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In vitro cytotoxicity of Indonesian stingless bee products against human cancer cell lines

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PEER REVIEW

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Comments

This is a valuable study work in which the authors threw light on the relationship between stingless bee products and human's most serious diseases, which is cancer using human cancer cell lines in a new way, and showed that the propolis and honey from some species of stingless bees should have anticancer activities.

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ABSTRACT

Objective: To screen crude extracts of propolis, bee pollen and honey from four stingless bee species [*Trigona incisa* (*T. incisa*)], *Timia apicalis*, *Trigona fusco-balteata* and *Trigona fuscibasis*) native to East Kalimantan, Indonesia for cytotoxic activity against five human cancer cell lines (HepG2, SW620, ChaGo-I, KATO-III and BT474).

Methods: All samples were extracted with methanol, and then subpartitioned with *n*-hexane and ethyl acetate. Each crude extract was screened at 20 μg/mL for *in vitro* cytotoxicity against the cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. In addition, four previously shown bioactive components from propolis (apigenin, caffeic acid phenyl ester, kaempferol and naringenin) and two chemotherapeutic drugs (doxorubicin and 5-fluorouracil) were used to evaluate the sensitivity of the cell lines.

Results: Overall, crude extracts from propolis and honey had higher cytotoxic activities than bee pollen, but the activity was dependent upon the extraction solvent, bee species and cell line. Propolis extracts from *T. incisa* and *Timia apicalis* showed the highest and lowest cytotoxic activity, respectively. Only the HepG2 cell line was broadly sensitive to the honey extracts. For pure compounds, doxorubicin was the most cytotoxic, the four propolis compounds the least, but the ChaGo-I cell line was sensitive to kaempferol at 10 μg/mL and KATO-III was sensitive to kaempferol and apigenin at 10 μg/mL. All pure compounds were effective against the BT474 cell line.

Conclusions: Propolis from *T. incisa* and *Trigona fusco-balteata* contain an *in vitro* cytotoxic activity against human cancer cell lines. Further study is required, including the isolation and characterization of the active antiproliferative agent(s).

KEYWORDS

Antiproliferative activity, Bee product, Cancer cell lines, Cytotoxicity, Ethyl acetate extract, *n*-Hexane, Honey, Methanol, Propolis

1. Introduction

Propolis, bee pollen and royal jelly are bee products that have been ascribed several medical properties in both traditional medicine and more recently in conventional medicine[1]. Bee pollen is a granular composite of pollen collected by bees from various flowers and then compacted with a sticky substance produced by the bee. Propolis, often called bee glue, is produced by worker bees from collected plant resins or exudates from phloem-feeding insects, and

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is used to assemble, protect, or repair the bee hives[2]. Royal jelly is secreted by nurse bees and fed to all bee larvae during their first 3 days of development, but the continuous feeding to larvae at sufficient levels thereafter promotes the developmental switch to queen and not worker bees[3].

In recent decades, propolis has attracted increasing attention and use in foods, beverages, supplements and cosmetics for both medicinal treatment and beneficial health reasons (preventative medicine). It is used to prevent or reduce some diseases or symptoms, such as inflammation, heart disease and cancer[4-6]. Propolis has been shown to have various biological activities, such as antibacterial, anti-inflammatory, antioxidant and anticancer properties, in support of its ancient use as a folk medicine in many regions of the world[7,8]. The chemical components of propolis depend on the resin from the vegetation within the foraging region of the bees and the plants the bees select for collection from, since honeybees preferentially target certain plants within range of their beehives as sources of propolis. Thus, propolis has been found to have a seasonal, geophysical regional and bee species specificity to its composition and bioactivity[9]. For example, *Apis mellifera* (*A. mellifera*) propolis collected in temperate regions contains many kinds of flavonoids and phenolic acid esters, particularly pinocembrin, pinobanksin, galangin, chrysin and caffeic acid phenyl ester (CAPE), as the main bioactive compounds[7,10]. The propolis from these regions was shown to have been collected from the bud exudates of members of the *Populus* genus[7,10]. However, the chemical composition of *A. mellifera* propolis has been found to be quite complicated with more than 300 identified compounds, such as polyphenols, phenolic aldehydes, sesquiterpene quinones, coumarins, amino acids, steroids and inorganic compounds, and to vary depending on the collecting location, time and plant source[4]. In contrast to *A. mellifera* propolis, the propolis from *Tetragonula carbonaria*, a stingless bee native to Australia, contained several isomers of pimaric acid and gallic acid as its main components[11,12]. Also, it was reported that eucalypt resin, especially that from *Corymbia torelliana*, shaped the chemical constituents in this stingless bee propolis[12].

Besides *A. mellifera* propolis, Sawaya *et al*. reported the antioxidant activity of propolis from three stingless species[13], which were *Scaptotrigona* spp., *Scaptotrigona depilis* and *Scaptotrigona bipunctata*. Samples were collected monthly over a one-year period. *Scaptotrigona* spp. propolis was collected from the northeastern region of Brazil, while the rest was collected from the southeastern region of the country. Using the 1,1-diphenyl-2-picrylhydrazyl free radical scavenging method (DPPH), the composition of the samples and the antioxidant activity was assayed and found to vary according to the bee species, geographic region and month of collection.

Recently, nine species of stingless bees were recorded in the Mulawarman University Botanical Garden, Samarinda, Indonesia [*Trigona apicalis* (*T. apicalis*), *Trigona drescheri*, *Trigona fuscibasis* (*T. fuscibasis*), *Trigona fuscobalteata* (*T. fuscobalteata*), *Trigona incisa* (*T. incisa*), *Trigona itama*, *Trigona laeviceps*, *Trigona melina* and *Trigona terminate*][14]. Thirty nine plant species from 13 families were reported to act as their pollen source, whilst 22 plant species from 17 families belonging to forest plants and crops were reported to act as their nectar source[14]. The products of those stingless bees were honey (15.4%), beebread (20.9%) and propolis (63.7%).

The existence of stingless bees in the Mulawarman University Botanical Garden is likely to be important in terms of the economics and ecology of the region, since these bees are essential in pollination and can also make useful bee products that can be harvested and applied in food products. However, no research on the bioactivities of products from stingless bees in this region has been reported, yet it is likely to be of interest given that the biological activities of propolis can vary greatly across different phytogeographical areas, time periods[4], and within the same region. Moreover, the bioactivity of bee products from different races or species of bees can also be different. For example, the propolis of *Apis mellifera caucasica*, *Apis mellifera anatolica* and *Apis mellifera carnica* collected from the same apiary in East Anatolia contained different chemical compositions and had different antimicrobial activities[15]. Hence, in this research, the *in vitro* cytotoxic activity of the propolis, bee pollen and honey from four stingless bees collected from within the same area (Mulawarman University Botanical Garden, Samarinda, Indonesia) was evaluated. Crude methanol, *n*-hexane and ethyl acetate extracts of those bee products were prepared and tested for their *in vitro* cytotoxic activity against five human cancer cell lines. In addition, the sensitivity of these five cell lines to four pure compounds (apigenin, CAPE, kaempferol and naringenin) previously reported to be some of the main bioactive components in *A. mellifera* propolis were evaluated in comparison with doxorubicin and 5-fluorouracil (5-FU), two standard chemotherapeutic drugs.

2. Materials and methods

2.1. Sample collection

Propolis, bee pollen and honey were each harvested from four stingless bee species [*T. incisa*, *T. apicalis*, *T. fuscobalteata* and *Trigona fuscibisca* (*T. fuscibisca*)] collected in Mulawarman University Botanical Garden, Samarinda, East Kalimantan, Indonesia in February, 2013. All samples were kept at -20 °C until used.

2.2. Sample preparation

Propolis was cut into small pieces and ground. Bee pollen was collected by sifting with a sieve. Honey was filtered to remove the residual comb and solid matter, such as bee remains, and then by a sifter with the honey liquid being used directly. The samples (Table 1 for amounts) were then separately extracted in three times the volume of 96% (v/v) methanol at room temperature (RT) with continuous shaking (7400 Tübingen; Edmun Buchler, Germany) at 100 r/min for 24 h. This process was repeated until the color of extract was