In vitro effects of Thai medicinal plants on human lymphocyte activity

Busarawan Sriwanthana¹, Weena Treesangsri², Bongkod Boriboontrakul³, Somchit Niumsakul⁴, and Pranee Chavalittumrong⁵

Abstract
Sriwanthana, B., Treesangsri, W., Boriboontrakul, B., Niumsakul, S., and Chavalittumrong, P. In vitro effects of Thai medicinal plants on human lymphocyte activity

We assessed the effects of Cleistocalyx nervosum var paniala, Gynostemma pentaphyllum, Gynura procumbens, Houttuynia cordata, Hyptis suaveolens, Portulaca grandiflora, Phytolacca americana and Tradescantia spathacea on lymphocyte proliferation and the effects of C. nervosum, G. pentaphyllum, H. suaveolens and P. grandiflora on natural killer (NK) cells activity. All of the extracts significantly stimulated human lymphocyte proliferative responses at various concentrations depending on each extract. The extracts of C. nervosum and H. suaveolens were significantly enhanced NK cells activity while those of G. pentaphyllum and P. grandiflora did not alter NK cells function. Our results suggested that the extracts of those plants have stimulating activity on human lymphocytes and could be clinically useful for modulating immune functions of the body.

Key words: Cleistocalyx nervosum, Gynostemma pentaphyllum, Gynura procumbens, Houttuynia cordata, Hyptis suaveolens, Portulaca grandiflora, Phytolacca americana, Tradescantia spathacea, lymphocyte proliferation, NK activity, immunostimulating agents

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Cleistocalyx nervosum var paniala is a plant in the Family Myrtaceae. High contents of polyphenols and flavonoids in C. nervosum were known to have antioxidant and anticarcinogenic properties (Leelapornpisit et al., 2004). It is now popular for functional health food, cosmetic ingredients and health drinks.

Gynostemma pentaphyllum Makino is a perennial climber in the Family Cucurbitaceae. It is used for treatment of inflammation, cough, hyperviscosity of sputum and chronic bronchitis (Jiang-Xu, 1979; Lin et al., 1993). Gypenosides, dammarane-type saponins (Piacente et al., 1995; Hu et al., 1996; Liu et al., 2005) isolated from G. pentaphyllum, are major bioactive principles which have been reported to have various in vitro activities such as reducing cholesterol (Kimura et al., 1983; Huang et al., 2005), anti-tumor (Chen et al., 1999; Zhou et al., 2000; Wang et al., 2002; Chiu et al., 2003; Chen et al., 2004) anti-mutagenicity (Kulwat et al., 2005), anti-gastric ulcer (Rujjanawate et al., 2004), anti-thrombotic (Li and Jin, 1989; Tan et al., 1993), immunopotentiating (Zhang et al., 1990; Li and Xing, 1992) and anti-inflammatory (Lin et al., 1993) activities. Several lines of evidence have indicated its efficacy in experimental animals and patients, such as reducing levels of serum triglyceride and cholesterol in rats and quail (la Cour et al., 1995), hepatoprotection in rats (Lin et al., 1993; Lin et al., 2000), cardiovascular protection in anesthetized guinea pigs (Circosta et al., 2005), recovering leukocyte counts and lymphocyte proliferation in cancer patients with radiotherapy or chemotherapy and in irradiated mice (Hou et al., 1991; Chen et al., 1996) and increasing immune responses to ovalbumin in mice (Sun and Zheng, 2005).

Gynura procumbens (Lour.) Merr. is an annual evergreen shrub in the Family Compositae. It has been traditionally used as a topical anti-inflammatory and anti-allergy agent in Thailand (Jiratchariyakul et al., 2000). The ethanol extract of G. procumbens was reported to have anti-hyperglycaemic and anti-hyperlipidaemic activities in streptozotocin-induced diabetic rats (Zhang et al., 2000), and to possess anti-herpes simplex viral activity in vitro (Jiratchariyakul et al., 2000). The

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ethyl acetate fraction of the ethanolic extract demonstrated anti-inflammatory in mice (Iskander et al., 2002). In addition, its aqueous extract could lower blood pressure in spontaneously hypertensive rats (Kim et al., 2006).

_Houttuynia cordata_ Thunb is a plant in the Family Saururaceae and has been traditionally used for treatment of dermatitis, urinary tract infection, and malaria (Bunyapaphatsara et al., 1999). _H. cordata_ was reported to have various _in vitro_ activities such as anti-cancer, anti-bacterial, anti-fungal, antiviral and anti-inflammatory activities (Chavalittumrong et al., 2003). Houttuynin sodium bisulphate, a mixture of sodium bisulphate and houttuynin, was used for the treatment of bovine mastitis (Hu et al., 1997). Sodium houttuynonate, a mixture of sodium bisulfite and houttuynin, was demonstrated to have effects on antibody production, macrophage activities, lymphocyte proliferation and IL-2 secretion in mice (Wang et al., 2002).

_Hyptis suaveolens_ (L.) Poit is a strong aromatic and mosquito-repellent plant in the Family Lamiaceae (Smitinand, 2001; Seyoum et al., 2002). It is used as carminative, antiseptic, sudorific and galactagogue agents (Saluja and Santani, 1993). The essential oil of _H. suaveolens_ inhibited the growth of Gram-positive and Gram-negative bacteria and had a mild inhibitory effect on _C. albicans_ and _Aspergillus nigers_ (Iwu et al., 1990). Methanol extract of _H. suaveolens_ inhibited the growth of _Candida albicans_, selected Gram-positive and Gram-negative bacteria (Rojas et al., 1992). The ethanolic extract of its leaves showed wound healing activity (Shirwaikar et al., 2003). Leaf powder of _H. suaveolens_ could inhibit aflatoxin B production (Krishnamurthy and Shashikala, 2006). Suaveolol and methyl suaveolate, isolated from leaves of _H. suaveolens_, possessed anti-inflammatory activities (Grassi et al., 2006).

_Portulaca grandiflora_ Hook. is a succulent plant in the Family Portulacaceae (Backer & Bakhuizen Van Den Brink, 1963; Liu & Chen, 1976). In oriental traditional medicine, _P. grandiflora_ is used for the relief of sore throat, skin rash and detoxification. _P. grandiflora_ was reported as an effective anti-HBsAg herb by ELISA technique for the recognition of anti-HBsAg capability (Zheng & Zhang, 1990). In addition, antimutagenic effect of _P. grandiflora_ on the mutation induced by aflatoxin B1 and cyclophosphamide in mice was demonstrated (Liu et al., 1990).

_Phytolacca americana_ L. is a plant in the Family Phytolaccaceae. Ethanolic extraction of its fresh roots is used as an emetic (Smitinand, 2001). Phytolacca mitogens, derived from the ethanolic extract of _P. americana_ roots, were found to have a stimulating effect on murine B and T lymphocytes (Yokoyama et al., 1976). Saponin extracts from _P. americana_ demonstrated antifungal (Shao et al., 1999) and anti-viral activities (Uckan et al., 2005).

_Tradescantia spathacea_ Kerr. is a succulent herb in the Family Commelinaceae. In Thai folk medicine, it is used to relieve fever, cough and bronchitis. It was reported to possess antimicrobial, insecticidal, anti-inflammatory, anticancer and anti-fertility activities (Bunyapaphatsara et al., 2000).

A large number of plants used in traditional medicines have been shown to possess non-specific stimulating activities on humoral and cell-mediated immune responses (Azuma, 1987). At present, crude or partial extracts of medicinal plants are widely consumed because they are believed to strengthen human health by boosting the immune system, but with limited scientific information. The present study was, therefore, aimed to screen and elucidate _in vitro_ effects of the above Thai medicinal plants on human lymphocyte proliferation and functions of natural killer (NK) cells.

### Materials and methods

**Plant material**

Plants investigated in this study are summarized in Table 1. These plants were identified and confirmed by comparing with voucher specimens of known identities in the Forest Herbarium (RFD and BKF), Royal Forest Department, and the Bangkok Herbarium (BK), Department of Agriculture, Bangkok, Thailand.
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Table 1. List of plants investigated

<table>
<thead>
<tr>
<th>Name</th>
<th>Origin of collection</th>
<th>Plant parts/solvent</th>
<th>Voucher specimen number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleistocalyx nervosum var paniala</td>
<td>Chiang Mai</td>
<td>fruit/water</td>
<td>BK 3013</td>
</tr>
<tr>
<td>Gynostemma pentaphyllum Makino.</td>
<td>Chiang Rai</td>
<td>aerial part/water</td>
<td>BKF 135338</td>
</tr>
<tr>
<td>Gynura procumbens (Lour.) Merr.</td>
<td>Bangkok</td>
<td>leaf/water</td>
<td>BKF 40176</td>
</tr>
<tr>
<td>Houttuynia cordata Thunb.</td>
<td>Pittsanulok</td>
<td>aerial part/water</td>
<td>BKF 37022</td>
</tr>
<tr>
<td>Hyptis suaveolens (L.) Poit.</td>
<td>Chantaburi</td>
<td>aerial part/water</td>
<td>BKF 082711</td>
</tr>
<tr>
<td>Portulaca grandiflora Hook.</td>
<td>Nonthaburi</td>
<td>aerial part/water</td>
<td>RFD 99057</td>
</tr>
<tr>
<td>Phytolacca americana L.</td>
<td>Pathumthani</td>
<td>root, leaf, fruit, stem/50% ethanol</td>
<td>BK 999</td>
</tr>
<tr>
<td>Tradescantia spathacea Kerr.</td>
<td>Nonthaburi</td>
<td>leaf/water</td>
<td>BK 15143</td>
</tr>
</tbody>
</table>

Extraction

Fresh roots (405 grams), leaves (396 grams), fruits (988 grams) and stems (145 grams) of *P. americana* were extracted with 50% ethanol using a reflux method for 2 hours. The supernatants were dried under vacuum in a rotary evaporator. The yields of dried extracts obtained from roots, leaves, fruits and stems were 7.21, 8.60, 10.84 and 7.96 % w/w, respectively.

Dried *C. nervosum* fruits (40 grams), *G. pentaphyllum* (15 grams), *H. suaveolens* (100 grams) and *P. grandiflora* (15 grams) aerial parts were ground and extracted with distilled water using a reflux method for 2 hours. Filtrates were collected and dried under vacuum in a rotary evaporator. The yields obtained were 8.5, 20, 13, and 20.93 % w/w for *C. nervosum*, *G. pentaphyllum*, *H. suaveolens* and *P. grandiflora*, respectively.

Fresh leaves of *G. procumbens* (100 grams) and *T. spathacea* (200 grams) were blended with water and filtrates were dried under vacuum in a rotary evaporator. The yields of the dried extracts obtained from *G. procumbens* and *T. spathacea* were 4.19 and 3.42 % w/w, respectively.

The fresh aerial part of *H. cordata* (580 grams) was blended with water and its filtrate was lyophilized to give a residue (yield: 2.91 % w/w).

All of the dried extracts were kept in a refrigerator until use. Each extract was dissolved in sterile distilled water for an hour prior to the experiments. It was noted that all extracts, except that of *C. nervosum*, contained very minute amount of non-dissolved residues after 1-hour dissolution.

Subjects

Fresh heparinized blood from healthy Thai donors of the National Blood Bank, the Thai Red Cross Society were collected with permission. Their ages ranged from 20-50 years. None had a history of hepatitis B infection or had a risk for HIV-1 exposure.

Preparation of mononuclear cells

Mononuclear cells were separated from heparinized blood using Ficoll-Hypaque density gradient (Boyum, 1966). The mononuclear cells were counted and adjusted to an appropriate concentration in complete RPMI 1640 (RPMI 1640 medium supplemented with 2 mM glutamine, 10 mM HEPES, 100 U/ml penicillin G, and 100 µg/ml streptomycin) containing 10% fetal bovine serum (FBS; Grand Island Biological Company, Grand Island, NY, USA) for further assays.

Lymphoproliferation assay

Lymphocyte proliferative response to the extract was performed as described (Sriwanthana and Chavalittumrong, 2001). Briefly, purified mononuclear cells (2x10^6 cells/ml) were cultured in triplicate in 96-well microtiter plates (Costar, Cambridge, MA, USA) with the extracts at final
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Concentrations of 1 ng/ml, 10 ng/ml, 100 ng/ml, 1 µg/ml, 5 µg/ml, 10 µg/ml and 100 µg/ml in complete RPMI 1640 containing 10% FBS. The cultures were incubated at 37°C with 5% CO2 for 72 hours. Lymphocyte proliferation was determined by uptake of 3H-thymidine at 18 hours before harvesting. The radioactivity was measured by a liquid scintillation counter (TopCount Microplate Scintillation & Luminescence Counter, Packard Instrumental Co., CT, USA). The degree of activation was expressed as a stimulation index [S.I., i.e., the ratio of the 3H-thymidine uptake in count per minute (CPM) of samples with extract to those without extract]. Phytohemagglutinin (Sigma, St. Louis, MO, USA) at 10 µg/ml was also added to the culture system to check for cell survival and used as a positive control of each assay.

Natural Killer (NK) cell activity assay

Peripheral blood mononuclear cells (PBMC) were washed, resuspended and adjusted to 2x10^6 cells/ml in complete RPMI 1640 containing 10% FBS. PBMC were incubated in the absence or presence of the extracts at the final concentrations of 10 ng/ml, 100 ng/ml, 1 µg/ml, 10 µg/ml and 100 µg/ml at 37°C for 18 hours. After incubation, the cultures were washed and then used as effector cells for the assay of NK cell activity. K 562 cells were used as target cells and were grown in complete RPMI 1640 containing 10% FBS. The target cells (2x10^5 cells) were labeled with 100 µCi of Na_2^{51}CrO_4 (specific activity 37.0 MBq/µg: Amersham, Buckinghamshire, UK) at 37°C 5% CO2 for 60 minutes, and washed 3 times with cold RPMI 1640 containing 10% FBS.

The cytotoxicity assay was performed as described (Sriwanthana and Chavalittumrong, 2001). In brief, 2x10^5 target cells/well and a PBMC effector-to-target cell ratios (E:T) of 90:1, 30:1, 10:1, and 3:1 were set up in triplicate in 96-well round-bottom microtiter plates (Corning Incorporated, Corning, NY, USA). The plates were incubated for four hours at 37°C with 5% CO2. After incubation, supernatants from each well (100 µl) were transferred into tubes and counted in a Gamma counter (Cobra Series Gamma Counter Systems, Packard Instrumental Co., CT, USA). The percentage of cytolysis was calculated according to the following formula: %cytolysis = (experimental release - spontaneous release) / (maximal release - spontaneous release). Spontaneous release was measured by lysis of target cells with medium alone, while maximal release was measured by lysis of target cells with 5% Triton X-100. NK cell activity was expressed as lytic units (LU)/10^7 PBMCs as determined by least squares analysis derived from the percentage of specific lysis of all E:T ratios. One LU was defined as the number of effector cells required for 20% specific lysis of 1x10^4 target cells.

Statistical analysis

Data were expressed as mean±SD. The significance of the results was calculated using Student's paired t-test and statistical significance was defined as P<0.05.

Results

Lymphocyte proliferation

The extract-induced proliferative responses were performed by culturing human lymphocytes in the presence or absence of each extract. It was shown that the responses were significantly elevated with the H. cordata and P. grandiflora extracts ranging from concentrations of 1 ng/ml to 100 µg/ml (Tables 2, 3). Our study showed that the water extract of G. pentaphyllum and G. procumbens significantly increased lymphocyte proliferation at the concentrations of 1 µg/ml to 100 µg/ml (Table 2). Tables 2 and 3 also demonstrate various patterns in stimulating effects of C. nervosum, H. suaveolens and T. spathacea on the proliferative responses of human lymphocytes.

P. americana roots significantly enhanced lymphocyte proliferation at the concentrations of 1 µg/ml to 100 µg/ml, while other parts of P. americana significantly stimulated or decreased lymphoproliferative responses at different concentrations as shown in Table 4.
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Table 2. Effect on lymphocyte proliferation of normal PBMC

<table>
<thead>
<tr>
<th>Concentration</th>
<th><em>C. nervosum</em> (n = 20)</th>
<th><em>G. pentaphyllum</em> (n = 14)</th>
<th><em>G. procumbens</em> (n = 10)</th>
<th><em>H. cordata</em> (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>1.00±0.00</td>
<td>1.00±0.00</td>
<td>1.00±0.00</td>
<td>1.00±0.00</td>
</tr>
<tr>
<td>1 ng/ml</td>
<td>1.87±0.56*</td>
<td>1.15±0.32</td>
<td>1.55±0.78</td>
<td>1.70±0.44*</td>
</tr>
<tr>
<td>10 ng/ml</td>
<td>1.43±0.40*</td>
<td>1.19±0.37</td>
<td>1.18±0.36</td>
<td>1.64±0.55*</td>
</tr>
<tr>
<td>100 ng/ml</td>
<td>0.91±0.32</td>
<td>1.19±0.33</td>
<td>1.13±0.25</td>
<td>1.12±0.23*</td>
</tr>
<tr>
<td>1 µg/ml</td>
<td>1.12±0.33</td>
<td>1.36±0.44*</td>
<td>1.34±0.34*</td>
<td>1.13±0.22*</td>
</tr>
<tr>
<td>5 µg/ml</td>
<td>2.37±0.82*</td>
<td>1.69±0.55*</td>
<td>1.75±0.47*</td>
<td>2.07±0.64*</td>
</tr>
<tr>
<td>10 µg/ml</td>
<td>2.32±1.00*</td>
<td>1.56±0.46*</td>
<td>1.75±0.46*</td>
<td>2.27±0.76*</td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>1.47±0.72*</td>
<td>1.80±0.66*</td>
<td>2.03±0.96*</td>
<td>1.58±0.55*</td>
</tr>
<tr>
<td>PHA</td>
<td>368.63±141.87*</td>
<td>116.82±69.01*</td>
<td>326.96±213.30*</td>
<td>365.90±154.96*</td>
</tr>
</tbody>
</table>

Each value represents Mean±SD
*p< 0.05

Table 3. Effect on lymphocyte proliferation of normal PBMC

<table>
<thead>
<tr>
<th>Concentration</th>
<th><em>H. suaveolens</em> (n = 20)</th>
<th><em>P. grandiflora</em> (n = 60)</th>
<th><em>T. spathacea</em> (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>1.00±0.00</td>
<td>1.00±0.00</td>
<td>1.00±0.00</td>
</tr>
<tr>
<td>1 ng/ml</td>
<td>1.20±0.24*</td>
<td>1.41±0.11*</td>
<td>1.82±1.55*</td>
</tr>
<tr>
<td>10 ng/ml</td>
<td>1.04±0.21</td>
<td>1.41±0.10*</td>
<td>1.41±0.27*</td>
</tr>
<tr>
<td>100 ng/ml</td>
<td>0.98±0.21</td>
<td>1.88±0.11*</td>
<td>0.95±0.19</td>
</tr>
<tr>
<td>1 µg/ml</td>
<td>1.26±0.38*</td>
<td>2.26±0.12*</td>
<td>1.06±0.19</td>
</tr>
<tr>
<td>5 µg/ml</td>
<td>1.56±0.49*</td>
<td>2.59±0.17*</td>
<td>1.70±0.40*</td>
</tr>
<tr>
<td>10 µg/ml</td>
<td>1.42±0.43*</td>
<td>2.64±0.19*</td>
<td>1.53±0.29*</td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>1.28±0.39*</td>
<td>2.86±0.23*</td>
<td>1.16±0.29*</td>
</tr>
<tr>
<td>PHA</td>
<td>106.61±50.48*</td>
<td>120.17±14.10*</td>
<td>335.80±92.60*</td>
</tr>
</tbody>
</table>

Each value represents Mean±SD
*p< 0.05

Table 4. Effect on lymphocyte proliferation of normal PBMC

<table>
<thead>
<tr>
<th>Concentration</th>
<th><em>P. americana</em> (root) (n = 18)</th>
<th><em>P. americana</em> (leaf) (n = 18)</th>
<th><em>P. americana</em> (fruit) (n = 18)</th>
<th><em>P. americana</em> (stem) (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>1.00±0.00</td>
<td>1.00±0.00</td>
<td>1.00±0.00</td>
<td>1.00±0.00</td>
</tr>
<tr>
<td>1 ng/ml</td>
<td>1.06±0.23</td>
<td>1.22±0.39*</td>
<td>1.05±0.22</td>
<td>1.17±0.27*</td>
</tr>
<tr>
<td>10 ng/ml</td>
<td>0.97±0.25</td>
<td>0.98±0.22</td>
<td>0.94±0.20</td>
<td>1.00±0.24</td>
</tr>
<tr>
<td>100 ng/ml</td>
<td>0.94±0.25</td>
<td>0.85±0.21*</td>
<td>0.79±0.22*</td>
<td>0.92±0.27</td>
</tr>
<tr>
<td>1 µg/ml</td>
<td>1.19±0.37*</td>
<td>1.08±0.29</td>
<td>1.06±0.37</td>
<td>1.05±0.22</td>
</tr>
<tr>
<td>5 µg/ml</td>
<td>1.68±0.67*</td>
<td>1.28±0.37*</td>
<td>1.25±0.5*</td>
<td>1.41±0.36*</td>
</tr>
<tr>
<td>10 µg/ml</td>
<td>1.74±0.67*</td>
<td>1.21±0.35*</td>
<td>1.27±0.65</td>
<td>1.15±0.39</td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>2.64±1.12*</td>
<td>1.53±0.52*</td>
<td>1.86±0.95*</td>
<td>1.56±0.76*</td>
</tr>
<tr>
<td>PHA</td>
<td>155.73±108.07*</td>
<td>167.59±118.86*</td>
<td>183.52±134.50*</td>
<td>156.74±100.85*</td>
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</tbody>
</table>

Each value represents Mean±SD
*p< 0.05
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Table 5. NK cell activity by normal PBMC

<table>
<thead>
<tr>
<th>Concentration</th>
<th>C. nervosum (n = 20)</th>
<th>G. pentaphyllum (n = 12)</th>
<th>H. suaveolens (n = 12)</th>
<th>P. grandiflora (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>70.65±39.21</td>
<td>46.12±36.46</td>
<td>46.58±26.09</td>
<td>53.64±34.07</td>
</tr>
<tr>
<td>10 ng/ml</td>
<td>72.00±35.94</td>
<td>42.35±29.81</td>
<td>49.11±32.37</td>
<td>55.28±45.07</td>
</tr>
<tr>
<td>100 ng/ml</td>
<td>70.53±34.12</td>
<td>43.66±29.41</td>
<td>50.55±26.77</td>
<td>57.54±44.31</td>
</tr>
<tr>
<td>1 µg/ml</td>
<td>72.56±36.20</td>
<td>44.51±34.12</td>
<td>50.01±30.14</td>
<td>57.25±42.03</td>
</tr>
<tr>
<td>10 µg/ml</td>
<td>85.52±37.55*</td>
<td>49.10±35.61</td>
<td>56.94±30.95*</td>
<td>52.76±31.82</td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>115.97±51.86*</td>
<td>42.25±31.69</td>
<td>54.35±27.68*</td>
<td>53.04±40.07</td>
</tr>
<tr>
<td>PHA</td>
<td>140.19±36.01*</td>
<td>105.66±48.55*</td>
<td>92.66±22.22*</td>
<td>97.14±27.99*</td>
</tr>
</tbody>
</table>

Each value represents Mean±SD

*p< 0.05

NK cell activity

Results from other pharmacological studies performed in our institutes suggested that C. nervosum, G. pentaphyllum, H. suaveolens and P. grandiflora had high potential for further development as medicines. We, then, examined the effect of these plants on NK cell activity. No significant changes in NK cell activity, as expressed in LU/10⁷ PBMC, were observed in the presence of the water extracts of G. pentaphyllum or P. grandiflora (Table 5). Both C. nervosum and H. suaveolens significantly enhanced NK cell activity, as expressed in LU/10⁷ PBMC, at the concentrations of 10 µg/ml to 100 µg/ml, respectively (Table 5).

The activities, expressed as % lysis, of each extract are illustrated in Figures 1A -1D. Each of the extract significantly altered the function of NK cells at different E:T ratios.

Discussion

A number of herbal medicines and products are claimed to modify or boost immunity without scientific support. In addition, plants cultivated in different parts of the world may not have pharmacological activities as previously reported (Bauer, 2000). Therefore, attempts were made to verify 8 kinds of Thai medicinal plants for in vitro stimulating human lymphocyte activity.

Several lines of evidence have indicated non-specific immunostimulating or immuno-modulating activities of a large number of medicinal plants in experimental animals. Those in vivo studies are cumbersome for initial screening of such activities. Using human lymphocytes in our studies, it was shown that the extracts of G. pentaphyllum, H. cordata and P. americana possess immunostimulating activities. These findings parallel reports from studies in mice (Li and Xing, 1992; Wang et al., 2002; Yokoyama et al., 1976; Zhang et al., 1990), suggesting that human lymphocytes could be used to determine effects of medicinal plants on the immune system. In addition, it may possibly indicate that G. pentaphyllum, H. cordata and P. americana cultivated in Thailand also possess immunostimulating activity. Furthermore, enhanced lymphocyte proliferation was demonstrated with the extracts of G. procumbens and P. grandiflora, suggesting possible roles of G. procumbens and P. grandiflora as putative immunostimulants.

The increase in lymphocyte stimulation, as quantified by stimulation index (S.I.), was also found in cultures containing the extracts of C. nervosum, H. suaveolens and T. spathacea. Significant alteration induced by these extracts, was not dose-dependent. This may possibly be due to variation in chemical constituents responsible for plant activities.

We assessed the effects of C. nervosum, G. pentaphyllum, H. suaveolens and P. grandiflora on NK cell activity, one of natural defense
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Figure 1. In vitro effects of *C. nervosum* (1A), *G. pentaphyllum* (1B), *H. suaveolens* (1C) and *P. grandifora* (1D) on NK activity. PBMC were cultured in the presence and absence of different concentrations of each extract for 18 hrs. NK cell activity was determined by culturing stimulated PBMC with ^51^Cr-K562 cells in triplicate at different E:T ratios for 4 hrs at 37°C. Supernatants were counted for radioactivity and % lysis calculated. Each bar represents mean±SD. *p< 0.05

mechanisms against a variety of infections and cancers (Kuby, 1997). There is no definite rule in reporting NK cell activities. The activities, reported as LU/10^7^ PBMC, apparently demonstrated the effects of each extract as compared with the ones reported in % lysis. Calculation as % lysis may not be comparable with other studies because of differences in E:T ratios used in each study. We, therefore, used activities in LU/10^7^ PBMC in order to assess the effects of each extract on NK cell
function. Our studies demonstrated that the extracts of *C. nervosum* and *H. suaveolens* stimulated NK cell activity. Different immunomodulating profiles may provide a rational basis and support for plant selection aimed at drug discovery.

Our data revealed that those plant extracts show promising lymphocyte stimulating activities on non-specific cell-mediated immune responses. Purification of active components may be required for potential and clinical applications as immunomodulators.

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