. The RAW 264.7 macrophages was pretreated by 10 µg/ml LPS. Experimental groups were divided into five and we measured PGE₂ production. Antioxidant activity was measured by the DPPH method. experimental groups were divided into four(AC 25, 50, 100 and 200 µg/ml) and we measured DPPH radical scavenging activity.

Results :

1. Viability of RAW 264.7 macrophages did not significantly decrease in 25, 50 and 100 µg/ml *Acanthopanax Cortex* hot aqueous extract compared to control group.
2. NO production in LPS-stimulated RAW 264.7 macrophages significantly inhibited in 100, 200 µg/ml *Acanthopanax Cortex* hot aqueous extract compared to control group.
3. PGE₂ production in LPS-stimulated RAW 264.7 macrophages significantly inhibited in 100, 200 µg/ml *Acanthopanax Cortex* hot aqueous extract compared to control group.
4. DPPH radical scavenging capability of *Acanthopanax Cortex* hot aqueous extract in RAW 264.7 macrophages had the high level in 100, 200 µg/ml.

Conclusion : According to the results, *Acanthopanax Cortex* hot aqueous extract has ability to suppress NO, PGE₂ production and improve DPPH free radical scavenging activity. So *Acanthopanax Cortex* hot aqueous extract may have an anti-inflammation effect and antioxidant activity.

Key words :

Acanthopanax Cortex;
 Anti-inflammation;
 Antioxidant activity;
 Hot aqueous extract;
 Korean medicine

Received : 2014. 02. 18.
 Revised : 2014. 02. 27.
 Accepted : 2014. 02. 27.
 On-line : 2014. 03. 20.

* Corresponding author : Department of Acupuncture & Moxibustion Medicine, Je-Cheon Hospital of Traditional Korean Medicine, Semyung University, 65, Semyung-ro, Jecheon-si, Chungcheongbuk-do, 390-711, Republic of Korea
 Tel : +82-43-649-1816 E-mail : wsrohmi@hanmail.net

This is an Open-Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

The Acupuncture is the Journal of Korean Acupuncture & Moxibustion Medicine Society. (<http://www.TheAcupuncture.or.kr>)
 Copyright © 2014 KAMMS. Korean Acupuncture & Moxibustion Medicine Society. All rights reserved.

I. Introduction

Acanthopanax Cortex(AC) is the bark of the roots and stem of acanthopanax tree. In Korean medicine, *Acanthopanax Cortex* has been widely used for treatment of orthopedic disease¹⁾. When the tissue have been damaged, inflammatory reaction is shown to combat the pathogen²⁾.The inflammatory response is a cause of pain in sprains, tendinitis, aponeurosis, gout and osteoarthritis³⁾.

Previous studies have been reported in the antioxidant⁴⁻⁶⁾, fat reduction^{7,8)}, antidiabetic⁹⁾, anticancer^{10,11)}, increasing cerebral blood flow¹²⁾, IL-8 product inhibition¹³⁾, blood making, immunodulatory¹⁴⁾ and alcoholysis¹⁵⁾ effect of *Acanthopanax Cortex*. Likewise, many studies have suggested that the *Acanthopanax Cortex* might be anti-inflammation effective. But *Acanthopanax Cortex* was not enough in researching of the anti-inflammation. Therefore, this study is to investigate the effects of *Acanthopanax Cortex* hot aqueous extract on Nitric Oxide(NO), Prostaglandin E₂(PGE₂) production and DPPH(1,1-diphenyl-2-picryl hydrazyl) radical scavenging activity in macrophages.

II. Experimental materials and methods

A. Materials

Acanthopanax Cortex used in the experiment were purchased from the Omniherb(Kyongbuk, Korea). Extracting method is as follows. *Acanthopanax Cortex* 200 g mixed with 2 L of distilled water, and boiled for 4 hours at 100 °C. The extract was filtered with filter paper, and centrifuged at 3,000 xg. And then secondary filtered with 0.03 mm filter paper (Nalgene, New York, USA). The filtrate was concentrated to 100 ml, and frozen at -80 °C. Freezing solution to be freeze-dried for 7 days through the freezing dryer system(Labconco, USA). 14 g *Acanthopanax*

Cortex extract was obtained. The yield was 7 %.

B. Cell culture

RAW 264.7 macrophages purchased in the ATCC (Manassas, USA) were used in this experiment. RAW 264.7 macrophages were placed in the Dulbecco's modified eagle's medium(DMEM) including 10% Fetal bovine serum(FBS) and were cultivated inside the incubator at 37 °C under 5 % CO₂.

C. Measuring cytotoxicity

In order to evaluate cytotoxicity, MTT assay was performed. RAW 264.7 macrophages were cultivated to concentration of 1×10^5 /well in a 96 well plate and were cultured for 16 hrs at 37 °C under 5 % CO₂. Experimental groups were divided into five(Control, AC 25, 50, 100 and 200 µg/ml). Stable cells were treated in the MTT reagent and absorbance was measured at 570 nm.

D. Measuring NO production

RAW 264.7 macrophages were cultivated to concentration of 1×10^5 /well in a 96 well plate and were cultured for 16 hrs at at 37 °C under 5 % CO₂. Experimental groups were divided into five(control, AC 25, 50, 100 and 200 µg/ml) and were treated with the 10 µg/ml Lipopolysaccharide(LPS) reagents. After mixed supernatant 100 µl and Griess reagent 100 µl, absorbance was measured at 540 nm. Griess reagent were prepared with 0.1 % naphthylethyleneamine dihydrochloride 50 µl and 1% sulfanilamide 50 µl dissolved in 5 % H₃PO₄.

E. Measuring PGE₂ production

PGE₂ was measured using the commercial competitive enzyme immunoassay kit(R & D systems,

Minneapolis, USA). Experimental groups were divided into five(control, AC 25, 50, 100 and 200 $\mu\text{g/ml}$) and were treated with the 10 $\mu\text{g/ml}$ LPS reagents. Treated RAW 264.7 macrophages were cultured for 18 hrs at at 37 °C under 5 % CO₂. Each culture fluid 100 ml loaded at goat anti-mouse coated 96 well plate, and then mixed primary antibody solution 50 μl and PGE₂ conjugate 50 μl . It was stabilized for overnight at 4 °C. Treated culture fluid mixed substrate solution 200 μl and waiting for 5~20 minutes, and then mix the stop solution 50 μl . Absorbance was measured at 450 nm.

F. Measuring the antioxidative effect

In order to investigate the antioxidative ability of *Acanthopanax Cortex*, DPPH free radical scavenging capability was measured. RAW 264.7 macrophages were cultivated to concentration of $1 \times 10^5/\text{well}$ in a 96 well plate and were cultured for 16 hrs at at 37 °C under 5 % CO₂. Experimental groups were divided into four(AC 25, 50, 100 and 200 $\mu\text{g/ml}$) and were diluted with MeOH(Methanol) and diluent 50 μl mixed with 0.15 mM DPPH(Sigma, USA) 80 μl in 96 well plate. Isolate the light at room temperature for 3 minutes. Absorbance was measured using the microplate reader(Tecan, Italy) at 520 nm. DPPH free radical scavenging capability was evaluated in the following way.

$$\text{DPPH free radical scavenging activity(\%)} = \frac{\text{Absorbance of Control} - \text{Experimental}}{\text{Absorbance of Control}} \times 100$$

G. Statistical analysis

With SPSS Window program(Ver. 10.0), experimental result were processed in terms of averages \pm standard deviations. Statistical significance was confirmed by using Student's *t*-test, the level of significance was $p < 0.05$.

III. Result

- Viability of RAW 264.7 macrophages did not significantly decrease in 25, 50 and 100 $\mu\text{g/ml}$ *Acanthopanax Cortex* hot aqueous extract compared to control group.
- NO production in LPS-stimulated RAW 264.7 macrophages significantly inhibited in 100, 200 $\mu\text{g/ml}$ *Acanthopanax Cortex* hot aqueous extract compared to control group.
- PGE₂ production in LPS-stimulated RAW 264.7 macrophages significantly inhibited in 100, 200 $\mu\text{g/ml}$ *Acanthopanax Cortex* hot aqueous extract compared to control group.
- DPPH radical scavenging capability of *Acanthopanax Cortex* hot aqueous extract in RAW 264.7 macrophages had the high level in 100, 200 $\mu\text{g/ml}$.

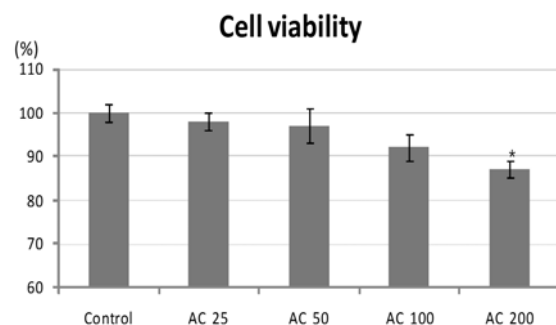


Fig. 1. This graph describes that concentrations of *Acanthopanax Cortex* hot aqueous extract make a difference in the viability of RAW 264.7 macrophage

Control : non-treated group.

AC 25 : 25 $\mu\text{g/ml}$ *Acanthopanax Cortex* hot aqueous extract treated group.

AC 50 : 50 $\mu\text{g/ml}$ *Acanthopanax Cortex* hot aqueous extract treated group.

AC 100 : 100 $\mu\text{g/ml}$ *Acanthopanax Cortex* hot aqueous extract treated group.

AC 200 : 200 $\mu\text{g/ml}$ *Acanthopanax Cortex* hot aqueous extract treated group.

Values are represented as mean \pm SD.

* : statistically significant difference from the normal group, as determined by the student's *t*-test as $p < 0.05$.

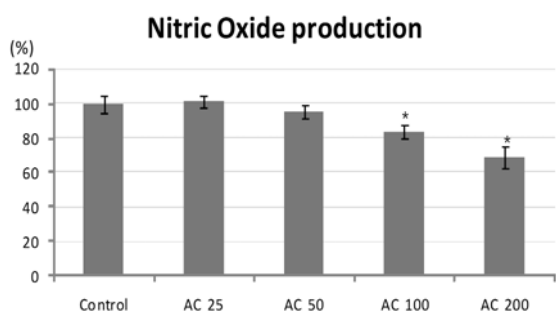


Fig. 2. This graph describes that concentrations of *Acanthopanax Cortex* hot aqueous extract make a difference in the NO production of RAW 264.7 macrophages

Control : 10 $\mu\text{g/ml}$ LPS treated group
 AC 25 : 10 $\mu\text{g/ml}$ LPS and 25 $\mu\text{g/ml}$ *Acanthopanax Cortex* hot aqueous extract treated group
 AC 50 : 10 $\mu\text{g/ml}$ LPS and 50 $\mu\text{g/ml}$ *Acanthopanax Cortex* hot aqueous extract treated group.
 AC 100 : 10 $\mu\text{g/ml}$ LPS and 100 $\mu\text{g/ml}$ *Acanthopanax Cortex* hot aqueous extract treated group.
 AC 200 : 10 $\mu\text{g/ml}$ LPS and 200 $\mu\text{g/ml}$ *Acanthopanax Cortex* hot aqueous extract treated group.
 Values are represented as mean \pm SD.
 * : statistically significant difference from the control group, as determined by the student's t -test as $p < 0.05$.

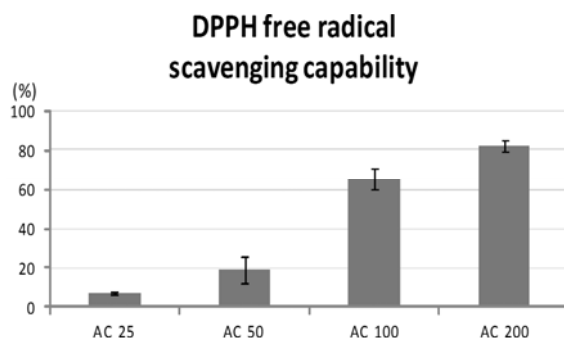


Fig. 4. This graph describes that concentrations of *Acanthopanax Cortex* hot aqueous extract make a difference in the DPPH free radical scavenging capability of RAW 264.7 macrophages

AC 25 : 25 $\mu\text{g/ml}$ *Acanthopanax Cortex* hot aqueous extract treated group
 AC 50 : 50 $\mu\text{g/ml}$ *Acanthopanax Cortex* hot aqueous extract treated group
 AC 100 : 100 $\mu\text{g/ml}$ *Acanthopanax Cortex* hot aqueous extract treated group
 AC 200 : 200 $\mu\text{g/ml}$ *Acanthopanax Cortex* hot aqueous extract treated group
 Values are represented as mean \pm SD.

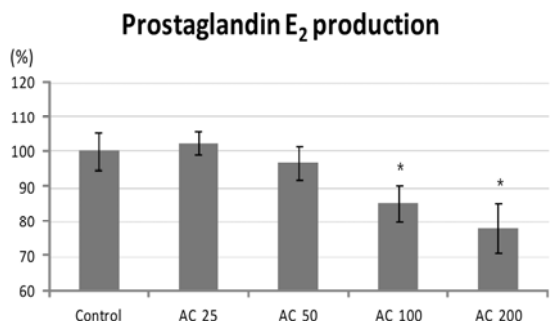


Fig. 3. This graph describes that concentrations of *Acanthopanax Cortex* hot aqueous extract make a difference in the PGE₂ production of RAW 264.7 macrophages

Control : 10 $\mu\text{g/ml}$ LPS treated group.
 AC 25 : 10 $\mu\text{g/ml}$ LPS and 25 $\mu\text{g/ml}$ *Acanthopanax Cortex* hot aqueous extract treated group.
 AC 50 : 10 $\mu\text{g/ml}$ LPS and 50 $\mu\text{g/ml}$ *Acanthopanax Cortex* hot aqueous extract treated group.
 AC 100 : 10 $\mu\text{g/ml}$ LPS and 100 $\mu\text{g/ml}$ *Acanthopanax Cortex* hot aqueous extract treated group.
 AC 200 : 10 $\mu\text{g/ml}$ LPS and 200 $\mu\text{g/ml}$ *Acanthopanax Cortex* hot aqueous extract treated group.
 Values are represented as mean \pm SD.
 * : statistically significant difference from the control group, as determined by the student's t -test as $p < 0.05$.

IV. Discussion

The effect of *Acanthopanax Cortex* stated in Simnongbonchokyung as follows. *Acanthopanax Cortex* can be used in diseases of the joints, skin, internal¹⁶⁾. In Seong's study¹⁷⁾, exhibit that *Acanthopanax Cortex* is effective in rheumatic pains, myalgia and neuralgia. In Lee's study¹⁸⁾, show that *Acanthopanax Cortex* is effective in anti-inflammation and antitumor.

In terms of oriental medicine, the inflammatory response is competing vital force and bad factor. Vital force means resisting power against disease. Bad factor means cause of disease¹⁹⁾. In terms of pathology, inflammatory response is process of restoring the damaged area. It is a variety of factors are involved. The reaction is active and, in this process, tissue damage is minimized²⁰⁾. Clinically, inflammatory response appear of redness, swelling, fever, pain and functional disability symptoms³⁾.

NO is freeradical. It is the transfer substances of cardiovascular, nervous system and the immune system. Its role is Intracellular homeostasis, transport of neurotransmitters, anticancer²¹⁾.

But Its excessive production is to cause another inflammatory and tissue damage. Persistent inflammatory response can result in myocarditis, chronic arthritis, glomerulonephritis, insulin dependent diabetes mellitus, and irritable bowel²²⁾. Therefore in recent various study, looking for ways to treat inflammation by effectively suppress the production of NO²³⁻²⁵⁾.

PGE₂ is also an important inflammatory mediator. It is synthesized by COX-2, and activated by macrophages. COX-2 not expressed in the resting state, but expression is induced when the inflammatory stimulation. Large excess of PGE₂ is lasting inflammatory reaction to cause rubefaction, edema, stiffness and pain^{26,27)}.

In the human body, oxidation promoting substance and oxidation inhibitors are in balance. When this balance was broken, the oxidative stress was generated and protein is decomposed, DNA synthesis is inhibited. Consequently, the harmful action of the cells and organs²⁸⁾. Reactive oxygen species(ROS) and reactive nitrogen species(RNS) are involved in this oxidative damage.

Free radical, such as superoxide(O₂⁻), nitric oxide(NO) and hydroxyl radical(HO⁻), and oxygen-derived species, such as singlet oxygen(¹O₂) and hydrogen peroxide(H₂O₂) are belong here²⁹⁾.

DPPH is free radical. It is purple, and has a light-absorbing at 520 nm. Generally it switch to DPPH-H and discolored by the proton-radical scavenger in various antioxidant mechanisms. Therefore, the results can be verified easily by the naked eye. In addition, it has the advantage of very stable at organic solvents such as alcohol³⁰⁾.

Result in cytotoxicity about *Acanthopanax Cortex* hot aqueous extract is that the control group 100 ± 2 %, *Acanthopanax Cortex* hot aqueous extract 25 µg/ml group 98 ± 2 %, 50 µg/ml group 97 ± 4 %, 100 µg/ml group 92 ± 3 % and 200 µg/ml group 87 ± 2 %. This result indicates that there is no significant toxicity up to 100 µg/ml.

Result of investigation NO production rate of the RAW 264.7 macrophages were treated *Acanthopanax Cortex* hot aqueous extract is that the control group 100.00 ± 5.00 %, *Acanthopanax Cortex* hot aqueous

extract 25 µg/ml group 101.52 ± 3.42 %, 50 µg/ml group 95.42 ± 4.06 %, 100 µg/ml group 83.62 ± 3.84 % and 200 µg/ml group 68.97 ± 6.36 %. The NO production rate was increased within an error range at *Acanthopanax Cortex* hot aqueous extract 25 µg/ml group. And NO Production rate was significantly decreased at *Acanthopanax Cortex* hot aqueous extract 100 µg/ml and 200 µg/ml group. In Yoon's study¹⁵⁾, shows that *Acanthopanax senticosus Cortex* hot aqueous extract inhibits the NO generation dose-dependently.

Result of investigation PGE₂ production rate of the RAW 264.7 macrophages were treated *Acanthopanax Cortex* hot aqueous extract is that the control group 100.00 ± 5.14 %, *Acanthopanax Cortex* hot aqueous extract 25 µg/ml group 102.35 ± 3.35 %, 50 µg/ml group 96.74 ± 4.72 %, 100 µg/ml group 85.09 ± 5.16 % and 200 µg/ml group 77.91 ± 7.24 %. The PGE₂ production rate was increased within an error range at *Acanthopanax Cortex* hot aqueous extract 25 µg/ml group. And PGE₂ production rate was significantly decreased at *Acanthopanax Cortex* hot aqueous extract 100 µg/ml and 200 µg/ml group.

Result of investigation scavenging ability of the RAW 264.7 macrophages were treated *Acanthopanax Cortex* hot aqueous extract is that the *Acanthopanax Cortex* hot aqueous extract 25 µg/ml group 6.99 ± 0.98 %, 50 µg/ml group 19.13 ± 6.84 %, 100 µg/ml group 65.50 ± 5.41 % and 200 µg/ml group 82.60 ± 2.92 %. *Acanthopanax Cortex* hot aqueous extract was 50 % or more of high erasure rate at 100 µg/ml and 200 µg/ml group. In Im's study⁴⁾ *Acanthopanax Cortex* Ethanol extract has antioxidant effect in 20 % and 40 % concentration.

Analyzing the results of this experiments, *Acanthopanax Cortex* hot aqueous extract has decreased NO and PGE₂ production rate and scavenging DPPH radical in 50 µg/ml, 100 µg/ml and 200 µg/ml concentration. Therefore, *Acanthopanax Cortex* hot aqueous extract is believed to have an anti-inflammatory and antioxidant effect. But in 25 µg/ml concentration, increased NO and PGE₂ production rate slightly. So it is possible that *Acanthopanax senticosus Cortex* hot aqueous increase inflammatory

reactions in low concentration. Therefore, various studies on *Acanthopanax senticosus* Cortex about concentration, extraction method and clinical study should be continued.

V. Conclusion

According to the results, *Acanthopanax Cortex* hot aqueous extract has ability to suppress NO, PGE2 production and improve DPPH free radical scavenging activity. So *Acanthopanax Cortex* hot aqueous extract may have an anti-inflammation effect and antioxidant activity.

VI. References

1. The Korea Society of Oriental Herbal medicine textbook compilation committee. Herbal medicine. Younglimsa. 2007 : 325-6.
2. Chapter of The Korea Society of Pathologists. Summarize Pathology. Jungmoongak. 2008 : 81
3. Song KY, Kim MK, Ji GK. Core Pathology. Korea medical books. 2013 : 45, 61-3, 97.
4. Im KR, Kim MJ, Jung TK, Yoon KS. Antioxidant activity of partially purified extracts isolated from *Acanthopanax sessiliflorum* Seeman. Korean Journal of Biotechnol & bioeng. 2008 ; 4 : 329-34.
5. Shim JS, Park KA, Cho JH, Jang YP, Oh MS. A Study on the Anti-oxidant Effects and TLC Analysis according to the Origins and Extract Methods of *Acanthopanax* species. Journal of Kyung Hee East-west Pharmacology. 2007 ; 35(1) : 7-14.0
6. Lim JA, Yun BW, Kang JI, Baek SH. Antioxidative Activity and Nitrite Scavenging Ability of Ethyl Acetate Extract from *Acanthopanax sessiliflorus*. Korean Journal of Physiology & Pathology. 2007 ; 21(4) : 955-60.
7. Park YS. Antioxidant Effects and Improvement of Lipid Metabolism of *Acanthopanax Cortex* Water Extract in Rats Fed High Fat Diet. Journal of East Asian Soc Dietary Life. 2010 ; 20(1) : 37-45.
8. Kim YK, Cho SI, Kim HW, Jeong HW, Jeon BG. Effects of *Acanthopanax Sessiliflorus* Seem Ethyl Alcohol Extract on the Body Weight and Serum Lipid Level in Mice. Korean Journal of Physiology & Pathology. 2006 ; 20(2) : 352-7.
9. Ham SH, Lim BL, Yu JH, Ka SO, Park BH. Fermentation Increases Antidiabetic Effects of *Acanthopanax*. Korean Journal of Physiology & Pathology. 2008 ; 22(2): 340-5.
10. Lee JH, Kim YC, Woo HJ. Study on Anti-Cancer of *Acanthopanax Sessiliflorus*. Journal of Korean Oriental Medicine.
11. Jeong HW, Rho YH, Lee GS, Kim CJ, Jeon BG. Experimental Effects of *Acanthopanax Cortex* Extract on the Immunity, Anti-Cancer and Obesity in Mice. Korean Journal of Physiology & Pathology. 2005 ; 19(2) : 389-97.
12. Jeong HW, Yoon YD, Kim YG, Jeon BG. Effects of *Acanthopanax Cortex* 50% Ethyle Alcohol Extracts on the Immunity, Anti-Cancer, Cerebral Blood Flow and Blood Pressure in Animal. Korean Journal of Physiology & Pathology. 2005 ; 19(5) : 1213-9.
13. Kim HC, Lee SI, An DK. Effect of *Acanthopanax Cortex* on the IL-8 Production in Human Monocyte as a Rheumatoid Arthritis Remedy. Journal of Herbology. 1995 ; 10(1) : 49-58.
14. Lim JK, Seo YB, Kim DH, Seol IC. The Experimental Studies on the Reinforcement Effects of *Acanthopanax Radicis Cortex* about Immunity Hematogenic Action. Journal of Herbology. 2000 ; 15(1) : 1-17.
15. Yoon TJ, Jo SY. Effect of *Acanthopanax senticosus* Extracts on Alcohol Degradation and Anti-Inflammatory Activity in Mice. Korea Journal of Food & Nutr. 2010 ; 23(4) : 542-8.
16. Dohongkyung, Sonseongyeon, Sonmailjib. Simnong-bonchogyeong. OJuchulpan. 1985 : 40-1.
17. Seong JH, Kyweon MH, Kim HK. Effects of Ogapi (*Acanthopanax senticosus*) Water Extract on the Functions of Murine Immunocytes for Antitumor Immunity. The journal of Wonkwang Medicine.

- 1995 ; 5(1) : 235–63.
18. Lee YC, Seo YB. The Theoretical Analysis on *Acanthopanax*. The Journal of Daejeon Oriental Medicine. 1999 ; 8(1) : 277–89.
 19. The Korea Society of Oriental Pathology textbook compilation committee. Oriental Pathology. Iljungsa. 2004 : 109–12.
 20. Korea Society of Pathologists. Pathology. Komoonsa. 1995 : 83–9.
 21. Lowenstein CJ, Snyder SH. Nitric oxide, a novel biologic messenger. Cell. 1992 ; 70(5) : 705–7.
 22. Nathan C, Xie QW. Nitric oxide synthases: Roles, tolls, and controls. Cell. 1994 ; 78(6) : 915–8.
 23. Jung HW, Park YK. Inhibitory effects of *Coptidis Rhizoma* on the LPS-induced production of nitric oxide and TNF- α in mouse macrophage cells. Journal of Herbology. 2006 ; 21(2) : 165–73.
 24. Choi YJ, Roh JD. Effects of *Angelicae Gigantis Radix* pharmacopuncture on Nitric Oxide and Prostaglandin E2 Production in Macrophage. Journal of Pharmacopuncture. 2011 ; 14(3) : 81–90.
 25. Lee DE, Lee JR, Kim YW et al. Inhibition of Lipopolysaccharide-Inducible Nitric Oxide Synthase, TNF- α , IL-1 β and COX-2 Expression by Flower and Whole Plant of *Lonicera japonica*. Korean Journal of Physiology & Pathology. 2005 ; 19(2) : 481–9.
 26. Rocca B, FitzGerald GA. Cyclooxygenases and prostaglandins: Shaping up the immune response. Int Immunopharmacol. 2002 ; 5(2) : 603–30.
 27. Kang GJ, Kang NJ, Han SC, Koo DH, Kang HK, Yoo BS, Yoo ES. The Chloroform Fraction of *Carpinus tschonoskii* Leaves Inhibits the Production of Inflammatory Mediators in HaCaT Keratinocytes and RAW264.7 Macrophages. Toxicol Res. 2012 ; 28(4) : 255–62.
 28. Imlay JA, Linn S. DNA damage and oxygen radical toxicity. Science. 1988 ; 240(3) : 1302–9.
 29. Bunn HF, Poyton RO. Oxygen sensing and molecular adaptation to hypoxia. Physiol Rev. 1996 ; 76(30) : 839–85.
 30. Blois MS. Antioxidant determination by the use of stable free radical. Nature. 1958 ; 18(1) : 1199–200.