RESEARCH ARTICLE

Antioxidant and Anti-cancer Cell Proliferation Activity of Propolis Extracts from Two Extraction Methods

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Abstract

Antioxidant activity, total phenolic, total flavonoid compounds and cytotoxicity to cancer cell lines of propolis extracts from two extraction methods were investigated in this study. Propolis was collected from Phayao province and extracted with 70% ethanol using maceration and sonication techniques. The antioxidant activity was evaluated by DPPH assay. Total phenolic and flavonoid compounds were also determined. Moreover, the cytotoxicity of propolis was evaluated using MTT assay. The percentage propolis yield after extraction using maceration (18.1%) was higher than using sonication (15.7%). Nevertheless, antioxidant and flavonoid compounds of the sonication propolis extract were significant greater than using maceration. Propolis extract from sonication showed antioxidant activity by 3.30±0.15 mg gallic acid equivalents/g extract. Total phenolic compound was 18.3±3.30 mg gallic acid equivalents/g extract and flavonoid compound was 20.49±0.62 mg quercetin/g extract. Additionally, propolis extracts from two extraction methods demonstrated the inhibitory effect on proliferation of A549 and HeLa cancer cell lines at 24, 48 and 72 hours in a dose-dependent manner. These results are of interest for the selection of the most appropriate method for preparation of propolis extracts as potential antioxidant and anticancer agents.

Keywords: Anticancer - antioxidant - flavonoid - maceration - phenolic - propolis - sonication

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Introduction

Propolis is the resinous mixture that bees collect from various parts of plants such as bark gum and use for sealing the pore and protecting the microbe in bee hive (Bankova et al., 2000). Propolis contains several compounds including phenolic, flavonoid, flavones, fatty acid, which have the therapeutic effects such as antimicrobial, antioxidant, immunostimulant and wound healing activities. The essential oil of propolis was found to inhibit the microbial infection and bee wax could supply moisture to human skin (De Castro, 2001). Moreover, propolis showed strong anti-free radical activity, which resulted from the components in propolis including caffeic acid, ferulic acid and caffeic acid phenethyl ester (Kumazawa et al., 2004). Propolis can inhibit the superoxide anion and hydroxyl radical, which are the important and dangerous reactive oxygen species (Nagai et al., 2001; 2003). Free radicals are normally generated from external source and biological process in human body and it can damage the biomolecule including protein, lipid, and genetic material. Moreover, free radicals disturb the homeostasis such as DNA repair, inflammation and cell proliferation (Kryston et al., 2011).

Several methods have been used for extraction of active component in propolis, for example maceration, soxlet extraction, ultrasonic extraction (sonication) and microwave extraction. For maceration technique, organic solvent is used to dissolve the component in propolis directly without producing heat so this technique is suitable for heat labile and heat stable substance (Cunha et al., 2004). Soxlet extraction is performed by heating and condensation to evaporate the organic solvent to concentrate the product. Thus, the advantage of this extraction is the use of low volume of solvent and the extraction is suitable for heat stable substance. Sonication technique by ultrasonic wave can reduce the time and solvent for extraction. This technique is used for dissociation and dissolving propolis. However, during sonication, the heat is produced so the sonication technique is suitable for heat stable substance. In addition, the microwave extraction uses the microwave energy to heat solvent for dissociation of propolis. Thus, using microwave extraction also reduces time and solvent (Trusheva et al., 2007).

The organic solvents were used to extract the chemical composition from propolis such as water, ethanol, methanol, hexane and acetone (Sun and Ho, 2005). The organic solvent can dissolve the different chemical

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compounds according to a polarity of substance. Ethanol was used to extract propolis to generate the fatty acid and flavonoids, while acetone extraction generated monosachride, glycerol and caffeic acid. Alkane, alcohol and bee wax were found in hexane fraction of propolis (Prytzyk et al., 2003). Beside, the extraction time, light and temperature affected propolis extraction (Cunha et al., 2004). Therefore, the aims of this study were to investigate and compare anti-free radical activity, phenolic compound, flavonoid and antiproliferative activity of propolis extracts from two extraction methods

Materials and Methods

Extraction of propolis by maceration

One hundred grams of propolis was cut into small pieces and frozen at -80°C. After that, propolis was ground and extracted with 70% ethanol with the ratio of 1:20. The solution was filtrated and macerated for 72 hrs in the dark. After maceration, the solution was kept in the refrigerator for wax removal overnight. Then, the solution was filtrated through Whatman filter No.1 and evaporated for removal of the organic solvent. The filtrate was lyophilized and dissolved in dimethyl sulfoxide (Trusheva et al., 2007).

Extraction of propolis by sonication

One hundred grams of propolis were cut into small pieces and frozen at -80°C. After that, propolis was ground and extracted with 70% ethanol with the ratio of 1:10. The solution was sonicated using ultrasonic bath at 25°C for 30 minutes in the dark. After sonication, the solution was kept in the refrigerator for wax removal overnight. The solution was filtrated through Whatman filter No.1 and evaporated to remove the organic solvent. The filtrate was lyophilized and dissolved in dimethyl sulfoxide (Trusheva et al., 2007).

Antioxidant activity of propolis extracts

Antioxidant activity of propolis extracts were evaluated by 2,2-diphenyl-1-picrylhydrazyl DPPH method. Propolis extract was dissolved in absolute methanol to obtain concentrations ranging from 0.1-1.0 mg/mL. Then, 500μL of dissolved propolis was mixed with 1.5 mL of 0.1 mM DPPH reagent and incubated at room temperature in the dark for 20 minutes. The absorbance of the reaction was measured at the wavelength of 517 nm and methanol was used as a blank. The antioxidant activity was calculated and compared to gallic acid as an antioxidant standard (Ghasemi et al., 2009).

Quantitation of total phenolic compound

Propolis extract was dissolved with absolute methanol at concentration of 1mg/mL. Then, 250μL of dissolved propolis was mixed with 1.25mL of distilled water, 250μL of ethanol and 125μL of 50% Folin-ciocalteu's reagent. After incubation at room temperature for 5 minutes, 250μL of 5% sodium carbonate was added and incubated at room temperature for 1 hour in the dark. The absorbance of reaction was measured at the wavelength 725nm using ethanol as a blank. The total phenolic compound was calculated from gallic acid standard curve (Ghasemi et al., 2009).

Quantitation of total flavonoid compound

Propolis extract was dissolved in absolute methanol at a concentration of 1 mg/mL and then 500μL of dissolved propolis was added to 1.5mL of methanol, 100μL of 10% aluminium chloride, 100μL of potassium acetate and 2.8mL of distilled water. After incubation at room temperature for 30 minutes, the absorbance was measured at wavelength 415nm. The total flavonoid content was calculated from quercetin standard curve (Ghasemi et al., 2009).

Cell culture

A549 human lung epithelial cells and HeLa cervical cancer cells were cultured in Dulbecco's modified eagle's medium. The cells were incubated at 37°C, 5%CO_2 in a humidified atmosphere and subcultured every 2-3 days.

Determination of cytotoxicity of propolis from two extraction methods

The cytotoxicity of propolis extracts was tested using MTT assay (Umthong et al., 2011). The cells were plated into 96-well plates and incubated at 37°C in 5%CO_2 incubator for 24 hours. After incubation, each concentration of propolis extracts was added. The plate was incubated at 37°C in 5%CO_2 incubator for 24, 48 and 72 hours. Then, the MTT solution was added and incubated for 4 hours. Finally, the blue formazan crystal was dissolved with dimethyl sulfoxide and absorbance was measured at 540 and 630 nm. The percentage of viability was calculated comparing to the cell control.

Statistical analysis

The data were represented as mean and standard deviation of triplicate and repeated in three independent experiments for each test. Anti-free radical activity, phenolic compound and flavonoid were compared between extracted propolis from maceration and sonication using independent sample t-test. The statistically significant differences were expressed with p value<0.05

Results

Physical appearance of propolis and propolis extract

In this research, propolis was collected from Phayao province. The physical appearance of propolis was dark brown color and sticky (Figure 1A). Then, propolis was extracted with 70% ethanol by maceration and sonication. The solution after extraction was dark yellow. After

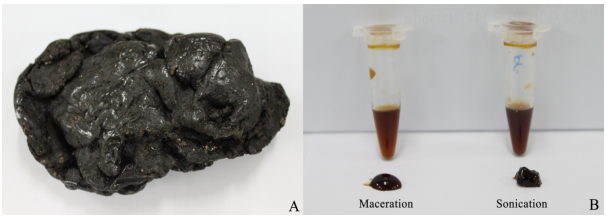

Figure 1. Propolis Collected from Phayao Province (A) and the Appearance of Propolis Extract after Evaporation and Lyophilization (B)

evaporation and lyophilization, propolis extract showed sticky appearance with brown to dark brown color (Figure 1B). The percentage of yields of propolis extract by maceration and sonication were 18.08 and 15.66%, respectively (Table 1).

The antioxidant activity of propolis extracts using 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Propolis extract was mixed with DPPH solution and then the absorbance of sample was compared to gallic acid standard curve for calculation of IC_{50} of propolis extract. After that, the antioxidant activity was calculated as follows: ***Antioxidant activity (mg GAE/g extract)=(IC_{50} of propolis extract ×1,000)/(IC_{50} of gallic acid)***

Propolis extracts from two extraction methods; maceration and sonication were analyzed. It was found that propolis from sonication showed significantly high antioxidant activity more than maceration techniques ($p<0.05$). The propolis from maceration showed the antioxidant activity by 2.69±0.18 mg GAE/g extract while propolis from sonication technique showed the antioxidant activity by 3.30±0.15 mg GAE/g extract (Table 1).

Quantitation of total phenolic compound

Propolis extract was mixed with 50% Folin-ciocalteu's solution and the absorbance of the sample was compared to gallic acid standard curve. Then, the phenolic compound content was calculated as follows: ***Total phenolic compound [mg gallic acid equivalents (GAE)/(g extract]=[(Abs.at 725 nm+0.016)×1,000]/11.15***

Propolis extracts from two methods; maceration and sonication were analysed. The propolis from maceration technique showed phenolic compound content by 17.17±2.19 mg GAE/g extract and propolis from sonication technique showed phenolic compound content by 18.27±3.30 mg GAE/g extract (Table 1).

Table 1. The Percentage of Yield, the Antioxidant Activity, Total Phenolic and Flavonoid Compound Content of Propolis from Maceration and Sonication Tecgniques

Extraction techniques	Yield (%)	Antioxidant activity**	Total phenolic compound**	Total flavonoid compound#
Maceration	18.08	2.69±0.18*	17.17±2.19	18.61±0.52*
Sonication	15.66	3.30±0.15*	18.27±3.30	20.49±0.62*

*p≤0.05 when compare propolis extract from two extraction methods; **mg GAE/g extract; #mg quercetin/g extract

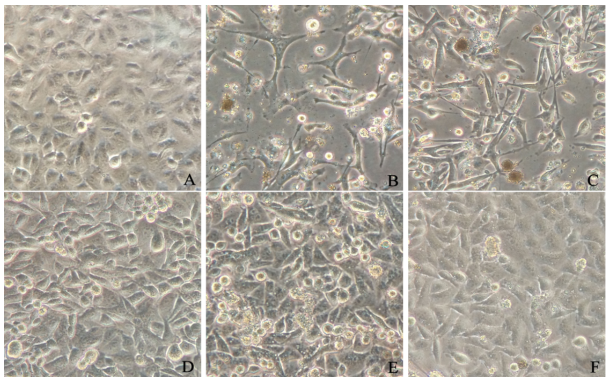

Figure 2. Morphogical Change of A549 and HeLa Cell Lines after Treatment with Propolis 48 Hours at IC_{50} Concentration. A) A549 cells control; **B)** A549 cells treated with propolis from maceration; **C)** A549 cells treated with propolis from sonication; **D)** HeLa cells control; **E)** HeLa cells treated with propolis from maceration; and **F)** HeLa cells treated with propolis from sonication

Quantitation of total flavonoid compound

Total flavonoid compound was analyzed in propolis extract. The propolis extract was mixed with aluminium chloride and potassium acetate, and then the absorbance was measured and compared to quercetin standard curve. After that, the total flavonoid compound content was calculated as follows: ***Total flavonoid compound (mg quercetin/g extract)=[(Abs.at 415 nm+0.007)×1,000]/6.061***

From two extraction method, propolis extract from sonication showed flavonoid compound significantly higher than maceration techniques ($p<0.05$). Propolis from maceration showed the total flavonoid compound content by 18.61±0.52 mg quercetin/g extract, while flavonoid compound extracted from sonication technique was 20.49±0.62 mg quercetin/g extract (Table 1).

The cytotoxicity of propolis on A549 and HeLa cell lines

After treatment A549 and HeLa cell lines with propolis extract from two extraction methods, the morphology of A549 and HeLa cell lines were abnormal comparing to untreated cell control. Propolis induced morphological change of treated cells such as cell shrinkage and floating in medium (Figure 2). The alteration of cell morphology was observed on A549 cell line more than HeLa cell line. The cytotoxicity of propolis to A549 and HeLa cell lines were evaluated using MTT assay and found that both A549 and HeLa cell lines could be inhibited after 24, 48 and 72 hours of treatment with propolis extracts from two extraction method in dose-dependent manner. Percentage of cell viability of A549 human lung epithelial and HeLa cell lines after treatment with propolis extract from maceration and sonication techniques was shown

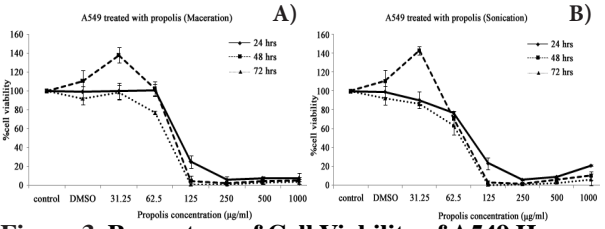

Figure 3. Percentage of Cell Viability of A549 Human Lung Epithelial Cell Lines after Treatment with Propolis Extract from A) Maceration technique; and B) Sonication technique

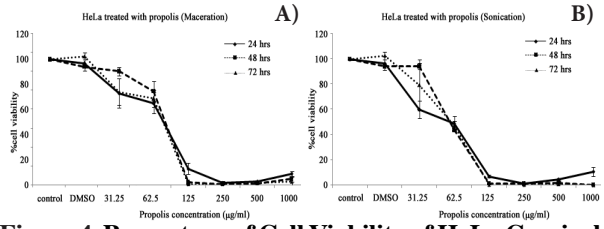

Figure 4. Percentage of Cell Viability of HeLa Cervical Cell Lines after Treatment with Propolis Extract from A) Maceration technique; and B) Sonication technique

Table 2. The 50% Inhibitory Concentration of Propolis Extract from Maceration and Sonication on A549 and HeLa Cell Lines after Treatement 24, 48 and 72 Hours

Propolis	50% Inhibitory concentration (IC_{50}; μg/ml)					
	A549 cell lines			HeLa cell lines		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Maceration	104.55	96.11	85.05	80.96	83.89	79.83
Sonication	93.96	81.99	76.02	58.77	58.66	59.61

in Figure 3-4. The 50% inhibitory concentration (IC_{50}) after treatment with propolis extract from maceration technique at 24, 48 and 72 hours on A549 cell lines were 104.55, 96.11 and 85.05 μg/ml, respectively, while IC_{50} values of propolis extract from sonication technique were 93.96, 81.99 and 76.02 μg/ml, respectively. The IC_{50} after treatment HeLa cell lines for 24, 48 and 72 hours with propolis extract from maceration technique were 80.96, 83.89 and 79.83 μg/ml, respectively whereas IC_{50} of propolis extract from sonication technique were 58.77, 58.66 and 59.61 μg/ml, respectively (Table 2).

Discussion

Propolis extraction can be performed by several techniques. Different components in propolis were shown from each extraction method (Trusheva et al., 2007). Extraction of propolis using maceration had the percentage of yield higher than sonication techniques. Whereas, propolis extraction by sonication techniques showed antioxidant activity, total phenolic and flavonoid compound content higher than maceration. Thus, in this study using sonication techniques was better than maceration for extraction of propolis. However, percentage of yield, antioxidant activity, total phenolic and flavonoid compound content in propolis from Phayao province was lower than the study by Trusheva et al. (2007). These may due to the difference of propolis source and active component of propolis as different geographic property affects chemical composition and biological activity of propolis (Kujumgiev et al., 1999). Propolis form Europe and China had high flavonoid and phenolic contens (Bankova et al., 2000). On the other hand, propolis from Brazil showed high terpenoid and cumaric acid derivative (Kumazawa et al., 2003). Moreover, propolis extracts by maceration and sonication showed the same contents of flavonoid and flavones compounds (Marghitas et al., 2007).

The ratio of propolis and organic solvent was selected at 1:10 or 1:20. It did not affect the extraction of active compound in propolis (Trusheva et al., 2007). In addition, the use of high concentration of ethanol did not affect percentage of extract yield. However, the use of ethanol at 70% in this study was better than the use of ethanol at concentration that lower than 50% or water since unrequired lipid wax was not extracted by 70% ethanol (Cunha et al., 2004). Increasing of ethanol concentration at 30, 40, 50, 70% affected total phenolic compound and DPPH activity due to polyphenolic compound in propolis could easily dissolve in ethanol more than water (Siripatrawan et al., 2013). Similarly to the study of Park et al. (1998) and Cvek et al. (2007) revealed that the use of ethanol at concentration more than 70% did not assist to extract the phenolic compound in propolis extraction process (Park et al., 1998 and Cvek et al., 2007).

The study of antiproliferative activity of propolis extract against A549 and HeLa cancer cell lines showed that the propolis from two extraction methods could inhibit the proliferative of A549 and HeLa cells in dose-dependent manner. The comparison between the extractions methods of propolis demonstrated that the IC_{50} of propolis from sonication less than maceration, which indicated that propolis extract from sonication technique had toxicity to cancer cells higher than extract from maceration technique. The results showed that the IC_{50} of HeLa cell treated with propolis less than IC_{50} of A549 cells. Thus, toxicity of propolis extract on HeLa more than A549 cells.

Moreover, other study of the toxicity of propolis on A549 cells revealed that propolis from Mexico inhibited the proliferation of A549 cells and the IC_{50} was 6.2 μM which lower than IC_{50} of anticancer drug (5-fluorouracil) (Li et al., 2010). Beside, the HeLa cells were inhibited with ethanolic extract of propolis from Brazil for 48 hours with IC_{50} of 7.45 μg/ml (Alencar et al., 2007). From our result, the propolis from sonication techniques, which had the higher flavonoid content also showed antiproliferative activity to cancer cells more than propolis extract by maceration technique. Thus, flavonoid content may be related to the antiproliferative activity of propolis on cancer cells. Barbaric et al. (2011) studied the propolis composition and the antiproliferative activity on HeLa cells. Propolis composed of flavonoids group including tectochrysin, galangin, pinocembrin and pinocembrin-7-methylether, and these substances could inhibit HeLa cells proliferation more than other substance (Barbaric et al., 2011). Moreover, the inhibition of cancer cells growth depended on the geography and source of propolis which affected the composition of propolis (Syamsudin et al., 2009). The studies of antiproliferation of propolis from *Apis melifera* found that propolis could inhibit the proliferation of breast, lung, oral and leukemic cancer cell lines (Kaewmuangmoon et al., 2012). Propolis form *Trigona laeviceps*, which is the stringless bee, could inhibit the proliferation of colon, breast, liver and lung cancer cell lines (Umthong et al., 2011). The cancer prevention of propolis was studied in mice treated with carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), that enhances the lung tumor. The result showed that the mice, which was fed with propolis before NNK demonstrated the reduction of lung tumor incidence when compared to NNK treated group (Sugimoto et al., 2003). Thus, propolis should be used as cancer prevention agent.

Several studies reported that propolis showed the antimicrobial activity against gram positive and negative bacteria such as *Staphylococcus aureus, Escherichia coli, Bacillus cereus, B. subtilis, Salmonella typhimurium, Enterococcus faecalis* and *Pseudomonas aeruginosa. Candida albicans, C. tropicalis, Tenia versicolor* and *Aspergillus flavus* were also inhibited by propolis. Moreover propolis inhibited viruses including avian influenza virus and herpes simplex virus type 1 (Amoros et al., 1994; Kujumgiev et al., 1999; Uzel et al., 2005; Choi et al., 2006; Ngatu et al., 2011; Kaewmuangmoon

et al., 2012).

The extraction of propolis using maceration and sonication affected biological properties of propolis especially antioxidant activity, antiproliferative activity, total phenolic and flavonoid compound contents. These two methods gave the different active compounds. Therefore, the data from this study can use as a guideline for selection of extraction method to evaluate biological properties of propolis. Moreover, these results will be applied for the selection of extraction method for preparation of propolis extract as potential antioxidant and anticancer agents. Further study of antiproliferative activity on A549 and HeLa cell lines should be performed to evaluate mechanism of anticancer activity of propolis extracts. Moreover, application of propolis to prevent cancer in animal model should be evaluated.

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References

Alencar SM, Oldoni TLC, Castro ML, et al (2007). Chemical composition and biological activity of a new type of Brazilian propolis: Red propolis. *J Ethanopharmacol*, **113**, 278-83.

Amoros M, Lurton E, Boustie J, et al (1994). Comparison of the anti-herpes simplex virus activities of propolis and 3-methyl-but-2-enylcaffeate. *J Nat Prod*, **57**, 644-7.

Bankova V, Castro SL, Marcucci MC (2000). Propolis: recent advances in chemistry and plant origin. *Apidologie*, **31**, 3-15.

Barbaric M, Miskovic K, Bojic M, et al (2011). Chemical composition of the ethanolic propolis extracts and its effect on HeLa cells. *J Ethnopharmacol*, **135**, 772-8.

Choi YM, Noh DO, Cho SY, et al (2006). Antioxidant and antimicrobial activities of propolis from several regions of Korea. *LWT - Food Sci Technol*, **39**, 756-61.

Cunha IBS, Sawaya AC, Caetano FM, et al (2004). Factors that influence the yield and composition of brazilian propolis extracts. *J Brazil Chem Soc*, **15**, 964-70.

Cvek J, Medic-saric M, Jasprica I, et al (2007). Optimization of an extraction procedure and chemical characterization of Croatian propolis tinctures. *Phytochem Analysis*, **18**, 451-9.

De Castro SL (2001). Propolis: biological and pharmacological activities. *ARBS*, **3**, 49-83.

Ghasemi K, Ghasemi Y, Ebrahimzadeh MA (2009). Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. *Pak J Pharm Sci*, **22**, 277-81.

Kaewmuangmoon J, Nonthapa P, Rattanawannee A, et al (2012). Preliminary screening for various bioactivities in honey and propolis extracts from Thai bees. *Eur J Med Plants*, **2**, 74-92.

Kryston TB, Georgiev AB, Pissis P, et al (2011). Role of oxidative stress and DNA damage in human carcinogenesis. *Mutat Res-Fund Mol M*, **711**, 193-201.

Kujumgiev A, Tsvetkova I, Serkedjieva Y, et al (1999). Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. *J Ethnopharmacol*, **64**, 235-40.

Kumazawa S, Yoneda M, Shibata I, et al (2003). Direct evidence for the plant origin of Brazilian propolis by the observation of honeybee behavior and phytochemical analysis. *Chem Pharm Bull*, **51**,740-2.

Kumazawa S, Hamasaka T, Nakayama T (2004). Antioxidant activity of propolis of various geographic origins. *Food Chem*, **84**, 329-39.

Li F, Awale S, Tezuka Y, et al (2010). Cytotoxicity of constituents from Mexican propolis against a panel of six different cancer cell lines. *Nat Prod Commun*, **5**, 1601-6.

Marghitas LAI, Dezmirean D, Laslo L, et al (2007). Validated method for estimatation of total flavonoids in Romanian propolis. *Bulletin USAMV-CN*, **64**, 164-9.

Nagai T, Sakai M, Inoue R, et al (2001). Antioxidative activities of some commercially honeys, royal jelly and propolis. *Food Chem*, **75**, 237-40.

Nagai T, Inoue R, Inoue H, et al (2003). Preparation and antioxidant properties of water extract of propolis. *Food Chem*, **80**, 29-33.

Ngatu NR, Saruta T, Hirota R, et al (2011). Antifungal efficacy of Brazilian green propolis extracts and honey on *Tinea capitis* and *Tinea versicolor*. *Eur J Integr Med*, **3**, 281-7.

Park YK, Ikegaki M (1998). Preparation of water and ethanolic extracts of propolis and evaluation of the preparations. *Biosci Biotechnol Biochem*, **62**, 2230-332.

Prytzyk E, Dantas AP, Salomao K, et al (2003). Flavonoids and trypanocidal activity of Bulgarian propolis. *J Ethnopharmacol*, **88**, 189-93.

Siripatrawan U, Vitchayakitti W, Sanguandeekul R (2013). Antioxidant and antimicrobial properties of Thai propolis extracted using ethanol aqueous solution. *Int J Food Sci Tech*, **48**, 22-7.

Sugimoto Y, Iba Y, Kayasuga R, et al (2003). Inhibitory effects of propolis granular A.P.C on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis in A/J mice. *Cancer Lett*, **193**, 155-9.

Sun T, Ho C (2005). Antioxidant activities of buckwheat extracts. *Food Chem*, **90**, 743-49.

Syamsudin SW, Simanjuntak O, Hellfen WL (2009). Chemical composition of propolis from different regions in Java and their cytotoxicity activity. *Am J Biochem Biotechnol*, **5**, 180-3.

Trusheva B, Trunkova D, Bankova V (2007). Different extraction methods of biologically active components from propolis: a preliminary study. *Chem Cent J*, **1**, 1-4.

Umthong S, Phuwapraisirisan P, Puthong S, et al (2011). In vitro antiproliferative activity of partially purified *Trigona laeviceps* propolis from Thailand on human cancer cell lines. *BMC Complem Altern M*, **11**, 1-8.

Uzel A, Sorkun KY, Oncag O, et al (2005). Chemical composition and antimicrobial activities of four different Anatolian propolis samples. *Microbiol Res*, **160**, 189-95.