

8-31-2021

Cytotoxic Evaluation of Malaysian Kelulut Honey on Human Gingival Fibroblast Cell Line using MTT Assay

Chee Zi Yun

School of Dental Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia, yun-223@hotmail.com

Nurul Hafizah Mohd Nor

School of Dental Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia, sakeena.nh@gmail.com

Zurairah Berahim

School of Dental Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia, zurairah@usm.my

Kannan Thirumulu Ponnuraj

School of Dental Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia; Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia, kannan@usm.my

Follow this and additional works at: <https://scholarhub.ui.ac.id/jdi>



Part of the [Alternative and Complementary Medicine Commons](#)

Recommended Citation

Zi Yun, C., Mohd Nor, N., Berahim, Z., & Thirumulu Ponnuraj, K. Cytotoxic Evaluation of Malaysian Kelulut Honey on Human Gingival Fibroblast Cell Line using MTT Assay. *J Dent Indones.* 2021;28(2): 88-93

This Article is brought to you for free and open access by the Faculty of Dentistry at UI Scholars Hub. It has been accepted for inclusion in Journal of Dentistry Indonesia by an authorized editor of UI Scholars Hub.

Cytotoxic Evaluation of Malaysian Kelulut Honey on Human Gingival Fibroblast Cell Line using MTT Assay

Cover Page Footnote

ACKNOWLEDGEMENTS The authors thank the staff of Craniofacial Sciences Laboratory, School of Dental Sciences, Universiti Sains Malaysia for their help and Assoc. Prof. Dr. Wan Muhamad Amir W Ahmad for the statistical assistance provided. The authors acknowledge the financial support provided by the Fundamental Research Grant Scheme, Ministry of Higher Education, Malaysia FRGS/203/PPSG/6171220.

ORIGINAL ARTICLE

Cytotoxic Evaluation of Malaysian Kelulut Honey on Human Gingival Fibroblast Cell Line using MTT Assay

Chee Zi Yun¹, Nurul Hafizah Mohd Nor¹, Zurairah Berahim¹, Kannan Thirumulu Ponnuraj^{1,2}

¹*School of Dental Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia*

²*Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia*

*Correspondence e-mail to: kannan@usm.my

ABSTRACT

Kelulut honey or stingless bee honey is a type of honey produced by stingless bees of the *Trigona* species where the nest is found in living trees. **Objective:** The aim of this study was to evaluate the cytotoxic potential of Malaysian Kelulut honey by employing MTT assay on a human gingival fibroblast cell line. **Methods:** Human gingival fibroblast cell line was cultured in minimal essential medium alpha (α -MEM) with 10% foetal bovine serum and 1% penicillin-streptomycin solution in a 5% CO₂ incubator at 37°C in a humidified atmosphere. The cells were seeded at a cell density of 5x10³ cells/well in a 96-well culture plate for 24 hours. The cells were treated with seven different concentrations (200, 100, 50, 25, 12.5, 6.25, and 3.125mg/ml) of Malaysian Kelulut honey and incubated in a CO₂ incubator. The negative control comprised cells treated with growth media alone. The cell viability was assessed using MTT assay at 24, 48, and 72 hours. The test plate was shaken using a microplate shaker and the absorbance of the solution was measured at 570nm using an ELISA reader with the Magellan software. Statistical analysis of the data was carried out using Kruskal-Wallis test and SPSS 24.0.0 for Windows. A p value <0.05 was considered as statistically significant. **Results:** There was no cytotoxic effect of Malaysian Kelulut honey on HGF-1 based on the MTT assay at different concentrations and at different time points tested as the cell viability was above 70%. The highest percentage of cell viability at all three different durations of treatment were observed at 3.125mg/ml, whereas the lowest cell viability was observed at 200mg/ml of Kelulut honey concentration. However, statistically significant differences were seen between some of the concentrations at various time points. **Conclusion:** Since the cell viability of HGF-1 treated with Malaysian Kelulut honey was more than 70% at all concentrations ranging from 3.125mg/ml to 200mg/ml at three different time points (24, 48 and 72 hours), Malaysian Kelulut honey can be considered as non-cytotoxic on human gingival fibroblasts based on MTT assay under the present test conditions.

Key words: cytotoxicity, human gingival fibroblasts, Kelulut honey, MTT assay

How to cite this article: Yun CZ, Nor NHM, Berahim Z, Ponnuraj KT. Cytotoxic evaluation of Malaysian kelulut honey on human gingival fibroblast cell line using MTT Assay. *J Dent Indones.* 2021;28(2):88-93.

INTRODUCTION

Kelulut honey or stingless bee honey is a type of honey produced by stingless bees of the *Trigona* species where the nest is found in living trees.¹ Kelulut honey is characterised by its significant amount of flavour and fragrant qualities. It consists of high sugar content i.e., glucose, fructose, sucrose and maltose, as well as non-aromatic organic acids such as D-gluconic acid, malic acid and citric acid. It also consists of large amounts of bioactive substances, namely flavonoids, glucose oxidase catalase, and phenolic acids.

Kelulut honey has been perceived as a therapeutic honey as it exhibits high antioxidant,¹ antimicrobial,² and wound healing properties.³ It is necessary to assess the cytotoxic nature of natural products as cytotoxicity is the characteristic of certain substances to be harmful to cell life. Cytotoxicity testing enables researchers to observe the growth, morphological effects, reproduction and proliferation of cells on treatment with substances having an unknown cytotoxic state.⁴ This test also offers many ways of

detecting cell damage like monitoring morphological changes, cell growth and measurement of metabolic properties.⁵⁻⁷ MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) is one of the cytotoxic assays widely used in assessing cell viability and proliferation assay.⁸⁻¹¹ Human gingival fibroblasts (HGFs) are important cellular components involved in periodontal tissue repair.

Due to the dearth of information on the cytotoxicity of Kelulut honey on human gingival fibroblasts, this preliminary study aimed to assess the cell viability of human gingival fibroblasts treated with different concentrations of Malaysian Kelulut honey at different time periods using MTT assay. Moreover, it is of utmost importance to thoroughly investigate its benefits so that Malaysian stingless bee honey could contribute to the health of humans through its potential proliferative action.

METHODS

Cell line and culture

Human gingival fibroblast cell line (HGF-1) obtained from ATCC®CRL-2014™ (USA) was employed in the current research. HGF-1 were cultured until confluence in complete media comprising minimal essential medium alpha (α -MEM) (Gibco, Life Technologies, USA) supplemented with 10% foetal bovine serum (FBS) (Gibco, Life Technologies, USA), 1% penicillin (10000 units/ml) - streptomycin (10000 μ g/ml) (Gibco, Life Technologies, USA) in a 5% CO₂ incubator (Nuair, USA) at 37°C in a humidified atmosphere. The original medium was discarded, and the cells were washed using a phosphate buffered saline (PBS) (Gibco, Life Technologies, USA) before trypsinization using one ml of TrypLE™ Express stable trypsin (Gibco, Life Technologies, USA) in a 25cm² flask (SPL, USA) for the passaging process. Cells were left for three to four minutes in an incubator for the dissociation process and then the cells were neutralised using complete media (one ml for 25cm² flask). The suspensions were spun at 1200rpm for five minutes, and media and trypsin were discarded. Then, 1000 μ l of fresh complete medium was added to the cells and dispensed at 1:3 subculture ratio. HGF-1 were sub-cultured in three flasks (25cm²) with four ml of culture medium. The cells were allowed to grow until it reached confluence and then the process of detaching the cells was repeated. The cells were seeded at a cell density of 5 x 10³ cells/well in a 96-well culture plate (Nunc™, Denmark) for 24 hours for cell attachment at 37°C, 5% CO₂ and 95% relative humidity.

Malaysian kelulut honey sample

The Kelulut honey used in the current study was the one commercially marketed by Syamille Agrofarm & Resort Sdn. Bhd. (Kuala Kangsar, Perak, Malaysia).¹² This honey was obtained from the nests of stingless

bees namely the *Trigona* species. Stingless bees after producing honey store it in resin pots in their hives.¹³ For commercial use, honey is collected from the pot using suction pump and subsequently filtered to remove the impurities.¹⁴

MTT assay

After 24 hours of incubation, the extraction medium was filtered through a 0.22 μ m Millipore membrane filter (TPP, Switzerland). The extraction medium was serially diluted (200, 100, 50, 25, 12.5, 6.25, 3.125 mg/ml) in seven different 15 ml centrifuge tubes (Eppendorf, Germany). The diluted extracts were added into three 96 well-plates containing 5 x 10³ HGF-1/well. The negative control comprised of cells treated with growth media alone. The 96 well plates containing the cells were then incubated in a 5% CO₂ incubator for 24, 48, and 72 hours at 37°C. Then, 10 μ l of MTT (Invitrogen, USA) with a concentration of 5 mg/ml was added to the culture medium and the plates were further incubated for four hours in a 5% CO₂ incubator at 37°C to enable the dye to be taken up by the remaining surviving cells. Total removal of the media containing the MTT solution was done and then cells were lysed with 100 μ l of dimethyl sulfoxide (DMSO) (Sigma Aldrich, USA) solution. The test plate was shaken using a microplate shaker for several minutes to solubilise the formazan crystals and the absorbance of the solution was measured at 570nm using an ELISA reader (Sunrise, TECAN, Austria) with the Magellan software. Three independent experiments were carried out with three replicates.

The cell viability was calculated using the following formula.

$$\% \text{ viable cells} = \frac{[A]_{\text{test}}}{[A]_{\text{control}}} \times 100$$

Where A = absorbance

Data analysis

Data were analysed using SPSS Statistics for Windows, Version 24.0. and pairwise comparisons were done using the Kruskal-Wallis test. Statistical significance was set at $p < 0.05$.

RESULTS

Cell viability of HGF-1 treated with different concentrations of Malaysian Kelulut honey

Cell viability of HGF-1 treated with Kelulut Honey at seven different concentrations (200, 100, 50, 25, 12.5, 6.25, and 3.125mg/ml) according to ISO 10993-12,¹⁵ and negative control (cells treated with growth media only) at three different time periods (24, 48 and 72 hours) using MTT assay are shown in Table 1. The highest percentage of cell viability at all three different durations of treatment was observed at 3.125 mg/ml, whereas the lowest cell viability was observed at 200 mg/ml of Kelulut honey concentration. Pairwise comparisons of cell viability of HGF-1 treated with

Table 1. Cell viability of human gingival fibroblasts (HGF-1) treated with Malaysian Kelulut honey using MTT assay

| Concentration (mg/ml) | Cell viability (%) Mean (SD) | | |
|-----------------------|------------------------------|---------------|---------------|
| | 24 hours | 48 hours | 72 hours |
| 200 | 75.87 (4.14) | 81.17 (6.18) | 77.47 (3.61) |
| 100 | 85.37 (2.02) | 83.53 (5.11) | 81.30 (0.96) |
| 50 | 88.30 (2.55) | 93.87 (6.99) | 85.63 (2.99) |
| 25 | 93.77 (1.23) | 102.87 (5.35) | 88.97 (3.19) |
| 12.5 | 98.93 (1.45) | 105.57 (7.37) | 95.37 (3.74) |
| 6.25 | 105.83 (6.91) | 108.13 (8.54) | 98.33 (3.45) |
| 3.125 | 113.30 (7.31) | 112.87 (6.40) | 117.13 (6.43) |
| Control | 100.00 (0.00) | 100.00 (0.00) | 100.00 (0.00) |

Table 2. Pairwise comparison of cell viability of human gingival fibroblasts (HGF-1) treated with Malaysian Kelulut honey for 24, 48 and 72 hours using MTT assay

| Variables | Median (IQR) | Chi square statistic (df) | p-value | Duration of incubation (hours) |
|----------------|--------------|---------------------------|---------|--------------------------------|
| Concentrations | 4.50 (4.50) | 42.567 (7) | 0.000 | 24 |
| Values | 0.22 (0.05) | | | |
| Concentrations | 4.50 (4.50) | 16.956 (7) | 0.018 | 48 |
| Values | 0.23 (0.06) | | | |
| Concentrations | 4.50 (4.50) | 37.434 (7) | 0.000 | 72 |
| Values | 0.22 (0.04) | | | |

Kruskal-Wallis test was performed as normality assumption was not met. The level of significance was set at 0.05 ($p < 0.05$). Pairwise comparison: 200-25, 200-12.5, 200-control, 200-6.25, 200-3.125, 100-6.25, 100-3.125, 50-3.125 mg/ml are statistically significant ($p < 0.05$), others are not significant at 24 hours of incubation. Pairwise comparison: 200-3.125 mg/ml is statistically significant ($p < 0.05$), others are not significant at 48 hours of incubation. Pairwise comparison: 200-12.5, 200-6.25, 200-3.125, 100-3.125 mg/ml are statistically significant ($p < 0.05$), others are not significant at 72 hours of incubation.

Malaysian Kelulut honey at three different time periods using MTT assay are presented in Table 2. However, statistical significance ($p < 0.05$) was observed for the cell viability between the concentrations at 24, 48, and 72 hours (Table 2).

DISCUSSION

The Kelulut Honey used in this study was a commercial one which is rich in flavonoids, furfural and terpenoid. There are limited publications available on Malaysian stingless bee honey.¹⁶ Most of the publications reported are on the physicochemical and antioxidant properties. The physicochemical properties of Kelulut honey that have been previously reported are moisture content (33.24 ± 2.54 g/100 g), water activity (0.76 ± 0.03), specific gravity (1.36 ± 0.04), viscosity (0.29 ± 0.18 Pa.s), pH (3.26 ± 0.15), free acidity (136.8 ± 7.6 meq/kg), electrical conductivity (1.08 ± 0.37 mS/cm) and colour intensity (990.3 ± 380.0 MAU).¹⁶ It has also been mentioned that the high water activity of Kelulut honey has a tendency of fermentation from bacteria and yeasts.¹⁷ The low pH of Kelulut honey contributed to a sourly taste and its high electrical conductivity is related to the high ash (mineral) content.¹⁶ The same authors also reported higher reducing power and also stronger antioxidant activity of Kelulut honey

based on their studies. With regard to the nutritional composition, Kelulut honey showed high content of carbohydrate ranging from 67.20 ± 0.11 to 73.01 ± 0.35 g/100 g, potassium (701.33 ± 26.27 mg/kg), calcium (292.67 ± 1.17 mg/kg), magnesium (51.61 ± 0.08 mg/kg) and zinc (5.33 ± 0.36 mg/kg), while the phytochemical analysis showed that the total flavonoids and phenolic compounds ranged from 53.81 ± 4.12 to 549.05 ± 9.74 mg rutin/kg and 357.14 ± 3.57 to 520.83 ± 4.49 mg gallic acid/kg respectively depending on the various solvents used for extraction.¹⁸

However, there is still a dearth of information on the cytotoxicity of Malaysian Kelulut honey on the normal cell line. Human gingival fibroblasts (HGFs) are the most abundant cell types in the periodontal connective tissues that function in repairing periodontal tissues and play a role in inflammatory periodontal diseases.¹⁹ Supraja et al. described that HGFs are one of the common cell types that are used in cell cultures as they are easy to culture following enzymatic digestion.²⁰ Giannopoulou and Cimasoni reported the simple establishment of primary gingival fibroblast culture as they adhere to the culture plates and spread well to allow good proliferation without requiring any specific culture conditions.²¹ Moreover, gingival fibroblasts are the major constituents of gingival tissues and function in maintenance as they are involved in the production

of extracellular matrix of tissue, both in disease and health.²²⁻²³ Egusa et al. reported that through the process of efficient reprogramming of gingival fibroblasts, it is possible to make the gingiva a good source for induced pluripotent stem (iPS) cells in drug screening applications and for autologous cell therapy in the dentistry field.²⁴ Therefore, this led to the choice of HGF-1 in the current study.

Some studies have been carried out to demonstrate the beneficial effects of stingless bee honey on fibroblast cell previously. A study carried out by Nordin and colleagues reported that at the low dose of 0.024µg/ml, stingless bee honey extract possessed a beneficial effect on the dermal fibroblast viability and proliferation.²⁵ Another study by Malik et. al. demonstrated that stingless bee honey beneficially increased collagen type I expression and decreased MMP-1 expression during cellular aging of human dermal fibroblast cells.²⁶ Malaysian propolis collected by the stingless honey bee *Trigona* spp showed an overall positive effect on both fibroblast migration and proliferation assays compared to the control, and it followed a concentration-dependent curve with 250µg/ml being the most optimum concentration for cell migration and 500µg/ml for cell proliferation.²⁷

In this study, the assessment of cell viability depended on the cytotoxic effect of Kelulut honey on HGF-1 cell line. A study on Sprague Dawley rats showed that Kelulut honey had chemopreventive properties against azoxymethane-induced colon cancer based on the reduction of total number of aberrant crypt foci and crypt multiplicity.²⁸ Similar studies were also performed by Pashinskiĭ and Gribel et al., who reported that honey possesses moderate anticolon cancer activity.²⁹ An in vitro study was also done to screen for the cytotoxic activity of different stingless bee products against five human cancer cell lines, namely, BT474 (ductal carcinoma and lung undifferentiated cancer), HepG29 (liver hepatoblastoma), KatoIII (gastric carcinoma), and SW620 (adenocarcinoma), where the crude extracts of stingless bee honey showed great cytotoxicity effect towards HepG2 cell line, while propolis crude extracts exhibited high cytotoxic effect towards all the human cancer cell lines.³⁰ A study was carried out by Nafi et al. on four stingless bee propolis ethanol extracts namely, *Heterotrigona itama* (*H. itama*), *Geniotrigona thoracica* (*G. thoracica*), *Lepidotrigona terminata* (*L. terminata*) and *Tetrigona apicalis* (*T. apicalis*) against triple negative breast cancer cells (MDA-MB-231), uterine leiomyosarcoma cells (SKUT-1) and cervical cancer cells (HeLa).³¹ They concluded that *H. itama* produced the most active extract in terms of cytotoxicity and had the potential to be an antioxidant agent compared to propolis produced by other Malaysian stingless bee species. According to Borrelli et al.³² caffeic acid phenethyl ester (CAPE) which is an important active component of honeybee

propolis that possesses a plethora of biological activities, inhibited the development of azoxymethane-induced colonic aberrant crypt foci in the colon of rats. Besides, another study based on human sporadic colon cancer cell line, HCT116 treated with CAPE at serial concentrations reported that CAPE had antioxidant effect, biological and pharmacological functions including immunoregulation, anti-inflammatory, antiviral, antibacterial, and antitumor activities.³³ It was shown that the local presence of CAPE resulted in a significant delay in tumour formation in a mice model as well as significantly suppressed the proliferation of human HeLa cervical carcinoma cells in vitro which they attributed to the cytotoxic nature of the polyphenolic compounds.³⁴

Compared to human cancer cell lines, there are also limited studies on the cytotoxicity of Kelulut honey on normal human cell lines. In the current study, the HGF-1 treated with Malaysian Kelulut honey showed high cell viability of more than 70% at all concentrations ranging from 3.125mg/ml to 200mg/ml and based on ISO 10993-5,³⁵ it can be inferred that Kelulut honey can was not cytotoxic at these concentrations. The statistical analyses found significant differences in the cell viability between concentrations of 200mg/ml - 3.125mg/ml at 24, 48 and 72 hours. The cell viability of the HGF-1 treated was generally higher at 48 hours than 72 hours. This could be due to the accumulation of waste products from the cells in the culture media over time. Moreover, a dose-dependent decrease in the cell viability was noticed; the higher the concentration of Kelulut honey, the lower the cell viability. The cell viability of HGF-1 was lowest at 200 mg/ml which were 75.87%, 81.17% and 77.47% at 24, 48 and 72 hours respectively, whereas the cell viability was highest at 3.125mg/ml which were 113.30%, 112.87% and 117.13% at 24, 48 and 72 hours respectively. The reduced cell viability at higher concentrations could be due to the increase in viscosity which could affect the medium and hinder the proliferation of cells. Moreover, it has been reported previously that different concentrations of honey could result in the change of osmolality of the media which can then negatively affect the growth of cells.³⁶ However, based on these fundamental results, further research needs to be carried out to exploit the potential of Malaysian Kelulut honey in clinical applications such as tissue engineering and regenerative medicine in the future.

CONCLUSION

This study showed that the cell viability of HGF-1 treated with Malaysian Kelulut honey was more than 70% at all concentrations ranging from 3.125mg/ml to 200mg/ml at three different time points, namely 24, 48, and 72 hours indicating that Malaysian Kelulut honey was not cytotoxic to HGF-1 cell line based on MTT assay under the test conditions.

ACKNOWLEDGEMENTS

The authors thank the staff of Craniofacial Sciences Laboratory, School of Dental Sciences, Universiti Sains Malaysia for their help and Assoc. Prof. Dr. Wan Muhamad Amir W Ahmad for the statistical assistance provided. The authors acknowledge the financial support provided by the Fundamental Research Grant Scheme, Ministry of Higher Education, Malaysia FRGS/203/PPSG/6171220.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

REFERENCES

1. Kek SP, Chin NL, Yusof YA, Tan, Chua LS. Total phenolic contents and colour intensity of Malaysian honeys from the *Apis* spp. and *Trigona* spp. Bees. *Agric Agric Sci Procedia*. 2014;2:150–5.
2. Zainol MI, Mohd Yusoff K, Mohd Yusof MY. Antibacterial activity of selected Malaysian honey. *BMC Complement Altern Med*. 2013;13:129.
3. Sabir A, Tabbu CR, Agustiono P, Sosroseno W. Histological analysis of rat dental pulp tissue capped with propolis. *J Oral Sci*. 2005;47(3):135–8.
4. Soenen SJ, Manshian B, Montenegro JM, Amin F, Meermann B, Thiron T. Cytotoxic effects of gold nanoparticles: a multi-parametric study. *ACS Nano*. 2012;6(7):5767-583.
5. Damas BA, Wheeler MA, Bringas JS, Hoen MM. Cytotoxicity comparison of mineral trioxide aggregates and EndoSequence bio-ceramic root repair materials. *J Endod*. 2011;37(3):372-5.
6. Kasper J, Hermanns MI, Bantz C, Maskos M, Stauber R, Pohl C. Inflammatory and cytotoxic responses of an alveolar-capillary coculture model to silica nanoparticles. Comparison with conventional monocultures. *Part Fibre Toxicol*. 2011;8(1):1-16.
7. Ubaldi C, Giudetti G, Broggi F, Gilliland D, Ponti J, Rossi F. Amorphous silica nanoparticles do not induce cytotoxicity, cell transformation or genotoxicity in Balb/3T3 mouse fibroblasts. *Mutat Res Genet Toxicol Environ Mutagen*. 2012;745(1):11-20.
8. Wang S, Yu H, Wickliffe JK. Limitation of the MTT and XTT assays for measuring cell viability due to superoxide formation induced by nanoscale TiO₂. *Toxicol In Vitro*. 2011;25(8):2147-51.
9. Buch K, Peters T, Nawroth T, Sanger M, Schmidberger H, Langguth P. Determination of cell survival after irradiation via clonogenic assay versus multiple MTT Assay-A comparative study. *Radiat Oncol*. 2012;7(1):1-6.
10. Mendes LP, Delgado JMF, Costa ADA, Vieira MS, Benfica PL, Lima EM. Biodegradable nanoparticles designed for drug delivery: The number of nanoparticles impacts on cytotoxicity. *Toxicol In Vitro*. 2015;29(6):1268-74.
11. Stepanenko A, Dmitrenko V. Pitfalls of the MTT assay: direct and off-target effects of inhibitors can result in over/underestimation of cell viability. *Gene*. 2015;574(2):193-203.
12. Madu Kelulut Syamille – Terbaik untuk anda. Viewed 19 July 2021. <http://kelulutmalaysia.blogspot.com/>.
13. Al-Hatamleh MAI, Boer JC, Wilson KL, Plebanski M, Mohamud R, Mustafa MZ. Antioxidant-based medicinal properties of stingless bee products: recent progress and future directions. *Biomolecules*. 2020;10(6):923.
14. Souza BA, Roubik DW, Barth OM, Heard TA, Enrquez E, Carvalho C, et al. Composition of stingless bee honey: setting quality standards. *Interciencia*. 2006;31:867–75.
15. ISO 10993-12. Biological evaluation of medical devices - Part 12: Sample preparation and reference materials. 2012;1-20.
16. Kek SP, Chin NL, Yusof YA, Tan SW, Chua LS. Classification of entomological origin of honey based on its physicochemical and antioxidant properties. *Int J Food Prop*. 2017;20:2723–38.
17. Vit P, Pedro SR, Roubik D. *Pot-Honey: A Legacy of Stingless Bees*; Springer: New York, USA. 2013;654.
18. Mohd Fadzelly AB, Shuaibu BS, Fazleen IAB, Ong JC, Zakbah M. Physicochemical and antioxidant potential of raw unprocessed honey from Malaysian stingless bees. *Pak J Nutr*. 2017;16: 888-94.
19. Lee I, Lee M, Jang H. The interrelationship between human gingival fibroblast differentiation and cultivating time. *Tissue Eng Regen Med*. 2013;10(2):60–4.
20. Supraja A, Dinesh MG, Rajasekaran S, Balaji TM, Rao SR. Effect of cyclosporin A and angiotensin II on cytosolic calcium levels in primary human gingival fibroblasts. *Dent Res J*. 2016;13(5):405-12.
21. Giannopoulou C, Cimasoni G. Functional characteristics of gingival and periodontal ligament fibroblasts. *J Dent Res*. 2011;75(3):895-902.
22. Bartold PM, Walsh LJ, Narayanan AS. Molecular and cell biology of the gingiva. *Periodontol*. 2000;24:28-55.
23. Poggi P, Rodriguez Baena R, Rizzo S, Rota MT. Mouthrinses with alcohol: cytotoxic effects on human gingival fibroblasts in vitro. *J Periodontol*. 2003;74(5):623-9.
24. Egusa H, Okita K, Kayashima H, Yu G, Fukuyasu S, Saeki M, et al. Gingival fibroblasts as a promising source of induced pluripotent stem cells. *PLoS One*. 2010;5(9):e12743.

25. Nordin A, Omar N, Sainik NQAV, Chowdhury SR, Omar E, Saim AB, et al. Low dose stingless bee honey increases viability of human dermal fibroblasts that could potentially promote wound healing. *Wound Med.* 2018;23:22–7.
26. Abdul Malik N, Mohamed M, Mustafa MZ, Zainuddin A. In vitro modulation of extracellular matrix genes by stingless bee honey in cellular aging of human dermal fibroblast cells. *J Food Biochem.* 2020;44:e13098.
27. Jacob A, Parolia A, Pau A. The effects of Malaysian propolis and Brazilian red propolis on connective tissue fibroblasts in the wound healing process. *BMC Complement Altern Med.* 2015;15:294.
28. Saiful Yazan L, Muhamad Zali MF, Mohd Ali R, Zainal NA, Esa N, Sapuan S, et al. Chemopreventive properties and toxicity of kelulut honey in sprague dawley rats induced with azoxymethane. *BioMed Res Int.* 2016;2016:4036926.
29. Pashinskiĭ VG, Gribel NV. The antitumor properties of honey. *Vopr Onkol.* 1990;36(6):704–9.
30. Kustiawan PM, Puthong S, Arung ET, Chanchao C. In vitro cytotoxicity of Indonesian stingless bee products against human cancer cell lines. *Asian Pac J Top Biomed.* 2014;4(7):549–56.
31. Nafi NE, Zin NB, Pauzi N, Khadar AS, Anisava AR, Badiazaman AA, et al. Cytotoxicity, antioxidant and phytochemical screening of propolis extracts from four different Malaysian stingless bee species. *Mal J Fund Appl Sci.* 2019;307-12.
32. Borrelli F, Izzo AA, Di Carlo G, Maffia P, Russo A, Maiello FM, et al. Effect of a propolis extract and caffeic acid phenethyl ester on formation of aberrant crypt foci and tumors in the rat colon. *Fitoterapia* 2002;73(1):38–43.
33. He YJ, Liu BH, Xiang DB, Qiao ZY, Fu T, He YH. Inhibitory effect of caffeic acid phenethyl ester on the growth of SW480 colorectal tumor cells involves β -catenin associated signaling pathway down-regulation. *World J Gastroenterol.* 2006;12(31):4981–5.
34. Orsolić N, Terzić S, Mihaljević Z, Sver L, Basić I. Effects of local administration of propolis and its polyphenolic compounds on tumor formation and growth. *Biol Pharm Bull.* 2005;28(10):1928–1933.
35. ISO 10993-5. Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity. 2009;1-34.
36. Mohamad MAM, Mazlan MA, Ibrahim M, Yusof AM, Shamsuddin SAA, Hassan NFN, et al. The effect of Malaysian stingless bee, *Trigona* spp. honey in promoting proliferation of the undifferentiated stem cell. *AsPac J Mol Biol Biotechnol.* 2018;27(1):10-9

(Received February 17, 2021; Accepted July 28, 2021)