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Therapeutic Evaluation of Ethanolic Bee Pollen Extract from Malaysian Stingless Bee in MCF-7 and MCF-10A Cell Lines

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ABSTRACT

Bee pollen is a complete food containing comprehensive nutrients and therapeutic properties that could eliminate free radicals. Three stingless bee species native to Malaysia were used to prepare bee pollen ethanolic extracts (BPE): *Geniotrigona thoracica*, *Heterotrigona itama*, and *Tetrigona apicalis*. The methodology used in this study was a trypan blue exclusion assay for cell proliferation activity in cultured breast adenocarcinoma human cell lines (MCF-7) and mammary epithelial human cell lines (MCF-10A). In addition, the therapeutic index (TI) was analyzed to assess the relative antiproliferative activity of BPE on cancer versus normal cells. *Geniotrigona thoracica* BPE exhibited the highest therapeutic index (TI = 3.12) compared to *H. itama* (TI = 1.16) and *T. apicalis* (TI = 0.90) BPE. Each species represents different bioactive compounds due to different pollen foraging activities. Therefore, the highest TI species (*G. thoracica*) could be a potential candidate to be developed as a potential chemotherapeutic agent.

Keywords: MCF-7, MCF-10A, stingless bee pollen, therapeutic index

INTRODUCTION

Environment, nutrition, and lifestyle factors play a significant role in influencing cancer development. Cancer could be considered a leading cause of mortality globally, including breast cancer. It is the most common cancer to cause mortality in women (Azamjah et al., 2019; Seely & Alhassan, 2018). Therefore, there is a lot of research on cancer chemoprevention and therapy using nutritional supplementation and

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traditional medicines to treat cancer using various natural compounds (Cha et al., 2005; Choudhari et al., 2013).

Compounds in natural products, such as herbal and bee products, offer many research opportunities in developing anticancer agents that have nutraceutical properties, are non-toxic, and safe for human health (Kuppusamy et al., 2014; Premratanachai & Chanchao, 2014; Wang et al., 2012). Natural compounds in bee products, including honey, propolis, and bee pollen, were found to significantly inhibit cell growth and reduce tumor cells proliferation (Ahmad et al., 2019; Choudhari et al., 2013; Franchi et al., 2012; Kustiawan et al., 2014; T-Johari et al., 2019). In Malaysia, 45 stingless bee species from 14 genera were documented (Norowi et al., 2010; Samsudin et al., 2018), including *Geniotrigona thoracica*, *Heterotrigona itama*, and *Tetrigona apicalis*, which are commonly domesticated for honey production.

Other than honey, stingless bee produces bee pollen that serves as a source of nutrients for both adult bees and larvae. It is known as a complete food since the food energy produced is relatively high, ranging from 396.4 to 411.1 kcal/100 g of pollen (Kocot et al., 2018). Bee pollen is also a popular health supplement for maintaining health and longevity. Studies have demonstrated that 70% of bee pollen compositions are biologically active and exhibit numerous benefits, including cardioprotection, hepatoprotection, antioxidation, anticarcinogen, antibacterial, antiosteoporosis, antiprostatitis, anti-anemia, anti-aging, anti-inflammatory, and immunostimulant (Campos et al., 2010; Rzepecka-Stojko et al., 2015; Yang et al., 2013).

In vitro antiproliferative activity of bee pollen extract can be determined based on the minimum effective concentrations (EC_{50}) in cancer cells and the minimum inhibitory concentrations (IC_{50}) in normal cells. EC_{50} and IC_{50}, however, are often used interchangeably. The terms determine the tested compounds or drugs with the desired properties and qualities (Sebaugh, 2011). EC_{50} is expressed as the effective concentration of a molecule where 50% of the bioactivity is observed and is commonly used to describe the stimulation of responses (MarÉchal, 2011). IC_{50}, on the other hand, is defined as the inhibitor concentration that decreases the response to 50% of its maximum. That maximum response (or binding) is inhibited due to an inhibitor's action that binds to a receptor (MarÉchal, 2011).

In drug development, the therapeutic index (TI) is expressed as the ratio of dosage with the maximum exposure that is not toxic (with fewer adverse effects) to the dosage that indicates the preferred pharmacological outcome (Muller & Milton, 2012). TI is a quantitative relationship between the drug's safety (toxicology) and efficacy (pharmacology). *In vitro* safety assays, such as cytotoxicity tests, determine the IC50 (minimum toxic concentrations), and *in vitro* efficacy assays, such as growth inhibition of tumor cells, determine the EC_{50} (minimum effective concentrations) (Muller

& Milton, 2012). IC_{50}/EC_{50} is typically used to measure the effectiveness of a compound in biochemical or biological studies in cells.

TI provides a semiquantitative evaluation of the concentrations used to attain the expected response with a tolerable level of adverse effects. It is expressed as the ratio of the IC_{50} of normal/non-tumor cells to the EC_{50} of tumor cells (Deepa et al., 2012). TI with a high value indicates more safety and specificity in targeting cancer cells (Abughazaleh & Tracy, 2014; Muller & Milton, 2012). The United States Food and Drug Administration (FDA) describes drugs with a low TI as less than a 2-fold difference in the IC_{50} and EC_{50} (Abughazaleh & Tracy, 2014). However, there is no clear FDA guidance on the use of TI for clinical trials, believing that each drug is unique.

In antiproliferative or cytotoxicity assay, *in vitro* cell cultures are frequently identified as alive or dead based on membrane integrity. Different assays can detect dead cells accumulation, including the measurement of cytoplasm's component leakage into the culture medium (by enzyme or fluorescent marker) or non-permeable dye penetration into cells with damaged membranes (trypan blue or fluorogenic DNA binding dyes) (Riss et al., 2019).

By using the trypan blue exclusion method, the toxicity of compounds and inhibition of tumor cells can be evaluated. This method observes cell membrane integrity and detects nonviable cells in non-dividing cell populations (Aslantürk, 2018). The number of viable cells can be determined based on the fundamental that intact membranes of living cells exclude trypan blue dye, exhibiting a perfect cytoplasm. Meanwhile, a nonviable cell exhibits blue ruptured cytoplasm.

Antiproliferative and cytotoxicity are terms that are being used interchangeably. The term cytotoxic refers to chemicals that cause cell toxicity, such as anticancer agents or chemotherapy treatments that aim to kill cancer cells and stop their growth (Kandaswami, 2014). On the other hand, the antiproliferative assay determines cell viability and cell proliferation, a measure of mammalian cell growth and survival (Kandaswami, 2014). Thus, the trypan blue exclusion method is better known as an antiproliferative viability assay that can estimate the rate of proliferation and the percentage of viable/nonviable cells (Strober, 2015).

This study evaluated the antiproliferative effect of three stingless bee pollen extracts, *G. thoracica*, *H. itama*, and *T. apicalis*, on two cell lines (MCF-7 and MCF-10A). In addition, the therapeutic index was analyzed to assess the relative antiproliferative activity of BPE on cancer versus normal cells.

MATERIALS AND METHODS

Preparation of Bee Pollen Extract (BPE)

Malaysian stingless bee species were collected from Syamille Agrofarm, Kuala Kangsar, Perak, Malaysia, i.e., *Geniotrigona thoracica* (*G. thoracica*), *Heterotrigona itama* (*H. itama*), and *Tetrigona apicalis*

(*T. apicalis*). The pollen samples were collected from two different colonies for each species. They were extracted in ethanol (10% w/v), sonicated in an ultrasound bath at 41°C (90 min), centrifuged at 2,800 × *g* (5 min), filtered, and dried in a rotary evaporator (EYELA OSB-2100, Japan). The BPE was then freeze-dried for four days (Martin Christ Alpha freeze dryer, Germany) and kept at 4°C to be used later in the experiment.

Cell Lines

Mammary epithelial human cell lines (MCF-10A) were maintained in a 75 cm^3 cell culture flask containing Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12) medium supplemented with serum from the horse (5% v/v), recombinant insulin from human (10 µg/mL), recombinant epidermal growth factor from human (hEGF, 20 ng/mL), hydrocortisone (0.5 µg/mL) and penicillin-streptomycin (1% v/v).

Breast adenocarcinoma human cell lines (MCF-7) were grown in a 75 cm^3 cell culture flask containing RPMI 1640 medium supplemented with serum from fetal bovine (10% v/v) and penicillin-streptomycin (1% v/v). All media and supplements were purchased from Sigma (USA) and Gibco (USA).

Cell lines were preserved in humidified air with 5% CO_2 at 37°C and subcultured every two to three days. Trypsin was used to detach cells with 80% viability for trypan blue exclusion assay. MCF-10A and MCF-7 ATCC cell lines were obtained from Advanced Medical and Dental Institute, Universiti Sains Malaysia (USM).

Trypan Blue Exclusion Assay

Antiproliferative activity was evaluated using the trypan blue dye exclusion assay. MCF-10A and MCF-7 cells were seeded at 5.0×10^4 cells/well in 24-well plates and allowed to adhere for 36 h to 48 h. After reaching 70% to 80% confluency, cells were treated for 24 h with different BPE concentrations ranging from 0.3 to 5.0 mg/mL dissolved in the growth medium, while in control cells, no BPE treatment was applied.

After 24 h treatment with BPE, phosphate-buffered saline (PBS) was used to wash the cells, followed by centrifuging the cells at 112 × *g* (5 min) and discarding the supernatant. PBS was used again to resuspend the cell pellet and mixed with trypan blue (0.4%, 10:10 µL) in a microplate well. Ten µL of the trypan blue/cell mixture was taken into a hemocytometer, and cells were observed under a microscope. The unstained (viable) and stained (nonviable) cells attached to the hemocytometer were calculated. The viable cells' total number was multiplied by the trypan blue dilution factor (×2) to obtain viable cells per mL. The percentage of viable cells was estimated as (viable cells number per mL/total number of cells per mL) × 100 (Strober, 2019).

A dose-response curve of sample concentration versus cell viability was consequently plotted. Finally, the 50% inhibitory concentration in MCF-10A cells (IC_{50}) and 50% effective concentration in

MCF-7 were calculated by interpolating the plotted graph using Microsoft Excel (version 16.37). All three experiments were done as independent experiments, each performed in duplicate.

Therapeutic Index

The therapeutic index (TI) was estimated as follows: TI = (IC_{50} non-neoplastic cell) / (EC_{50} neoplastic cell) (Deepa et al., 2012). MCF-10A was non-neoplastic (normal) cells and MCF-7 was neoplastic (cancer) cells. A compound with a high therapeutic index is potent compared with a low therapeutic index compound.

Statistical Analysis

All experiments were duplicated and presented as mean ± standard deviation (SD). The significant differences between experimental groups were assessed by Student's *t*-test using IBM SPSS Statistics version 24.0. (IBM Corp., USA). Statistically significant data was presented with *P* values ≤ 0.05.

RESULTS

Antiproliferative Activity of *T. apicalis* BPE in MCF-7 and MCF-10A Cells

Different concentrations of *T. apicalis* BPE on the viability of MCF-7 and MCF-10A cells treated at 24 h were demonstrated in Figure 1. The effect of BPE from *T. apicalis* showed a dose-dependent increase in MCF-7 cell inhibition. Both colonies of *T. apicalis* BPE inhibited MCF-7 cells at EC_{50} of 1.60 + 0.10 mg/mL (Figure 1a). Meanwhile, the IC_{50} of *T. apicalis* in MCF-10A cells was 1.46 + 0.51 mg/mL (Figure 1b).

When the EC_{50} and IC_{50} values were evaluated using Student's *t*-test, no significant difference was seen with *p*-values of 0.161 and 0.567 for both colonies of *T. apicalis* (i) and (ii), respectively. In addition, the result showed no difference in EC_{50} value in MCF-7 cells compared with the IC_{50} value in MCF-10A cells.

Antiproliferative Activity of *H. itama* BPE in MCF-7 and MCF-10A Cells

Extrapolation from Figure 2a showed that the EC_{50} for MCF-7 cells was 1.72 + 0.28 mg/mL for *H. itama*. In MCF-10A cells, the IC_{50} was 1.91 + 0.72 mg/mL for *H. itama* as depicted in Figure 2b. Comparing IC_{50} of MCF-10A and EC_{50} of MCF-7 in two colonies of *H. itama* did not show any statistically significant difference [$p = 0.212$ in *H. itama* (i) and $p = 0.172$ in *H. itama* (ii)].

Antiproliferative Activity of *G. thoracica* BPE in MCF-7 and MCF-10A Cells

Geniotrigona thoracica BPE showed the MCF-7 EC_{50} mean value of 1.61 + 0.30 mg/mL (Figure 3a). While in MCF-10A, the IC_{50} was 4.93 + 0.81 mg/mL for colony i and the IC_{40} was 4.93 + 0.51 mg/mL for colony ii (Figure 3b).

The IC_{40} value was used in the second colony of *G. thoracica* because the IC_{50} value was outside the data range (Figure 3b). All variables were standardized in each experiment, where the maximum

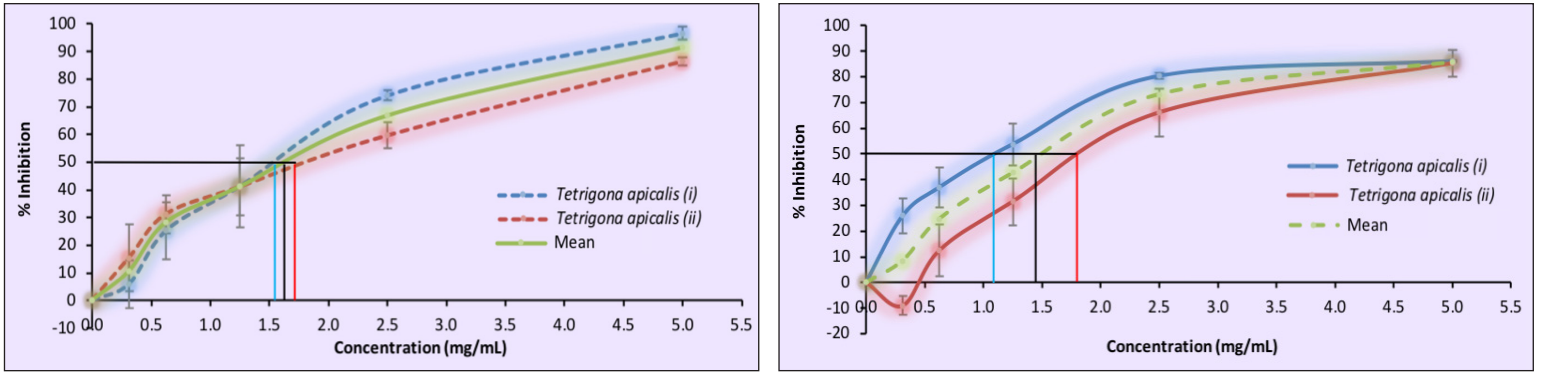


Figure 1. Antiproliferation of *T. apicalis* BPE against MCF-7 and MCF-10A cells

Note. (a) Antiproliferation of *T. apicalis* BPE against MCF-7 cells at 24 h treatment, mean = 1.60 mg/mL; (b) Antiproliferation of *T. apicalis* BPE against MCF-10A cells at 24 h treatment, mean =1.46 mg/mL. Each point indicates three independent experiments' mean values ± SD

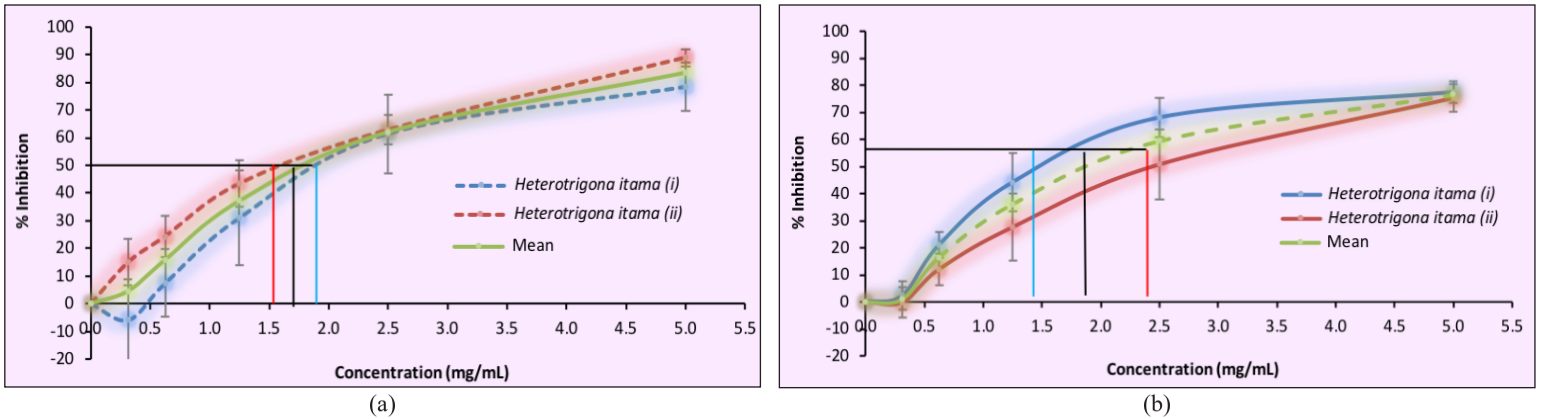
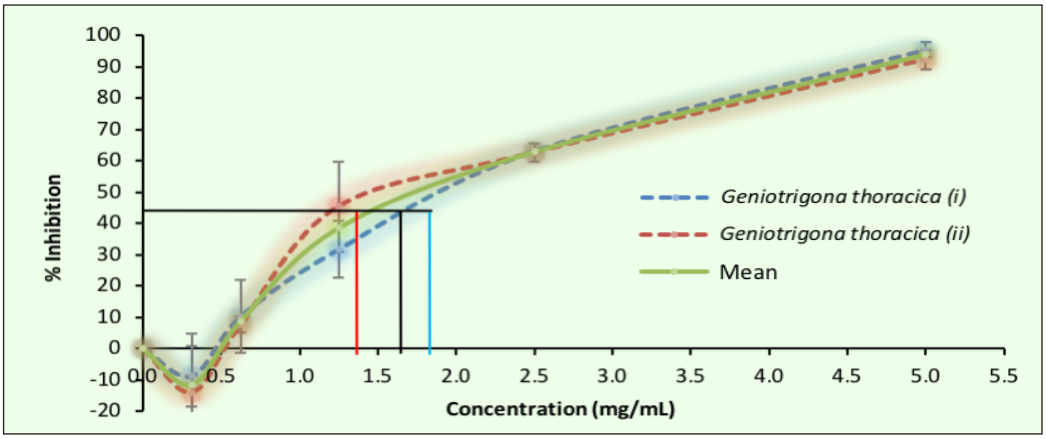
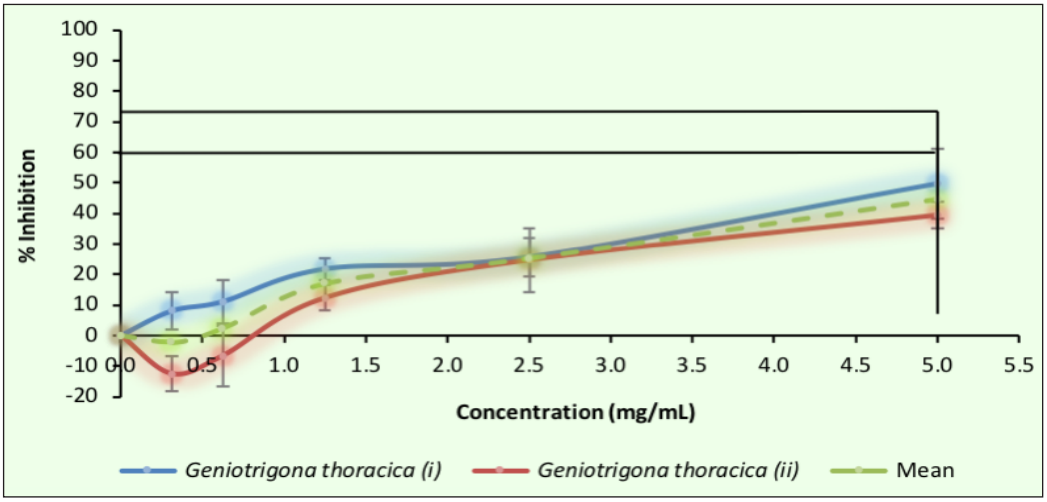


Figure 2. Antiproliferation of *H. itama* BPE against MCF-7 and MCF-10A cells

Note. (a) Antiproliferation of *H. itama* BPE in MCF-7 cells, mean =1.72 ± 0.28 mg/mL; (b) Antiproliferation of *H. itama* BPE in MCF-10A cells at 24 h treatment, mean = 1.91 ± 0.72 mg/mL. The results presented are means of three separate experiments ± SD



(a)



(b)

Figure 3. Antiproliferation of *G. thoracica* BPE against MCF-7 and MCF-10A cells
Note. (a) Antiproliferation of *G. thoracica* BPE in MCF-7 cells, mean = 1.61 ± 0.30 mg/mL; (b) Antiproliferation of *G. thoracica* BPE in MCF-10A cells at 24 h treatment, mean = 4.93 ± 0.51 mg/mL. The results presented are means of three separate experiments ± SD

concentration was 5.0 mg/mL. Both IC_{50} and IC_{40} values were applicable and consistent with treatment doses of less than 50% inhibition used in the clinical setting (Stordal et al., 2006).

A significant difference was found in EC_{50} values in MCF-7 cells compared with the IC_{50}/IC_{40} values in MCF-10A cells for both colonies of *G. thoracica* species. In colony i, the *p*-value was 0.018, while in colony ii, the *p*-value was 0.001. Only *G. thoracica* species indicated a significant result compared to the other two species.

Therapeutic Index (TI)

The therapeutic index is a ratio of concentration that inhibits 50% proliferation of normal cells (IC_{50}) to the concentration

that inhibits 50% proliferation of tumor cells (EC_{50}). The TI values of each colony of BPE species differed from each other. The order of TI values is as follows: *G. thoracica* > *H. itama* > *T. apicalis*.

The average of two colonies of each species was calculated, where *G. thoracica* showed the highest TI value of 3.12, followed by *H. itama* (1.16), and *T. apicalis* (0.90) (Figure 4).

DISCUSSION

Antiproliferative Assay

In this study, a trypan blue exclusion assay has been used to assess the antiproliferative activity of *T. apicalis*, *H. itama*, and *G. thoracica* BPE in MCF-7 and MCF-10A cell lines. The minimum effective concentrations (EC_{50}) in MCF-7 cells and the minimum inhibitory concentrations (IC_{50}) in MCF-10A cells were determined from the graph based on extrapolation from the antiproliferative curve after 24 h treatment.

MCF-7, the breast adenocarcinoma human cell line, was developed in 1970, and since then, more than 25,000 publications have been reported (Lee et al., 2015). In addition, MCF-7 is a frequently studied cell line that serves as a valuable model system in hormone-receptor-positive breast cancer research (Lee et al., 2015).

Among the commonly used normal breast cells as an *in vitro* model is MCF-10A, the mammary epithelial human cell line that was isolated in 1984 (American Type Culture Collection [ATCC], n.d.). These cells originated from benign immortalized breast tissue proliferation, no estrogen receptors expression, and exhibited some features of normal breast epithelium (Qu et al., 2015).

Antiproliferative Activity of BPE

In the present study, the antiproliferative activity revealed that three BPE species demonstrated the capacity to decrease

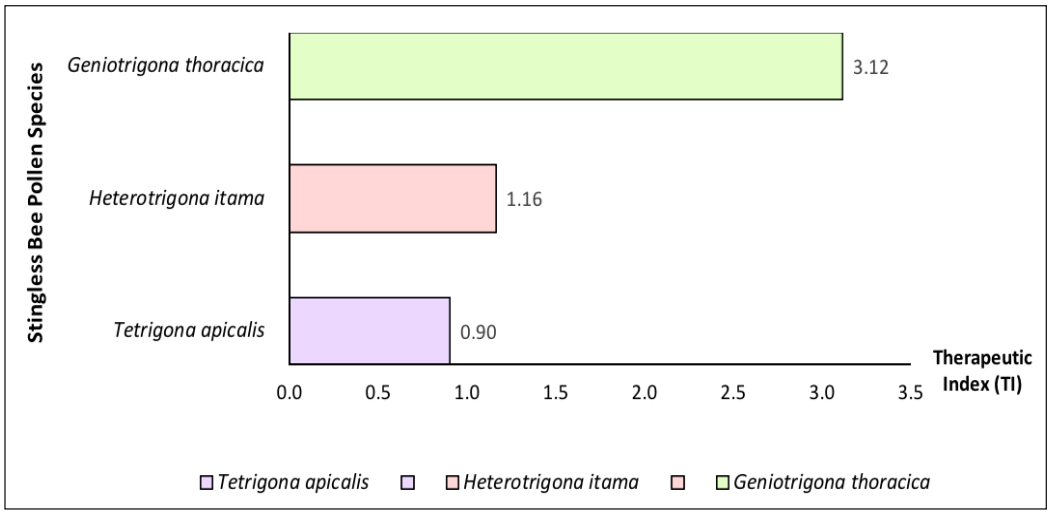


Figure 4. Comparison of therapeutic index (TI) in different BPE species with respect to MCF-10A cells
Note. Bars illustrate the TI values of three BPE species

the MCF-7 and MCF-10A cell viability in a dose-dependent manner. However, *T. apicalis* (EC_{50} = 1.60 mg/mL, IC_{50} = 1.46 mg/mL, TI = 0.90) and *H. itama* (EC_{50} = 1.72 mg/mL, IC_{50} = 1.91 mg/mL, TI = 1.16) showed a lower antiproliferative effect compared to *G. thoracica* (EC_{50} = 1.61 mg/mL, IC_{50} = 4.93 mg/mL, TI = 3.12).

The antiproliferative effect of *G. thoracica* (colonies i and ii) strongly increased by reaching a maximum of 92-95% inhibition in MCF-7 and only 39-49% inhibition in MCF-10A cell lines. Among the three species, *G. thoracica* showed a significant result ($p < 0.05$) with the highest antiproliferative effect on MCF-7 and the least antiproliferative activity seen in MCF-10A [$p = 0.018$ in *G. thoracica* (i), $p = 0.001$ in *G. thoracica* (ii)]. According to FDA, the TI value of more than a 2-fold difference in the IC_{50} and EC_{50} gives the distinction that *G. thoracica* is better than *T. apicalis* and *H. itama*; thus, *G. thoracica* could act as a potent antiproliferative agent (Tamargo et al., 2015).

Kustiawan et al. (2014) showed that the proliferation of five cancer cell lines was inhibited by four different species of stingless bee products, i.e., honey, bee pollen, and propolis (Kustiawan et al., 2014). The result showed BPE from n-hexane and ethyl acetate (EtOAc) extract from two species gave <50% relative viable cell number after 48 h treatment on two cancer cell lines. In their study, *T. apicalis* was the least cytotoxic species with a lower antiproliferative effect on cancer cells than other species. Our data showed a similar finding (EC_{50} of *T. apicalis* = 1.60 mg/mL, TI = 0.90).

BPE's ability to inhibit the growth of cancer cells depends on the bee species and cell line in *in vitro* study, representing different bioactive compounds due to different pollen foraging activities displayed by each species. In addition, it was previously shown that BPE contained different antioxidant activity and phenolic/flavonoid content (Harif Fadzilah et al., 2017), which could contribute to its bioactivity and antiproliferative effects on cell lines tested in this study.

CONCLUSION

Geniotrigona thoracica BPE showed a strong antiproliferative effect on MCF-7 cells, and less antiproliferative activity was seen on MCF-10A cells. The calculated therapeutic index in this study showed that the specificity of *G. thoracica* BPE was more effective in killing MCF-7 cells with less toxicity to MCF-10A cells compared with *T. apicalis* and *H. itama*. The therapeutic index of *G. thoracica* BPE was the highest, potentially developing as a chemotherapeutic agent.

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REFERENCES

Abughazaleh, R. D., & Tracy, T. S. (2014). *Therapeutic index*. Wiley StatsRef: Statistics Reference Online. https://doi.org/10.1002/9781118445112.stat07121

Ahmad, F., Seerangan, P., Mustafa, M. Z., Osman, Z. F., Abdullah, J. M., & Idris, Z. (2019). Anti-cancer properties of *Heterotrigona itama* sp. honey via induction of apoptosis in malignant glioma cells. *Malaysian Journal of Medical Sciences*, *26*(2), 30–39. https://doi.org/10.21315/mjms2019.26.2.4

American Type Culture Collection. (n.d.). *MCF 10A (CRL-10317™)*. ATCC. https://www.atcc.org/products/all/CRL-10317.aspx#history

Aslantürk, Ö. S. (2018). *In vitro* cytotoxicity and cell viability assays: Principles, advantages, and disadvantages. In M. L. Larramendy & S. Soloneski (Eds.), *Genotoxicity - A predictable risk to our actual world*. IntechOpen. https://doi.org/10.5772/intechopen.71923

Azamjah, N., Soltan-Zadeh, Y., & Zayeri, F. (2019). Global trend of breast cancer mortality rate: A 25-year study. *Asian Pacific Journal of Cancer Prevention*, *20*(7), 2015–2020. https://doi.org/10.31557/APJCP.2019.20.7.2015

Campos, M. G. R., Frigerio, C., Lopes, J., & Bogdanov, S. (2010). What is the future of Bee-Pollen?. *Journal of ApiProduct and ApiMedical Science*, *2*(4), 131–144. https://doi.org/10.3896/ibra.4.02.4.01

Cha, M. C., Lin, A., & Meckling, K. A. (2005). Low dose docosahexaenoic acid protects normal colonic epithelial cells from araC toxicity. *BMC Pharmacology*, *5*, 7. https://doi.org/10.1186/1471-2210-5-7

Choudhari, M. K., Haghniaz, R., Rajwade, J. M., & Paknikar, K. M. (2013). Anticancer activity of Indian stingless bee propolis: An *in vitro* study. *Evidence-Based Complementary and Alternative Medicine*, *2013*, 928280. https://doi.org/10.1155/2013/928280

Deepa, P. R., Vandhana, S., Jayanthi, U., & Krishnakumar, S. (2012). Therapeutic and toxicologic evaluation of anti-lipogenic agents in cancer cells compared with non-neoplastic cells. *Basic and Clinical Pharmacology and Toxicology*, *110*(6), 494–503. https://doi.org/10.1111/j.1742-7843.2011.00844.x

Franchi, G. C., Moraes, C. S., Toreti, V. C., Daugsch, A., Nowill, A. E., & Park, Y. K. (2012). Comparison of effects of the ethanolic extracts of brazilian propolis on human leukemic cells as assessed with the MTT assay. *Evidence-Based Complementary and Alternative Medicine*, *2012*, 918956. https://doi.org/10.1155/2012/918956

Harif Fadzilah, N., Jaapar, M. F., Jajuli, R., & Wan Omar, W. A. (2017). Total phenolic content, total flavonoid and antioxidant activity of ethanolic bee pollen extracts from three species of Malaysian stingless bee. *Journal of Apicultural Research*, *56*(2), 130–135. https://doi.org/10.1080/00218839.2017.1287996

Kandaswami, C. C. (2014). *Re: What is the difference between an antiproliferative assay and a cytotoxicity assay?*. ResearchGate. https://www.researchgate.net/post/What-is-the-difference-between-an-antiproliferative-assay-and-a-cytotoxicity-assay/53a079e2d4c1188e0a8b4626/citation/download

Kocot, J., Kiełczykowska, M., Luchowska-Kocot, D., Kurzepa, J., & Musik, I. (2018). Antioxidant potential of propolis, bee pollen, and royal jelly: Possible medical application. *Oxidative Medicine and Cellular Longevity*, *2018*, 7074209. https://doi.org/10.1155/2018/7074209

Kuppusamy, P., Yusoff, M. M., Maniam, G. P., Ichwan, S. J. A., Soundharrajan, I., & Govindan, N. (2014). Nutraceuticals as potential therapeutic

agents for colon cancer: A review. *Acta Pharmaceutica Sinica B*, *4*(3), 173–181. https://doi.org/10.1016/j.apsb.2014.04.002

Kustiawan, P. M., Puthong, S., Arung, E. T., & Chanchao, C. (2014). *In vitro* cytotoxicity of Indonesian stingless bee products against human cancer cell lines. *Asian Pacific Journal of Tropical Biomedicine*, *4*(7), 549–556. https://doi.org/10.12980/APJTB.4.2014APJTB-2013-0039

Lee, A. V., Oesterreich, S., & Davidson, N. E. (2015). MCF-7 cells - Changing the course of breast cancer research and care for 45 years. *Journal of the National Cancer Institute*, *107*(7), djv073. https://doi.org/10.1093/jnci/djv073

MarÉchal, E. (2011). Measuring bioactivity: KI, IC50 and EC50. In E. Marechal, S. Roy, L. Lafanechère (Eds.), *Chemogenomics and chemical genetics* (pp. 55–65). Springer. https://doi.org/10.1007/978-3-642-19615-7_5

Muller, P. Y., & Milton, M. N. (2012). The determination and interpretation of the therapeutic index in drug development. *Nature Reviews Drug Discovery*, *11*(10), 751–761. https://doi.org/10.1038/nrd3801

Norowi, M. H., Mohd, F., Sajap, A. S., Rosliza, J., & Suri, R. (2010). *Conservation and sustainable utilization of stingless bees for pollination services in agricultural ecosystems in Malaysia*. ResearchGate. https://www.researchgate.net/publication/318338675

Premratanachai, P., & Chanchao, C. (2014). Review of the anticancer activities of bee products. *Asian Pacific Journal of Tropical Biomedicine*, *4*(5), 337–344. https://doi.org/10.12980/APJTB.4.2014C1262

Qu, Y., Han, B., Yu, Y., Yao, W., Bose, S., Karlan, B. Y., Giuliano, A. E. & Cui, X. (2015). Evaluation of MCF10A as a reliable model for normal human mammary epithelial cells. *PLOS One*, *10*(7), e0131285. https://doi.org/10.1371/journal.pone.0131285

Riss, T., Niles, A., Moravec, R., Karassina, N., Vidugiriene, J., Markossian, S., Grossman, A., Brimacombe, K., Arkin, M., Auld, D., Austin, C., Baell, J., Chung, T. D. Y., Coussens, N. P., Dahlin, J. L., Devamarayan, V., Foley, T. L., Glicksman, M., Hass, J. V., … Xu, X. (Eds.). Cytotoxicity assays: *In vitro* methods to measure dead cells. In *Assay guidance manual*. Eli Lilly & Company and the National Center for Advancing Translational Sciences. https://pubmed.ncbi.nlm.nih.gov/31070879/

Rzepecka-Stojko, A., Stojko, J., Kurek-Górecka, A., Górecki, M., Kabała-Dzik, A., Kubina, R., Moździerz, A., & Buszman, E. (2015). Polyphenols from bee pollen: Structure, absorption, metabolism and biological activity. *Molecules*, *20*(12), 21732–21749. https://doi.org/10.3390/molecules201219800

Samsudin, S. F., Mamat, M. R., & Hazmi, I. R. (2018). Taxonomic study on selected species of stingless bee (Hymenoptera: Apidae: Meliponini) in Peninsular Malaysia. *Serangga*, *23*(2), 203–258.

Sebaugh, J. L. (2011). Guidelines for accurate EC50/IC50 estimation. *Pharmaceutical Statistics*, *10*(2), 128–134. https://doi.org/10.1002/pst.426

Seely, J. M., & Alhassan, T. (2018). Screening for breast cancer in 2018 — What should we be doing today?. *Current Oncology*, *25*(s1), 115–124. https://doi.org/10.3747/co.25.3770

Stordal, B. K., Davey, M. W., & Davey, R. A. (2006). Oxaliplatin induces drug resistance more rapidly than cisplatin in H69 small cell lung cancer cells. *Cancer Chemotherapy and Pharmacology*, *58*(2), 256–265. https://doi.org/10.1007/s00280-005-0148-7

Strober, W. (2015). Trypan blue exclusion test of cell viability. *Current Protocols in Immunology*, *111*, A3.B1-A3.B3. https://doi.org/10.1002%2F0471142735.ima03bs111

Tamargo, J., Le Heuzey, J. Y., & Mabo, P. (2015). Narrow therapeutic index drugs: A clinical

pharmacological consideration to flecainide. *European Journal of Clinical Pharmacology*, *71*(5), 549–567. https://doi.org/10.1007/s00228-015-1832-0

T-Johari, S. A. T., Hashim, F., Ismail, W. I. W., & Ali, A. M. (2019). Combinatorial cytotoxic effects of gelam honey and 5-fluorouracil against human adenocarcinoma colon cancer HT-29 cells *in vitro*. *International Journal of Cell Biology*, *2019*, 9050626. https://doi.org/10.1155/2019/9050626

Wang, H., Khor, T. O., Shu, L., Su, Z., Fuentes, F., Lee, J.-H., & Kong, A.-N. T. (2012). Plants against cancer: A review on natural phytochemicals in preventing and treating cancers and their druggability. *Anti-cancer Agents in Medical Chemistry*, *12*(10), 1281–1305. https://doi.org/10.2174%2F187152012803833026

Yang, K., Wu, D., Ye, X., Liu, D., Chen, J., & Sun, P. (2013). Characterization of chemical composition of bee pollen in China. *Journal of Agricultural and Food Chemistry*, *61*(3), 708–718. https://doi.org/10.1021/jf304056b