

Immunomodulatory effects of edible Chinese black ant, *Polyrhachis vicina* Roger extract by using supercritical CO₂

Qingfeng Tang¹ & Yin Dai^{2*}

¹Institute of Insect Resources Development and Utilization, Department of Entomology, Anhui Agricultural University, Hefei, Anhui Province-230036, China

²Institute of Animal Husbandry & Veterinary Medicine, Anhui Academy of Agricultural Sciences, Hefei, Anhui Province, 230031, China

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The Chinese black ant, *Polyrhachis vicina* Roger has been used as a traditional Chinese medicine and nutritional ingredient. In order to take full advantage of *P. vicina* resources, the supercritical fluid CO₂ (SF-CO₂) *P. vicina* extraction on the immune systems of mice was carried out. The effects of SF-CO₂ *P. vicina* extraction on spleen index, thymus index and carbon expurgation experiments of mice, indicated that *P. vicina* extraction did not affect visceral index, but enhanced observably carbon expurgatory index, phagocytic index. *P. vicina* extraction could stimulate response of delayed hypersensitivity. The proliferation of ConA induced mitogenic response for spleen lymphocyte was also increased. The amount of hemolytic antibody in mice serum increased compared with those of the control group mice. The half of hemolysis values, acid phosphatase and alkaline phosphatase activity in serum of treated mice increased compared to the control group. Furthermore, serum NO content in all treatment groups was higher than that of the control group. Our findings suggest that SF-CO₂ *P. vicina* extraction has potential as a health food supplement.

Keywords: Food supplement, Immunological function, Insect utilization, Supercritical CO₂ fluid extraction, Traditional medicine

The Chinese black ant, *Polyrhachis vicina* Roger, belongs to Formicidae, Hymenoptera, Insecta in zootaxy, and is widely distributed in subtropical southeast China, India, Malaysia, Srilanka and Bangladesh^{1,2}. *P. vicina* have been widely used as a traditional Chinese medicine for the treatment of rheumatoid and osteoarthritis, inflammatory diseases and diabetes³. It has been used as a nutritional ingredient and processed into various tonics. Its products available in Chinese market include powder products, drinks such as wines and capsules^{4,5}. More than 30 ant-containing health products have been approved by the State Food and Drug Administration or State Health Ministry of China since 1996. Some ant products have been exported to Japan, South Korea, Thailand and other Southeast Asian countries.

Polyrhachis vicina has long been used as an important ingredient of health foods for centuries in other countries of Asia, Africa and Latin America. There were numerous literatures to report that *P. vicina* are rich in nutrients, such as protein, lipid, fatty acids, vitamins and superoxide dismutase,

especially essential amino acids, unsaturated fatty acids and minerals⁶⁻⁸.

Supercritical carbon dioxide (CO₂) extraction as a green technology is certainly alternative method to replace or to complement conventional industrial process, such as pressing and solvent extraction. Supercritical fluid extraction technique has many advantages over traditional methods, especially in preservation of thermo sensitive compounds using low temperatures, which results in reduced energy consumption⁹⁻¹¹. Supercritical fluids, like supercritical CO₂, are characterized by high mass transfer rates, liquid like density, and variable selectivity (achievable by variation of temperature and pressure). By variation of supercritical CO₂ selectivity extracts of desirable content and concentration of certain compounds can be produced¹¹. One of the important characteristics of this extraction technique is production of extracts without residues of extraction solvent — “solvent free extracts”. Supercritical CO₂ fluid extraction was used to extract the organic compounds from *P. vicina*. The content of organic compounds was higher in the extracted with supercritical CO₂ than that obtained by conventional water extraction¹¹. The analyses performed indicated that supercritical CO₂ fluid extraction of *P. vicina* was

*Correspondence:

Phone: +86 551 62160568; Fax: +86 551 62160568

E-Mail: 913678797@qq.com

effective for improving the activity of natural compounds¹.

Modulation of immune responses to alleviate diseases has been of interest for many years. The immune system is involved in the etiology as well as pathophysiologic mechanisms of various diseases and its function and efficiency is influenced by many exogenous and endogenous factors, such as food, pharmaceuticals, physical and psychological stress, resulting in either immunosuppression or immunostimulation¹². A number of insects used in Chinese traditional medicines and food systems for rejuvenation therapy have been demonstrated to modulate immune responses¹³. The anti-inflammatory, hepatoprotective, immunoregulatory, and analgesic activities displayed by the Chinese black ant are well documented¹⁴. However, supercritical CO₂ fluid extraction of *P. vicina* that was responsible for immunomodulatory effects were largely unknown. In this study, we report immunomodulatory activity of supercritical CO₂ fluid extraction of *P. vicina* in normal mice. We tried to explain the scientific basis for comprehensive utilization of the insect, improve the insect utilization efficiency, and evaluate the potential for future applications.

Materials and Methods

Insect material

The whole bodies of *Polyrhachis vicina* adults used were obtained from the laboratory stocks. The adults, which were removed from culture and held in clean giant bottle without food for 24 h, were burnt to death by boiling water and dried to constant weight following sun drying of ant. The dried ant samples were mechanically milled into powder with flat-hammer grinding mill and sifted through a 60-mesh screen. Lastly, the powder was stored in sealed aluminum foil bag at 4°C before analysis.

Supercritical CO₂ fluid extraction of the ant soluble organic compounds

Supercritical CO₂ fluid system (SF-CO₂, HL-1/32-50MPa, SFT-110XW, Supercritical Fluids Technology Inc., USA.) was used to extract the organic soluble compounds. The temperatures and pressures of the extraction and separation vessels were 50°C and 30 MPa, and 45°C and 8 MPa, respectively. The linear velocity of CO₂ was maintained at 8 kg/h.

Animals

Normal KunMing mice were purchased from the laboratory animal center of Anhui Institute of Medical Science and the animals were kept in air controlled rooms. The experiments were conducted on female KunMing mice weighing 20±2 g maintained at 25±2°C with normal mouse chow and water *ad libitum*. The animals were housed, five per cage and maintained on 12 h day and night cycle. The animals were divided into four groups comprising ten rats each: Group I, control animals (distilled water control); and Groups II-IV, animals fed with SF-CO₂ extraction @ 1.89 (low), 3.78 (moderate) and 7.56 g kg⁻¹ d⁻¹ (high-dosage), respectively.

Mice were orally administered a dose of 0.025 mL g⁻¹ body weight of larvae supercritical CO₂ fluid extraction once daily for four consecutive weeks. The control mice were given distilled water only by the same method. After four weeks oral administration, the immunomodulatory effect of SF-CO₂ *P. vicina* extraction was obtained from each experimental group by the method described previously.

Immune organ weight of mice

Mice were sacrificed 24 h after last administration, and then the body, thymus and spleen were collected and weighed. The weight index of thymus and spleen can be described by mg g⁻¹ body weight.

$$\text{Thymus Index} = \frac{\text{Weight of thymus (mg)}}{\text{Body weight (g)}}$$

$$\text{Spleen Index} = \frac{\text{Weight of spleen (mg)}}{\text{Body weight (g)}}$$

Carbon clearance test

To test the function of the macrophages in treated and control animals, experimental mouse was injected with Indian ink which was used as granular foreign body. The Indian ink was phagocytized and cleared by mononuclear macrophage after it went into circulation. After four consecutive weeks' oral administration, each mouse was injected with Indian ink 0.1 mL/10 g body wt. from mouse tail vein. In the second minute (t₁) and tenth minute (t₂) after injection, 20 µL blood was drawn from the orbital venous plexus of mice' eye sockets with suction tube in which was moistened with heparin solution and diluted in 2 mL 0.1% sodium carbonate solution. Mice were sacrificed and the body, thymus and spleen were collected and weighed.

The optical density (OD) values of blood solution were assayed with a 721 spectrophotometer at a wavelength of 600 nm, and the K (representing the capability of carbon granule clearance from mouse blood) and α (representing the phagocytosis activity of macrophage) values were calculated according to the formula.

$$K = \frac{\lg \text{OD at 2 min} - \lg \text{OD at 10 min}}{t_2 - t_1}$$

$$\alpha = \frac{\text{Body weight}}{\text{Spleen weight} + \text{Thymus weight}} \times \sqrt[3]{K}$$

Phagocytosis of peritoneal macrophages

Chicken blood was put into a sterile flask containing crystal ball and shaken to remove the fiber. The solution was rinsed thrice with saline before centrifugation at 2000 rpm for 10 min. The supernatant was abandoned and the chicken red blood cells (CRBC) were prepared.

Phagocytosis of mice was detected using the method described by Lin *et al.*¹⁵ with slight modification. Briefly, on the last administration day, 1 mL of 20% (v/v) CRBC was intraperitoneally injected into each mouse, and the mice were euthanized 30 min later. Two milliliters saline was injected into the abdominal cavity and 1 mL fluid was then collected to make a smear for each mouse. The smears were incubated at 37°C for 30 min in a wet box, fixed with acetone-methanol (1/1, v/v) solution, and then stained by 4% (v/v) Giemsa-phosphoric acid dye. The number of macrophage ingesting CRBC out of a total of at least 100 cells was calculated by direct visual enumeration using a light microscope. The phagocytic rate (PR) and phagocytic index (PI) were calculated using the following formula:

$$\text{PR} (\%) = \frac{\text{Number of macrophage ingesting CRBC}}{\text{Number of total macrophage}} \times 100\%$$

$$\text{PI} = \frac{\text{Number of total ingested CRBC}}{\text{Number of total macrophage}}$$

Serum hemolysin assay

Sheep blood was put into a sterile flask containing crystal ball and shaken to remove the fiber. The solution was rinsed three times with saline before centrifugation at 2000 rpm for 10 min. The supernatant was abandoned and the sheep red blood cells (SRBC) were prepared.

After 24 days oral administration, 0.2 mL of 20% (v/v) SRBC was intraperitoneally injected into each mouse. Five days later, blood was drawn from the orbital venous plexus of mice' eye sockets with

suction tube and serum was isolated from normal mice' blood as well. Serum samples were diluted with normal saline to 1/400. The value of absorbance at the 50% hemolytic dose (HC₅₀) was determined by colorimetric method with a 721 spectrophotometer at a wavelength of 540 nm.

$$\text{HC}_{50} = \frac{\text{Value of samples absorbance}}{\text{Value of absorbance at SRBC 50\% hemolytic dose}} \times \text{Dilution of serum}$$

Delayed-type hypersensitivity reaction to dinitrofluorobenzene

After 24 days oral administration, mice were sensitized to dinitrofluorobenzene (DNFB) by placing 100 μ L 1% (w/v) DNFB in acetone-castor oil on the shaved abdominal of recipients with the area of 3 \times 3 cm. Five days later, 20 μ L 1% (w/v) DNFB solution was used to challenge the right ear of the mice. The antigen challenge was evaluated by the methods described with some modification. Twenty-four hours later, a piece of left and right ear with the diameter of 8 mm was obtained with a stiletto. The delayed-type hypersensitivity reaction (DTH) was calculated using the following formula:

$$\text{DTH} = \text{Weight of right ear (diameter 8 mm)} - \text{Weight of left ear (diameter 8 mm)}$$

Lymphocyte-proliferation assays

After four consecutive weeks' oral administration, spleens were removed aseptically from mice and teased in cold (4°C) phosphate-buffered saline (PBS, pH 7.2) and pressed through a nylon sieve to liberate the cells. The suspension was allowed to sediment for several minutes and the supernatant was centrifuged (400 g, 10 min, 4°C). The pellet was suspended in 2 mL 0.17 M ammonium chloride to disintegrate erythrocytes (2 min). The remaining cells were washed thrice with cold PBS. The last pellet was suspended in Roswell Park Memorial Institute Medium 1640 (RPMI 1640, Sigma USA). Under sterile conditions, the spleen cells were adjusted to a concentration of 3 \times 10⁶ cells/mL in 2 mL RPMI 1640, dispensed into 24-well tissue culture plates (1 mL/well). The cell viability was measured by using the trypan blue dye exclusion method. Spleen cells were incubated at 37°C in an atmosphere comprising 5% CO₂/95% air in 24-well tissue culture plates with 50 μ L Concanavalin A (Con A, Sigma USA) solution or pure medium for 72 h. Lymphocyte proliferation was determined using a Methylthiazolotetrazolium (MTT) assay. At 4 h before the end of the incubation period,

the 0.7 mL of supernatant was removed and 0.7 mL of RPMI 1640 medium was added to all wells of an assay. Subsequently, 50 μ L of MTT solution (Sigma USA) was added to all wells of an assay, and plates were incubated for another 4 h under the same conditions. 1 mL isopropanol was added to all wells and mixed thoroughly to dissolve the dark blue crystals. After a few minutes, the plates were read on a Universal Microplate Spectrophotometer, using a test wavelength of 570 nm. The results are presented as optical density (O.D.) \pm SD.

Antibody producing cells assay

The number of plaque forming cells from the spleen was determined by the Jerne's Plaque assay¹⁶. After 24 days oral administration, 0.2 mL of 2 % (v/v) sheep red blood cells (SRBC) was intraperitoneally injected into each mouse. Five days later, spleens were removed aseptically from mice and teased in cold (4°C) PBS (pH 7.2) and pressed through a nylon sieve to liberate the cells. The suspension was allowed to sediment for several minutes and the supernatant was centrifuged (400 g, 10 min, 4°C). The pellet was suspended in 2 mL 0.17 M ammonium chloride to disintegrate erythrocytes (2 min). The remaining cells were washed three times with cold PBS. The last pellet was suspended in RPMI 1640 medium. Under sterile conditions, the spleen cells were adjusted to a concentration of 5×10^6 cells/mL in 5 mL RPMI 1640. About 25 μ L of spleen cell suspension and 50 μ L of 10% SRBC (v/v) were mixed with equal volumes of Hank's solution (pH 7.2), and incubated at the slides which were spread by molten agarose in an atmosphere comprising 5% CO₂ /95% air for 1.5 h. Subsequently, number of plaques was counted using a colony counter.

Acid phosphatase and alkaline phosphatase assay

After four consecutive weeks' oral administration, blood was drawn from the orbital venous plexus of mice' eye sockets with suction tube. The centrifuge tubes remained relatively static for 10 min and were incubated at 4°C in the refrigerator overnight, fixed with the blood from which serum precipitated.

According to the method which Reagent Kit (Nanjing jiancheng Bioengineering Institute, China) provided, the activity of acid phosphatase and alkaline phosphatase was measured.

Serum NO assay

After four consecutive weeks' oral administration, blood was drawn from the orbital venous plexus of mice' eye sockets with suction tube. The centrifuge tubes remained relatively static for 10 min and were incubated at 4°C in the refrigerator overnight, fixed with the blood from which serum precipitated. According to the method which Reagent Kit (Nanjing jiancheng Bioengineering Institute, China) provided, the content of NO was measured.

Statistical analysis

The results are reported as mean \pm standard deviations. Statistical evaluation of the data was done using Student's *t*-test. A probability value of <0.05 was considered significant.

Results

Effect of SF-CO₂ *P. vicina* extraction on immune organ weights

Effect of supercritical CO₂ fluid *P. vicina* extraction administration on immune organ weight of mice is given in Table 1. No significant difference on weights was found in groups supplemented with different dosages of *P. vicina* compared with distilled water treated group.

Effect of SF-CO₂ *P. vicina* extraction on the carbon particles clearance of mice

Effect of supercritical CO₂ fluid *P. vicina* extraction administration on the carbon particles clearance of mice is given in Table 2. Supercritical CO₂ fluid *P. vicina* extraction administration was found to enhance the Expurgatory index (*K*) and Phagocytic index (α). It was dose-dependent to potentiate the capability of carbon granule clearance and the phagocytosis activity of macrophage in mice. Expurgatory index was significant increased from 0.011 in negative control to 0.021 and 0.031, respectively in group III and IV treated with 3.75 and

Table 1 — Effect of SF-CO₂ *P. vicina* extraction on the immune organ weights of mice (n=10, $\bar{X} \pm$ SD)

Group	Dosage (g kg ⁻¹ d ⁻¹)	Thymus index (mg g ⁻¹ body weight)	Spleen index (mg g ⁻¹ body weight)
Negative control	0	3.01 \pm 0.17 ^a	6.02 \pm 0.21 ^a
Low-dosage group	1.87	3.03 \pm 0.13 ^a	5.98 \pm 0.22 ^a
Moderate-dosage group	3.75	2.98 \pm 0.11 ^a	6.09 \pm 0.24 ^a
High-dosage group	7.50	3.04 \pm 0.09 ^a	6.04 \pm 0.26 ^a

[Values in the same column with the same letters did not differ significantly at 0.05 level. The same for Table below]

7.50 g kg⁻¹ body weight *P. vicina*. The Phagocytic index was significant increased from 4.98 in negative control to 6.14, in group IV treated with 7.50 g kg⁻¹ body weight *P. vicina*.

Effect of SF-CO₂ *P. vicina* extraction on phagocytosis of peritoneal macrophages

Phagocytic rate was significant increased from 19.84% in negative control to 23.86% and 25.92%, respectively in moderate and higher dosage groups treated with 3.75 g and 7.50 g kg⁻¹ body weight *P. vicina* (Table 3). The phagocytic index was 0.17 in negative control, which was significantly increased to 0.28, 0.38 and 0.49, respectively in groups treated with 1.87 (Gr. II), 3.75 (Gr. III) and 7.50 g kg⁻¹ body weight (Gr. IV) *P. vicina*.

Effect of SF-CO₂ *P. vicina* extraction on the hemolysin of mice

The effect of supercritical CO₂ fluid *P. vicina* extraction on humoral immune response in mice was tested. As shown in Table 4, difference in half of hemolysin values (HC₅₀) was significant increased from 110.32 in negative control to 123.78 and 135.58, in group treated with 3.75 and 7.50 g kg⁻¹ body weight *P. vicina*. No significant differences were found in group II (1.87 g kg⁻¹ body weight) compared

to negative control. However, it was dose-dependent to potentiate the humoral immune response in mice.

Evaluation of SF-CO₂ *P. vicina* extraction on cellular immune response

The effect of supercritical CO₂ fluid *P. vicina* extraction on the DTH reaction in mice was tested. As shown in Table 5, difference in ear thickness was significant increased from 2.32 mg in negative control to 3.98 mg and 4.02 mg, in group III & IV treated with 3.75 and 7.50 g kg⁻¹ body weight *P. vicina*. However, no significant differences were found in group II (1.87 g kg⁻¹ body weight) compared to negative control. Therefore, it was dose-dependent to potentiate the cellular immune response in mice.

The O.D. value of lymphocyte proliferation induced by ConA was significant increased from 0.184 in negative control to 0.285, respectively in group IV treated with 7.50 g kg⁻¹ body weight *P. vicina* (Table 5).

Evaluation of SF-CO₂ *P. vicina* extraction on humoral immune response

Supercritical CO₂ fluid *P. vicina* extraction administration was found to significantly enhance the number of antibody producing cells in spleen. As shown in Table 6, the number of plaques was significant increased from 62.48×10³ in negative control to 81.24×10³ and 98.87×10³, respectively in group III & IV treated with 3.75 and 7.50 g kg⁻¹ body weight *P. vicina*. It was dose-dependent to potentiate the humoral immune response in mice.

Effect of SF-CO₂ *P. vicina* extraction on the acid phosphatase and alkaline phosphatase activity of mice

Effect of supercritical CO₂ fluid *P. vicina* extraction administration on the acid phosphatase and

Table 2—Effect of SF-CO₂ *P. vicina* extraction on the carbon particles clearance of mice (n=10, $\bar{X} \pm SD$)

Group	Dosage (g kg ⁻¹ d ⁻¹)	Expurgatory index (K)	Phagocytic index (α)
Negative control	0	0.011±0.004 ^c	4.98±1.28 ^b
Low-dosage group	1.87	0.013±0.005 ^c	5.02±1.05 ^b
Moderate-dosage group	3.75	0.021±0.009 ^b	5.29±1.25 ^b
High-dosage group	7.50	0.031±0.012 ^a	6.14±1.27 ^a

Table 3—Effect of SF-CO₂ *P. vicina* extraction on the nonspecific immune response assessed by phagocytic rate and phagocytic index (n=10, $\bar{X} \pm SD$)

Group	Dosage (g kg ⁻¹ d ⁻¹)	Phagocytic rate (%)	Phagocytic index
Negative control	0	19.84±1.69 ^c	0.17±0.05 ^d
Low-dosage group	1.87	20.15±2.11 ^c	0.28±0.03 ^c
Moderate-dosage group	3.75	23.86±2.27 ^b	0.38±0.05 ^b
High-dosage group	7.50	25.92±2.53 ^a	0.49±0.06 ^a

Table 4—Effect of SF-CO₂ *P. vicina* extraction on the hemolysin of mice (n=10, $\bar{X} \pm SD$)

Group	Dosage (g kg ⁻¹ · d ⁻¹)	Half of hemolysin values (HC ₅₀)
Negative control	0	110.32±8.54 ^c
Low-dosage group	1.87	112.17±7.98 ^c
Moderate-dosage group	3.75	123.78±8.97 ^b
High-dosage group	7.50	135.58±9.64 ^a

Table 5—Effect of SF-CO₂ *P. vicina* extraction on the cellular immune response assessed by DTH reaction and lymphocyte proliferation (n=10, $\bar{X} \pm SD$)

Group	Dosage (g kg ⁻¹ d ⁻¹)	Difference in ear thickness (mg)	The O.D. value of lymphocyte proliferation induced by ConA
Negative control	0	2.32±1.06 ^b	0.184±0.017 ^b
Low-dosage group	1.87	2.51±1.15 ^b	0.191±0.018 ^b
Moderate-dosage group	3.75	3.98±1.04 ^a	0.202±0.012 ^b
High-dosage group	7.50	4.02±1.21 ^a	0.285±0.014 ^a

alkaline phosphatase activity of mice is given in Table 7. *P. vicina* extraction administration was found to enhance the acid phosphatase and alkaline phosphatase activity. It was dose-dependent to potentiate the activity of the acid phosphatase and alkaline phosphatase in mice. The acid phosphatase activity was significant increased from 5.07 U/100 mL in negative control to 7.45 U/100 mL and 9.87 U/100 mL, in group III & IV treated with 3.75 and 7.50 g kg⁻¹ body weight *P. vicina*. The alkaline phosphatase activity was significant increased from 7.98 King unit /100 mL in negative control to 13.86 King unit /100 mL, in group IV treated with 7.50 g kg⁻¹ body weight *P. vicina*. However, no significant differences were found in group II & III (1.87 and 3.75 g kg⁻¹ body weight) compared to negative control.

Effect of SF-CO₂ *P. vicina* extraction on the serum NO content of mice

The effect of supercritical CO₂ fluid *P. vicina* extraction on the serum NO content in mice was tested. As shown in Table 8, the serum NO content of mice was significant increased from 7.98 µmol/L in negative control to 10.45, 15.56 and 18.48 µmol/L, in group treated with 1.87, 3.75 and 7.50 g kg⁻¹ body weight *P. vicina*.

Table 6—Effect of SF-CO₂ *P. vicina* extraction on the humoral immune response assessed by antibody producing cells (n=10, $\bar{X} \pm SD$)

Group	Dosage (g kg ⁻¹ d ⁻¹)	Number of plaques (×103/spleen cells)
Negative control	0	62.48±8.81 ^c
Low-dosage group	1.87	63.12±7.59 ^c
Moderate-dosage group	3.75	81.24±9.25 ^b
High-dosage group	7.50	98.87±8.62 ^a

Table 7—Effect of SF-CO₂ *P. vicina* extraction on the acid phosphatase and alkaline phosphatase activity of mice (n=10, $\bar{X} \pm SD$)

Group	Dosage (g kg ⁻¹ · d ⁻¹)	Acid phosphatase activity (U/100 mL)	Alkaline phosphatase activity (King unit/100 mL)
Negative control	0	5.07±1.01 ^c	7.98±2.19 ^b
Low-dosage group	1.87	5.12±1.35 ^c	8.08±1.97 ^b
Moderate-dosage group	3.75	7.45±1.24 ^b	8.59±2.87 ^b
High-dosage group	7.50	9.87±1.38 ^a	13.86±2.64 ^a

Table 8—Effect of SF-CO₂ *P. vicina* extraction on the serum NO content of mice (n=10, $\bar{X} \pm SD$)

Group	Dosage (g kg ⁻¹ d ⁻¹)	NO content (µmol/L)
Negative control	0	7.98±1.56 ^d
Low-dosage group	1.87	10.45±1.81 ^c
Moderate-dosage group	3.75	15.56±2.15 ^b
High-dosage group	7.50	18.48±2.59 ^a

Discussion

The insects are thought to be one of the biggest biological resources that have not been fully exploited by human being. Immunoregulation is a complex balance between regulatory and effector cell and any imbalance in the immunological mechanism can lead to pathogenesis^{17,18}. Immunity has been shown to be suppressed in cancer. Chemotherapy and radiation therapy, useful in cancer treatment were found to deteriorate the immunity. In fact, essential nutrients and an array of phytonutrients have been shown to affect almost every aspect of the immune system¹². Supercritical CO₂ fluid *P. vicina* extraction contained 77000 IU/100 g of SOD, 56.6 g/100 g protein, 9.0 g/100 g fat, 13.2 g/100 g volatile oil, 6.0 g/100 g moisture, 1.6 g/100 g total acid and 6.3 g/100 g ash. There were 18 amino acids, of which, glutamic acid, glycine, aspartic acid, alanine, leucine, proline and tyrosine were predominant. Among the 16 minerals, K, Ca, P, Mg, Fe, Mn and Zn were predominant. More than 20 organic components were identified, the main ones were 9-octadecenoic acid, ethyl oleate, cholesterol and n-hexadecanoic acid. Six of the compounds found, i.e. hexadecanoic acid, ethyl ester, linoleic acid, ethyl oleate, oleic acid and cholesta-3,5-diene, have been reported by supercritical CO₂ fluid extraction¹. In view of this, the present study was carried out to investigate the effect of supercritical CO₂ fluid *P. vicina* extraction on some immunological parameters.

It is well known that macrophages are important cells in the immune response, especially in the anti-infection immunity. Administration of supercritical CO₂ fluid *P. vicina* extraction was found to increase the carbon expurgatory index and phagocytic index significantly indicating that the extract could stimulate the nonspecific phagocytic function. The humoral immune response is the aspect of immunity that is mediated by secreted antibodies produced in the cells of the B lymphocyte lineage. Humoral immunity is so named because it involves substances found in the humours, or body fluids. Through the determination of hemolysin in serum content, we can know the immune response and its intensity¹⁹⁻²¹. The extract was found to enhance half of hemolysin values indicating that the extract could stimulate humoral immune response.

Macrophages are cells produced by the differentiation of monocytes in tissues. Macrophages function in both non-specific defense (innate immunity) as well as help initiate specific defense

mechanisms (adaptive immunity) of vertebrate animals. Their role is to phagocytose cellular debris and pathogens, either as stationary or as mobile cells²²⁻²⁴. They also stimulate lymphocytes and other immune cells to respond to pathogens. Phosphatase is an important biological detoxification enzyme systems and its activity reflects the activated degree of macrophages. NO is the important medium and the initial factor that play an important cell functions, involved in a series of immune regulation effects²⁵⁻²⁷. Supercritical CO₂ fluid *P. vicina* extraction administration was found to enhance the serum NO content, acid phosphatase and alkaline phosphatase activity, indicating that *P. vicina* can effectively protect the biological immune system of mice, and enhance non-specific immune function.

Conclusion

In conclusion, nonspecific, cellular and humoral immune response are three of dominant indexes to evaluate the immunomodulatory activity of the test samples, which were determined to be efficient with the results positive. Therefore, Supercritical CO₂ fluid *P. vicina* extraction enhanced all of three indexes compared with negative control. The results indicated that *P. vicina* had a potency to potentiate the immune responses in mice. At present we do not know which compounds are responsible for the immunostimulatory activity produced by this extract. Further studies using isolated compounds are in progress.

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References

- Shen LR, Li D, Feng FQ & Ren YC, Nutritional composition of *Polyrhachis vicina* Roger (Edible Chinese black ant). *Songklanakarinn J Sci Technol*, 28 (2006) 107.
- Vijaykumar PV & Pandharinath SP, Scummy Insect in my Stomach! *Sci Rep*, 51 (2014) 22.
- Huang NJ & Xiao SX, Study on *Polyrhachis vicina*. *Res Pract Chin Med*, 17 (2003) 60.
- Zhon JH, A preliminary review on the present states of ant health product exploration in China. *Shanghai Med Info*, 2 (1996) 27.
- Shen LR & Ren YC, Advances on exploration and utilization of the edible and medical ants in China. *Entomol Knowl*, 36 (1999) 251.
- Tang JJ, Fang P, Xia HL, Tu ZC, Hou BY, Yan YM, Di L, Zhang L & Cheng YX, Constituents from the edible Chinese black ants (*Polyrhachis dives*) showing protective effect on rat mesangial cells and anti-inflammatory activity. *Food Res Int*, 67 (2015) 163.
- Tang XS, Ant's chemical ingredients and its pharmacology research. *Special Wild Econ Anim Plant Res*, 2 (2002) 60.
- Jones TH, Garraffo HM, Spande TF, Andriamaharavo NR, Gorman JST, Snyder AJ, Jeter AW, Torres JA, Snelling RR & Daly JW, Caste-specific tyramides from myrmicine ants. *J Nat Prod*, 73 (2010) 313.
- Moslavac T, Jokić S, Šubarić D, Aladić K, Vukoja J & Prce N, Pressing and supercritical CO₂ extraction of *Camelina sativa* oil. *Ind Crops Prod*, 54 (2014) 122.
- Tasnuva ST, Sahena F, Qamar UA, Kashif G, Juliana J & Zaidul ISM, *Mimosa pudica* L.: A comparative study via in vitro analysis and GC Q-TOF MS profiling on conventional and supercritical fluid extraction using food grade ethanol. *Indian J Natur Prod & Resour*, 8 (2017) 54.
- Nautiyal OH, Process optimization of sandalwood (*Santalum album*) oil extraction by subcritical carbon dioxide and conventional techniques. *Indian J Chem Technol*, 21 (2014) 290.
- Geetha S, Singh V, Ram MS, Ilavazhagan G, Banerjee PK & Sawhney RC, Immunomodulatory effects of seabuckthorn (*Hippophae rhamnoides* L.) against chromium (VI) induced immunosuppression. *Mol Cell Biochem*, 278 (2005) 101.
- Zhang C & Xu WH, *Resource insects* (Shanghai Science and Technology Press, Shanghai) 1990.
- Chen J, Liu JS, Fan CX, Wu FL, Zhang MZ, Tan L, Zhu JQ & Sun WQ, Effect of alcohol extract of *Polyrhachis vicina* Roger on immune function in mice with tumor. *J Jilin Univ (Med Edi)*, 30 (2004) 543.
- Lin P, Feng Z, Pan J, Zhang D & Xiao L, Effects of artesunate on immune function in mice. *Acta Pharmacol Sin*, 16 (1995) 441.
- Jerne NK & Nordin AA, Plaque formation in agar by single antibody producing cells. *Science*, 140 (1963) 405.
- Davis L & Kuttan G, Immunomodulatory activity of *Withania somnifera*. *J Ethnopharmacol*, 71 (2000) 193.
- Witczak P, Pietrzak A, Wódcz K & Brzezińska-Błaszczyk E, Mast cells generate cysteinyl leukotrienes and interferon- as well as evince impaired IgE-dependent degranulation upon TLR7 engagement. *Indian J Exp Biol*, 52 (2014) 589.
- Zheng JX, *Functional food* (China Light Industry Press, Beijing) 1995.
- Jin ZL, *Health food function evaluation and development* (China Light Industry Press, Beijing) 2001.
- Si CP, *Medical immunology experiment* (People's Medical Publishing House, Beijing) 1999.
- Sharma U, Bala M, Saini R, Verma PK, Kumar N, Singh B, Munshi RK & Bhalerao S, Polysaccharide enriched immunomodulatory fractions from *Tinospora cordifolia*

- (Willd) miers ax hook. F. & Thoms. *Indian J Exp Biol*, 50 (2012) 612.
- 23 Paradkar PH, Mishra LS, Joshi JV, Dandekar SP, Vaidya RA & Vaidya AB, *In vitro* macrophage activation: A technique for screening anti-inflammatory, immunomodulatory and anticancer activity of phytomolecules. *Indian J Exp Biol*, 55 (2017) 133.
- 24 More P & Pai K, Effect of *Tinospora cordifolia* (Guduchi) on the phagocytic and pinocytic activity of murine macrophages *in vitro*. *Indian J Exp Biol*, 55 (2017) 21.
- 25 He HQ & Sun F, Studies on the characteristics of acid and alkaline phosphatases in Chinese shrimp, *Penaeus chinensis*. *Oceanol Limnol Sin*, 23 (1992) 555.
- 26 Ding TM, Chen J & Zhang ZX, The methods for determination of nitric oxide *in vivo* and their applications. *Prog Pharm Sci*, 29 (2005) 221.
- 27 Tripathi YB & Pandey RS, *Semecarpus anacardium* L, nuts inhibit lipopolysaccharide induced NO production in rat macrophages along with its hypolipidemic property. *Indian J Exp Biol*, 42 (2004) 432.