Immunomodulating Activity of Thai Rejuvenating Plants

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Abstract

This research aimed to find the relationship between rejuvenating remedy and ICR mouse immune system. Screening of the methanolic extract of fifteen rejuvenating plants widely used in Thailand was conducted using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Among the plants studied, *Butea superba* Roxb, *Plumbago indica* L. and *Michelia champaca* L. were the three most active by MTT assay. They were further tested on lymphocyte proliferation with mitogen (lipopolysaccharide (LPS), pokeweed mitogen (PWM), phytohemagglutinin (PHA) and Concanavalin A (Con A)) as well as macrophage phagocytosis. The results showed that *B. superba* extracts (4 mg/ml) with LPS, PWM, PHA and Con A presented similar Stimulation Index (SI) value of about 1.3, suggesting moderate activation on both T- and B-lymphocyte proliferation. *P. indica* extracts with PHA showed the maximum SI value of about 1.6, suggesting major activation on T-lymphocyte proliferation using the same mechanism as PHA. Although *M. champaca* extract did not markedly stimulate lymphocyte proliferation, it slightly increased lysosomal enzyme production, suggesting a mild effect on the degranulation of phagocytosis while *B. superba* and *P. indica* did not cause any changes. These observations revealed credible relation of rejuvenating remedy and the murine immune system.
Keywords: ICR mice; Immunomodulating activity; Macrophage phagocytosis; Lymphocyte proliferation; Rejuvenating plant

Introduction

Plants have played a significant role in preserving human health and improving the quality of human life for thousands of years. The World Health Organization estimates that nearly 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts or their active ingredients (Craig, 1999). Nowadays, traditional plants for health promotion, disease prevention and rejuvenation approaches are gaining greater attention in many parts of the worlds.

Several rejuvenating plants with immunomodulating activity have been previously reported. Echinacea extract stimulated phagocytosis (Stotzem et al., 1992; Wagner & Jurcic, 1991), cytokine production from macrophages (Burger et al., 1997) and improved natural killer function of human polymorphonuclear cells (See et al., 1997). The aqueous extract of *Panax quinquefolium* increased immunoglobulin production and peritoneal macrophage function (Wang et al., 2001). Cytotoxic effects of *P. ginseng* on a wide range of tumor cell lines without major histocompatibility complex restriction have been reported (Lee et al., 1997). Allicin, the major ingredient of garlic (*Allium sativum*) induced tumoricidal activity and increased TNF-α and nitric oxide (NO) production in a dose-dependent manner (Kang et al., 2001).

In Thailand, there are several medicinal plants claimed for health-promoting properties. In this study, fifteen Thai plants used in traditional rejuvenating remedy

from different parts of Thailand (Ingkaninan et al., 2003) were explored whether they affect immune system. Mitogen-induced lymphocyte proliferation and peritoneal macrophage phagocytosis of ICR mice were studied.

Materials and Methods

Chemicals

The 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), nitroblue tetrazolium (NBT) dye, *p*-nitrophenyl phosphate (*p*-NPP), phytohemagglutinin (PHA), Concanavalin A (Con A), lipopolysaccharide (LPS), pokeweed mitogen (PWM), Zymosan A, phosphate buffer saline (PBS) tablet, dimethyl sulfoxide (DMSO), RPMI-1640 media, antibiotic solution (100 U penicillin, 100 μg streptomycin and 0.25 μg/ml amphotericin B) were all purchased from Sigma (Germany). 2-Mercaptoethanol and Triton X-100 were obtained from Pharmacia (Sweden). Fetal bovine serum (FBS) was from Biochem KG (Germany).

Preparation of extracts

The plant materials collected from the north and central of Thailand were authenticated by Associate Professor Wongsatit Chuakul, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. The voucher specimens were deposited at the Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok and PBM herbarium, Faculty of Pharmacy, Mahidol University, Bangkok. The plants were reduced into small pieces and dried in a hot air oven at 55 ^{o}C. The dried materials were macerated in methanol two times for 3 days and filtered. The filtrate was evaporated under reduced pressure until dryness. For preparation of tested samples, extracts were dissolved in 0.1% DMSO in PBS solution, and the 0.1% DMSO in PBS was used as control in all experiments. All extract solutions were sterilized by a Millipore filter (0.2 μm pore size). The concentration ranges provided in this study were in correlation with average traditional doses.

Animals

Female ICR mice (5-6 weeks old) were from the National Laboratory Animal Center, Mahidol University, Bangkok, Thailand. The animals were housed under standard conditions at 25$\pm$2 ^{o}C and fed with standard pellets and tap water. The experiments were conducted under the surveillance of the Ethics Committee of Naresuan University, Thailand.

Preparation of mouse splenocytes

Mice were sacrificed before their spleens were removed aseptically. Splenocyte suspensions were prepared as previously described (Manosroi et al., 2003). After centrifugation at 300 g, 25 ^{o}C for 10 min, the cell pellets were washed twice and re-suspended in complete RPMI-1640 medium [RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 50 μM 2-mercaptoethanol, 100 U penicillin, 100 μg streptomycin and 0.25 μg/ml amphotericin B]. The cell number was adjusted to $1x10^{7}$ cells/ml by counting in a hemocytometer. The cell viability was evaluated using trypan-blue dye exclusion technique.

Screening of fifteen extracts on splenocyte proliferation assay

Effect of plant extracts (0.1, 1 and 10 mg/ml) on splenocyte proliferation was screened according to MTT method (Mosmann, 1983). Briefly, splenocyte suspensions ($1x10^{6}$ cells/well)

and plant extracts were cultured in 96-well plates for 48 h at 37 ^{o}C in humidified 5% CO_2 atmosphere. After incubation, 5 mg/ml MTT was added, then cell cultures were further incubated for 4 h. The culture medium was removed by aspiration before 0.04 M HCl in isopropyl alcohol was added to lyse the cells. Distilled water was added to dilute the solution before the absorbance was measured at 570 nm using microplate reader (ETL Testing Laboratory Inc., New York). The Stimulation Index (SI) was calculated by the following equation:

$$SI = \left(\frac{\text{OD sample}}{\text{OD control}} \right).$$

The extract of *Allium sativum* was defined as positive control (SI 1.5). The plant was reported to be effective in enhancing the immune system and possess various pharmacological activities (Spellman et al., 2006). In this study, extracts which presented the SI value $\geq$ 1.5 were determined as active.

For further studies, the three most active extracts were reassayed on mitogen-induced lymphocyte proliferation, macrophage phagocytosis on nitroblue tetrazolium (NBT) dye reduction and cellular lysosomal enzyme activity assays. Extract at the concentrations of 0.25, 0.5, 1, 2 and 4 mg/ml were tested.

Mitogen-induced lymphocyte proliferation assay

Mitogen-induced lymphocyte proliferation assay was carried out according to the MTT method as previously described (Mosmann, 1983). The optimum dose (5 μg/ml) of LPS, PWM, PHA or Con A was used as mitogen. The absorbance was measured at 570 nm and the SI value was calculated.

Preparation of peritoneal mouse macrophages

FBS was administered by intraperitoneal injection in mice. Three days later, the peritoneal exudates were collected by lavage with RPMI-1640 medium. The exudates were centrifuged at 300 g, 25 ^{o}C for 20 min, and the cell pellets were washed twice and re-suspended in complete RPMI-1640 medium. The cell number was adjusted to $1x10^7$ cells/ml by counting in a hemocytometer. The cell viability was determined using trypan-blue dye exclusion technique.

Nitroblue tetrazolium (NBT) dye reduction assay

The phagocytic activity was measured using NBT dye reduction described previously (Rainard, 1986). Macrophages ($1x10^5$ cells/well) were treated with plant extracts for 24 h at 37 ^{o}C in 5% CO_2 humidified incubator. Zymosan A ($5x10^6$ particles/well) and 1.5 mg/ml of NBT were added in cultures, and the cultures were incubated for 60 min. The macrophages were rinsed vigorously with RPMI medium and washed four times with methanol. After air-dried, 2 M KOH and DMSO were added and the absorbance was measured at 570 nm using microplate reader. The Phagocytic Index (PI) was calculated according to the following equation:

$$PI = \left(\frac{\text{OD sample}}{\text{OD control}} \right).$$

Cellular lysosomal enzyme activity assay

The acid phosphatase activity in macrophages was determined as previously described (Suzuki et al., 1988). Macrophage suspensions ($1x10^5$ cells/well) were treated with plant extracts for 24 h at 37 ^{o}C in the 5% CO_2 incubator. The medium was removed

by aspiration prior to the addition of 0.1% Triton X-100, 10 mM *p*-NPP solution and 0.1 M citrate buffer (pH 5.0). The cultured cells were further incubated for 30 min, 0.2 M borate buffer (pH 9.8) was then added. The absorbance was measured at 405 nm using microplate reader. The PI value was calculated as stated previously.

Statistical analysis

All experiments were performed in triplicate. Results were expressed as mean±S.E. Statistical significance was analyzed using Student's *t*-test. A *P* value of less than 0.05 was considered statistically significant.

Results and Discussion

This *in vitro* study was undertaken to prove whether Thai traditional rejuvenating plants could have immunomodulating properties. Splenocyte proliferation of the methanolic extracts of fifteen plants (0.1, 1 and 10 mg/ml) were screened according to colorimetric MTT method without mitogen. MTT has several desirable properties for testing cell survival and proliferation. All living, metabolically active cells cleaved MTT dye and produced MTT formazan in direct proportion to the cell number (Mosmann, 1983).

As shown in Table 1, stimulation effect was clearly observed at high concentration (10 mg/ml). The results showed that extracts of *B. superba*, *P. indica* and *M. champaca* caused high activation (%A ≥80). Moderate activation (50 ≤ %A < 80) was observed for extracts of *T. triandra*, *D. scandens*, *T. crispa*, *E. antiquorum*, *P. acidus*, *G. oppositifolius* and *A. sativum* while others indicated low activity (%A <50). It might be assumed that traditional rejuvenating remedy could be related to immunomodulating properties with different potency.

Table 1. Stimulation Index (SI) and % activation (%A) of Thai traditional rejuvenating plant (10 mg/ml) by splenocyte proliferation assay

Activity	Plants	Family	Part of use	SI	%A [a]
High	*Michelia champaca* L.	Magnoliaceae	Leaf	2.0	100
	Plumbago indica L.	Plumbaginaceae	Root	2.0	100
	Butea superba Roxb.	Leguminosae	Root bark	1.9	90
Moderate	*Tiliacora triandra* (Colebr.) Diel	Menispermaceae	Root	1.7	70
	Derris scandens (Roxb.) Benth.	Leguminosae	Stem	1.6	60
	Tinospora crispa (L.) Miers ex Hook.f. & Thomson	Menispermaceae	Stem	1.6	60
	Euphorbia antiquorum L.	Euphorbiaceae	Stem	1.5	50
	Phyllanthus acidus (L.) Skeels	Euphorbiaceae	Leaf	1.5	50
	Glinus oppositifolius (L.) A. DC.	Molluginaceae	Whole plant	1.5	50
	Allium sativum L.[b]	Alliaceae	Bulb	1.5	50
Low	*Vitex trifolia* L.	Labiatae	Root	1.4	40
	Piper chaba Hunter	Piperaceae	Fruit	1.3	30
	Acanthus ebracteatus Vahl.	Acanthaceae	Aerial part	1.3	30
	Abutilon indicum L.	Malvaceae	Whole plant	1.2	20
	Musa sapientum L.	Musaceae	Fruit	1.2	20

[a] % A = [(SI extract - SI control) / SI control] x 100; SI control = 1; 20 ≤ %A < 50: low activity; 50 ≤ %A < 80: moderate activity; %A ≥ 80: high activity

[b] positive control

The three most active extracts (*B. superba*, *P. indica* and *M. champaca*) at 0.25, 0.50, 1, 2 and 4 mg/ml were further tested on lymphocyte proliferation with mitogen (LPS, PWM, PHA or Con A). Macrophage phagocytosis on NBT dye reduction and lysosomal enzyme activity were also assayed. The presence of mitogen in colorimetric

MTT system can postulate the possible activation pathway of the extracts (Nakamura et al., 1986). LPS and PWM were used for the stimulation of B-cell proliferation through T-cell independent and T-cell dependent pathways, respectively, while PHA and Con A were used for the activation of different subtypes of T-cell proliferation.

As presented in Figure 1, *B. superba* extract at 4 mg/ml with mitogen (LPS, PWM, PHA and Con A) equally increased lymphocyte proliferation with the SI value of about 1.3 compared to mitogen alone. This suggested mild stimulation on both T- and B-lymphocyte proliferation. *B. superba* has been traditionally consumed among Thai males for rejuvenating, as well as preserving sexual performance (Suntara, 1931). Immunomodulating activity of this plant has never been reported. However, its antitumor (Konoshima et al., 1997) and cytotoxic effects on KB and BC cell lines (Ngamrojanavanich et al., 2007) could support our findings. Flavonoid and flavonoid glycosides, which are major components in *B. superba* as well as many plant species, might be responsible for these activities (Roengsamran et al., 2000).

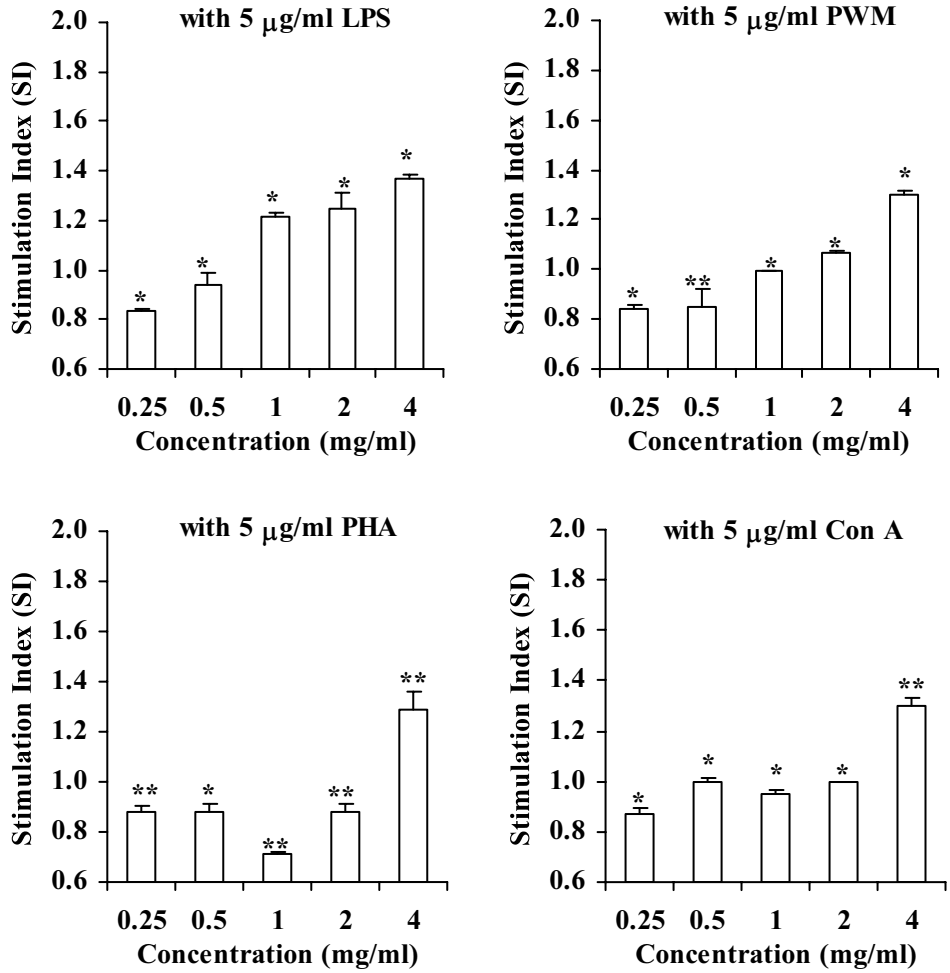

Figure 1. Effects of *Butea superba* Roxb. extract on ICR mouse splenocyte proliferation with mitogen (5 μg/ml LPS, PWM, PHA and Con A). Each value (Stimulation Index, SI) represents the mean ± S.E. of triplicates compared to the control or mitogens (SI = 1); $^{*}P<0.01$, $^{**}P<0.05$.

P. indica extracts (4 mg/ml) with PHA activated lymphocyte proliferation with the maximum SI value of about 1.6. On the other hand, *P. indica* extracts at 0.25, 0.5 and 1 mg/ml with Con A showed slight suppression (Figure 2). The results suggested that *P. indica* extracts at different concentrations had both stimulation and suppression effects on different

T-lymphocyte subpopulation using the same mechanism as PHA and Con A, respectively. These were supported by the anticancer activity and modulation of cellular proliferation by plumbagin (Sandur et al., 2006) and other isolated naphthaquinones from *P. indica* and other plants of the family Plumbaginaceae (Dinda & Chel, 1992). Moreover, these observations were in agreement with previous reports indicating that different concentrations of extracts could either enhance or suppress splenocyte proliferation (Amirghofran et al., 2000; Swamy & Tan, 2000). Differences in sensitivity to T- and B-mitogenic stimuli have also been described (Mookerjee et al., 1986; Hirano et al., 1989).

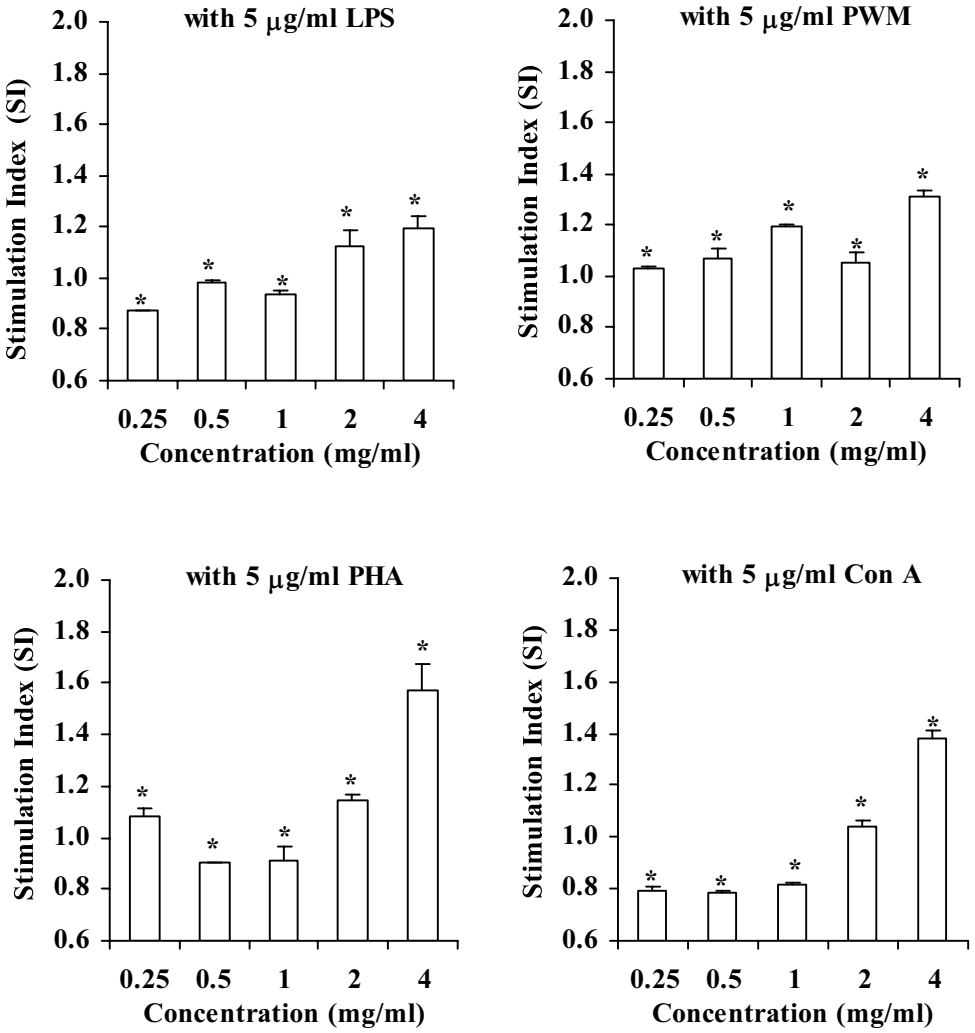

Figure 2. Effects of *Plumbago indica* L. extract on ICR mouse splenocyte proliferation with mitogen (5 μg/ml LPS, PWM, PHA and Con A). Each value (Stimulation Index, SI) represents the mean± S.E. of triplicates compared to the control or mitogens (SI = 1); $^{*}P<0.01$.

Effects of several sesquiterpene lactones, the major components derived from the root bark of *M. champaca* (Sethi et al., 1984) and other herbs, on humoral and cellular mediated immunity have been discussed. Artemisinin, dihydroartemisinin and arteether isolated from *Artemisia annua* displayed humoral immunosuppressive activity on hemolytic plaque assay (Tawfik et al., 1990). Sesquiterpene quinones derived from sponge *Smenospongia* sp. revealed both inhibitory and stimulatory activities on mitogen-induced lymphocyte proliferation (Bourquet-Kondracki et al., 1991). The findings from our study showed that *M. champaca* extract in the presence of mitogen showed only a little

stimulation at the tested concentration ranges. These suggested its ineffectiveness (data not shown) in contrast to what have been previously reported. Exact significance of these observations needed further investigation.

It is a common knowledge that macrophages play an important role in the defense mechanism against host infection and the killing of tumor cells. Modulation of antitumor properties of macrophages by various biological response modifiers is an area of active interest for cancer chemotherapy (Kang et al., 2002), closely related to immunomodulating activity. The higher reduction in NBT dye reduction assay represented higher activity of oxidase enzyme, reflecting phagocytosis stimulation in proportion to intracellular killing (Rainard, 1986). For lysosomal enzyme activity, transformation of *p*-NPP to the colored compound by the acid phosphatase of the stimulated macrophages could be correlated to degranulation in phagocytosis (Suzuki et al., 1990).

No markedly different phagocytic activity on both NBT dye reduction and lysosomal enzyme activity of *B. superba* and *P. indica* extracts were observed in these studies. The augmentation of macrophage bactericidal activity by plumbagin has been previously discussed. The mechanism of action relied on the potentiation of the oxyradical release at low concentration, whereas at higher concentration its activity was inhibitory (Abdul & Ramchender, 1995). However, phagocytic activity of *B. superba* has never been reported. Only *M. champaca* leaf extract (4 mg/ml) slightly increased lysosomal enzyme activity compared to the control (PI 1.2) (data not shown), suggesting a mild effect on the degranulation process of phagocytosis. Our result might be supported by the report on anticancer activity of sesquiterpene from the heartwood of *Cryptomeria japonica*, which induced human monocyte-derived dendritic cells (Takei et al., 2006).

Although the present results yield an incomplete picture on the effects of rejuvenating plants on immune system. It could be noticed that rejuvenating remedy was associated with both stimulation and suppression of lymphocyte proliferation which were closely related to cellular- and humoral- mediated immunity. These indicated the effects of rejuvenating plants on various modes of action of the immunity. Further investigations (in particular, the possible action of the extracts in interfering with cell signaling and cytokine production) are being carried out to permit a better understanding of their mechanisms of action.

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References

Abdul, K. M., & Ramchender, R. P. (1995). Modulatory effect of plumbagin (5-hydroxy -2-methyl-1,4-naphthoquinone) on macrophage functions in BALB/c mice. I. Potentiation of macrophage bactericidal activity. *Immunopharmacology*, *30*, 231-236.

Amirghofran, Z., Azadbakht, M., & Karimi, M. H. (2000). Evaluation of the immunomodulatory effects of five herbal plants. *Journal of Ethnopharmacology*, *72*, 167-172.

Bourquet-Kondracki, M-L., Longeon, A., Morel, E., & Guyot, M. (1991). Sesquiterpene quinones as immunomodulating agents. *International Journal of Immunopharmacology*, *13*, 393-399.

Burger, R. A., Torres, A. R., Warren, R. P., Caldwell, V. D., & Hughes, B. G. (1997). Echinacea-induced cytokine production by human macrophages. *International Journal of Immunopharmacology*, *19*, 371-379.

Craig, W. J. (1999). Health-promoting properties of common herbs. *The American Journal of Clinical Nutrition*, *70*, 491s-499s.

Dinda, B., & Chel, G. (1992). 6-Hydroxyplumbagin, a naphthoquinone from *Plumbago indica. Phytochemistry*, *31*, 3652-3653.

Hirano, T., Oka, K., Kawashima, E., & Akiba, M. (1989). Effects of synthetic and naturally occurring flavonoids on mitogen-induced proliferation of human peripheral-blood lymphocytes. *Life Sciences*, *45*, 1407-1411.

Ingkaninan, K., Temkitthawon, P., Chuenchom, K., Yuyaem, T., & Thongnoi, W. (2003). Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. *Journal of Ethnopharmacology*, *89*, 261-264.

Kang, N. S., Moon, E. Y., Cho, C. G., & Pyo, S. (2001). Immunomodulating effect of garlic component, allicin, on murine peritoneal macrophages. *Nutrition Research*, *21*, 617-626.

Kang, N. S., Park, S. Y., Lee, K. R., Lee, S. M., Lee, B. G., Shin, D. H., et al. (2002). Modulation of macrophage function activity by ethanolic extract of larvae of *Holotrichia diomphalia*. *Journal of Ethnopharmacology*, *79*, 89-94.

Konoshima, T., Takasaki, M., Kozuka, M., Tokuda, H., Nishino, H., Matsuda, E., et al. (1997). Anti-tumor promoting activities of isoflavonoids from *Wistaria brachybotrys*. *Biological Pharmaceutical Bulletin*, *20*, 865-868.

Lee, Y. S., Chung, I. S., Lee, I. R., Kim, K. H., Hong, W. S., & Yun, Y. S. (1997). Activation of multiple effector pathways of immune system by the antineoplastic immunostimulator acidic polysaccharide ginseng isolated from *Panax ginseng. Anticancer Research*, *17*, 323- 332.

Manosroi, A., Saraphanchotiwitthaya, A., & Manosroi, J. (2003). Immunomodulatory activities of *Clausena excavata* Burm.f. wood extracts. *Journal of Ethnopharmacology*, *89*, 155-160.

Mookerjee, B. K., Lee, T. P., Logue, G. P., Lippe, H. A., & Middleton, E. (1986). *Plant flavonoids in biology and medicine: Biochemical, pharmacological and structural-activity relationships*. New York: Alan R. Liss.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, *65*, 55-63.

Nakamura, A., Nagai, K., Suzuki, S., Ando, K., & Tamura, G. (1986). A novel method of screening for immunomodulating substances, establishment of an assay system and its application to culture broths of micro-organisms. *Journal of Antibiotics*, *39*, 1148-1154.

Ngamrojanavanich, N., Loontaisong, A., Pengpreecha, S., Cherdshewasart, W., Pornpakakul, S., Pudhom, K., et al. (2007). Cytotoxic constituents from *Butea superba* Roxb. *Journal of Ethnopharmacology*, *109*, 354-358.

Rainard, P. (1986). A colorimetric microassay for opsonins by reduction of NBT in phagocytosing bovine polymorphs. *Journal of Immunological Methods*, *90*, 197-201.

Roengsumran, S., Petsom, A., Ngamrojanavanich, N., Rugsilp, N., Sittiwichieanwong, P., Korphueng, P., et al. (2000). Flavonoid and flavonoid glycoside from *Butea superba* Roxb. and their cAMP phosphodiesterase inhibitory activity. *Journal of Scientific Research Chulalongkorn University*, *25*, 169-176.

Sandur, S., Ichikawa, H., Sethi, G., Ahn, K. S., & Aggarwal, B. (2006). Plumbagin (5-Hydroxy-2-methyl-1,4-naphthoquinone) suppresses NF-κB activation and NF-κB-regulated gene products through modulation of p65 and IκBα kinase activation, leading to potentiation of apoptosis induced by cytokine and chemotherapeutic agents. *Journal of Biological Chemistry*, *281*, 17023-17033.

See, D. M., Broumand, N., Sahl, L., & Tilles, J. G. (1997). *In vitro* effects of echinacea and ginseng on natural killer and antibody-dependent cell cytotoxicity in healthy subjects and chronic fatigue syndrome or acquired immunodeficiency syndrome patients. *Immunopharmacology*, *35*, 229-235.

Sethi, V. K., Thappa, R. K., Dhar, K. L., & Atal, C. K. (1984). Constituents of *Michelia champaca* and lewis acid catalyzed transformations of parthenolide into guaianolides. *Planta Medica*, *50*, 364.

Spellman, K., Burns, J., Nichols, D., Winters, N., Ottersberg, S., & Tenborg, M. (2006). Modulation of cytokine expression by traditional medicines: A review of herbal immunomodulators. *Alternative Medicine Review*, *11*, 128-150.

Stotzem, C. D., Hungerland, U., & Mengs, U. (1992). Influence of echinacea purpurea on the phagocytosis of human granulocytes. *Medical Science Research*, *20*, 719-720.

Suntara, A. (1931). *The remedy pamphlet of Kwao Krua tuber of Lung Anusarnsuntarakromkarnpiset*. Chiang Mai: Upatipong Press.

Suzuki, I., Tanaka, H., Adachi, Y., & Yadomae, T. (1988). Rapid measurement of phagocytosis by macrophages. *Chemical and Pharmaceutical Bulletin*, *36*, 4871- 4875.

Suzuki, I., Tanaka, H., Kinoshita, A., Oikawa, S., Osawa, M., & Yadomae, T. (1990). Effect of orally administered β-glucan on macrophages function in mice. *International Journal of Immunopharmacology*, *12*, 675-684.

Swamy, S. M. K., & Tan, B. K. H. (2000). Cytotoxic and immunopotentiating effects of ethanolic extract of *Nigella sativa* L. seeds. *Journal of Ethnopharmacology*, *70*, 1-7.

Takei, M., Umeyama, A., & Arihara, S. (2006). T-cadinol and calamenene induce dendritic cells from human monocytes and drive Th1 polarization. *European Journal of Pharmacology*, *537*, 190-199.

Tawfik, A. F., Bishop, S. J., Ayalp, A., & El-Feraly, F. S. (1990). Effects of artemisinin, dihydroartemisinin and arteether on immune responses of normal mice. *International Journal of Immunopharmacology*, *12*, 385-389.

Wagner, H., & Jurcic, K. (1991). Immunologic studies of plant combination preparations. In vitro and in vivo studies on the stimulation of phagocytosis. *Arzneimittel Forschung*, *41*, 1072-1076.

Wang, M., Guilbert, L. J., Ling, L., Li, J., Wu, Y., Xu, S., et al. (2001). Immunomodulating activity of CVT-E002, a proprietary extract from North American ginseng (*Panax quinquefolium*). *Journal of Pharmacy and Pharmacology*, *53*, 1515 -1523.