EVALUATION OF THE ANTI-PROLIFERATIVE AND ANTIMICROBIAL ACTIVITIES OF THREE TYPES OF *Trigona spp*. HONEY

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by

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LIST OF ABBREVIATIONS

%	Percent
ºC	Degree Celcius
®	Registered trademark
µg/ml	Microlitre per mililitre
AC	Aberrant crypts
ACF	Aberrant crypt foci
BRCA	Breast Cancer gene
BSC	Biological safety cabinet
C. albicans	*Candida albicans*
cells/ml	Cells per mililitre
CFU	Colony per unit
cm	Centimetre
CO_2	Carbon dioxide
CT	Computed tomography
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal Bovine Serum

FDG	Fluoro-2-deoxy-D-glucose
g	Gram
GAE	Gallic acid equivalent
H. itama	*Heterotrigona itama*
HC	Hemorrhagic colitis
HCV	Hepatitis C virus
HeLa	Human cervical cancer cell line
HIV	Human immunodeficiency virus
HO	Hydroxide
HPV	Human papillomavirus
HUS	Hemolytic-uremic syndrome
IC_{50}	50 % inhibitory concentration
kg	Kilogram
L	Litre
MR	Magnetic resonance
MBC	Minimum Bactericidal Concentration
MCF-7	Human breast cancer cell line
Me	Methyl
MFC	Minimum Fungicidal Concentration
MH	Mueller-Hinton
MIC	Minimum Inhibition Concentration
mL	Mililitre
mm	Milimetre

MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium
nm	Nanometre
O	Oxygen
PBS	Phosphate-buffered saline
PenStrep	Penicillin-streptomycin
PET	Positron emission tomography
pH	Power of the concentration of Hydrogen ion
Ph	Phenyl
Rpm	Rate per minute
RPMI	Roswell Park Memorial Institute
S. aureus	*Staphylococcus aureus*
spp.	Species
STEC	Shiga toxin-producing *E. coli*
SVG	Normal human glial cell
T. laeviceps	*Tetragonula laeviceps*
UV	Ultraviolet
v/v	Volume per volume

Penilaian Aktiviti Anti-proliferatif dan Antimikrob Tiga Jenis Madu *Trigona spp.*

ABSTRAK

Trigona spp. yang dikenali sebagai Kelulut di Malaysia, adalah lebah kecil dari suku *Meliponini.* Madu lebah ini menghasilkan madu yang digunakan dalam penyembuhan luka, meningkatkan sistem immunisasi tubuh badan, membunuh bakteria, rawatan kahak bronkial, menghilangkan sakit tekak, batuk, dan demam. Dalam kajian ini, madu *Trigona spp*. telah diuji untuk menentukan aktiviti anti-proliferatif terhadap sel kanser MCF-7, sel kanser servikal HeLa dan sel glial SVG menggunakan ujian MTT. Aktiviti antimikrobial madu *Trigona spp*. telah diuji dengan menentukan kepekatan penghalang minimum (MIC) terhadap *E. coli*, *S. aureus* dan *C. albicans* dengan menggunakan kaedah mikrodilution. Nilai kepekatan bakterisida minimum (MBC) atau nilai kepekatan fungisida minimum (MFC) ditentukan oleh sub kultiviti kandungan nutrien yang digunakan untuk ujian MIC pada agar nutrien dengan menggunakan gelang steril dan membuat caringan pada media untuk melihat pertumbuhan bakteria dengan inkubasi 37 ° C selama 24 jam. Tiada pertumbuhan *E. coli* dan *S. aureus* yang ditunjukkan oleh dari madu ialah MBC manakala MFC ditentukan oleh ketiadaan pertumbuhan *C. albicans*. Akhirnya, ujian pembunuhan masa ditentukan pada kepekatan 0.5 x MIC, 1 x MIC dan 2 x MIC dan diinkubasikan untuk jangka masa yang berlainan (0, 2, 4, 24 jam). Jumlah sel hidup (CFU / mL) bagi setiap sampel ditentukan menggunakan kaedah kiraan plat agar. Hasilnya, kedua-dua madu *H. itama* dan

madu *T. laeviceps* menunjukkan perencatan dalam pertumbuhan sel kanser payudara MCF-7 dan sel kanser servik HeLa. Walau bagaimanapun, madu *T. laeviceps* mempunyai aktiviti anti-proliferatif yang lebih baik pada sel MCF-7 tetapi aktiviti anti-proliferatif yang lemah pada sel HeLa. Sebaliknya, madu *H. itama* menunjukkan aktiviti anti-proliferatif yang lebih baik pada sel HeLa tetapi lebih lemah pada sel sel MCF-7. Selain itu, madu *Trigona spp*. adalah tidak toksik kepada sel normal manakala toksik kepada sel kanser. Madu *H. itama* yang tidak dicairkan (kepekatan 100%) mempromosikan percambahan sel normal. Untuk aktiviti antimikrob, nilai MIC madu *Trigona spp*. terhadap *E. coli* berkisar 3.125% hingga 6.25% manakala terhadap *S. aureus* berkisar 3.125% hingga 12.5%. Selain itu, *C. albicans* menunjukkan kerentanan rendah terhadap semua jenis madu. Ujian masa membunuh menunjukkan aktiviti bakteria terhadap *E.coli* dan *S. aureus*. Madu lebah tanpa sengat menunjukkan pengurangan *E.coli* selepas 24 jam manakala pengurangan *S. aureus* ditunjukkan selepas rawatan 2 jam. Aktiviti anti-proliferatif dan antimikrobium madu dikaitkan dengan kandungan fenol total mereka, aktiviti hidrogen peroksida, keasidan dan hyper-osmolarity madu. Kami membuat kesimpulan bahawa madu *Trigona spp*. menunjukkan kesan perencatan yang berlainan pada sel-sel kanser yang berbeza dan aktiviti antibakteria spektrum yang lebih luas tetapi lemah dalam aktiviti antikulat. Walau bagaimanapun, siasatan selanjutnya terhadap *Trigona spp*. mekanisme tindakan madu, dalam kajian vivo, dan kesan toksikologi diperlukan untuk menetapkan keselamatan mereka sebagai agen antikanker dan antimikrobial.

Evaluation of The Anti-proliferative and Antimicrobial Activities of Three Types of *Trigona spp*. Honey

ABSTRACT

Trigona spp. known as Kelulut in Malaysia, was a small stingless bee from tribe *Meliponini*. This stingless bee produced honey which was used in wound healing, increase immune system, killing bacteria, treatment of bronchial phlegm, relieving sore throat, cough, and cold. In this study, the *Trigona spp*. honeys were tested to determine the anti-proliferative activity against MCF-7 breast cancer cell lines, HeLa cervical cancer cell and SVG normal glial cell using MTT assay. The antimicrobial activities of *Trigona spp*. honey were tested by determination of minimum inhibitory concentration (MIC) against *E. coli*, *S. aureus* and *C. albicans* using microdilution method. Then minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) value were determined by sub culturing the contents of nutrient broth used for MIC tests on nutrient agar using sterile wire loop and making a strike on the media to see bacteria growth after incubating at 37 °C for 24 hours. Absence of growth of *E. coli* and *S. aureus* were indicated the MBCs of the honeys while MFC were determined by absence growth of *C. albicans*. Lastly, the time kill assay were determined at concentrations of 0.5 x MIC, 1 x MIC and 2 x MIC and were incubated for varied time intervals (0, 2, 4, 24 hours). The number of living cells (CFU/mL) of each sample was determined using the agar plate count method. As the result, both *H. itama* honey and *T. laeviceps* honey were showed inhibition in the growth of

MCF-7 breast cancer cell line and HeLa cervical cancer cell lines. However, the *T. laeviceps* honey has better anti-proliferative activity on MCF-7 cell lines but weak anti-proliferative activity on HeLa cell line. In contrast, *H. itama* honey showed better anti-proliferative activity on HeLa cell line but much weaker on MCF-7 cell lines. Besides, *Trigona spp.* honeys were non-toxic to the normal cell line while toxic to the cancer cell line. The undiluted *H. itama* honey (100 % concentration) promoted normal cell proliferation. For antimicrobial activity, MIC values of the *Trigona spp.* honey against *E. coli* were ranged 3.125 % to 6.25 % while against *S. aureus* were ranged 3.125 % to 12.5 %. Besides, *C. albicans* showed low susceptibility to all types of honeys. Time kill assay showed that have bactericidal activity against *E.coli* and *S. aureus*. The stingless bee honey showed the reduction of *E.coli* after 24 hours while the reduction of *S. aureus* was showed after 2 hours of treatment. The anti-proliferative and antimicrobial activities of the honey were correlated with their total phenols content, hydrogen peroxide activity, acidity and hyper-osmolarity of the honey. We concluded that the *Trigona spp.* honey showed varied inhibitory effects on different cancer cell lines and broader-spectrum antibacterial activity but weak in antifungal activity. However, further investigation of *Trigona spp*. honey mechanisms of action, *in vivo* studies, and toxicological effects will be necessary to establish their safety as anticancer and antimicrobial agents.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Stingless bee known as Kelulut in Malaysia is come from subfamily *Meliponinae*. Commonly stingless bees are important for pollination of crops and wild plants at South America, Australia, Asia and Africa (Garibaldi, 2013). This bee is different than honey bee (*Apis*) because do not have sting but release irritant chemical to defense from disrupter (David, 2006). Stingless bee consists of two tribes which are *Trigona* and *Melipona*. Variety of stingless bee species can be found in Malaysia including *Trigona itama*, *Trigona thoracica*, *Trigona terminata*, *Trigona laeviceps*, and *Hypotrigona scintillans* (Kelly *et al.*, 2014).

Stingless bee produce powerful honey that stored in clusters of small resin pots in their nest (Latifah *et al.*, 2016). The beneficial of the honey as medicinal and food for human health is well known worldwide. Usually, the honey is taken twice daily in the morning and at the night (Latifah *et al.*, 2016). The stingless bee honey also used traditionally for anti-ageing, enhancing libido and immune system, killing bacteria, treatment of bronchial phlegm, and relieving sore throat, cough, and cold (Barakhbah, 2007). Besides that, the honey also use as antiseptic to avoid infection on eyes and cutaneous injuries (Alejandro *et al.*, 2014). This honey is believed to reduce

cardiovascular risk in both healthy patients and in those with potential risk factors (Pasupuleti *et al.*, 2016).

The pharmacological properties of the stingless bee honey included anti-inflammatory, antioxidant, anti-ageing, and antibacterial properties (Latifah *et al.*, 2016). Based on Alejandro *et al.*, (2014), anti-inflammatory properties of the honey are proved to treat muscle fatigue, sprains, injuries and mauls by dabbing the honey on affected areas. A lot of studies reported the Kelulut honey have antibacterial properties. According to DeMera and Angert, (2004), the honey showed antimicrobial activity against several different bacterial strains, including *Bacillus cereus* (Gram positive bacteria) and *Pseudomonas aeruginosa* (Gram negative bacteria), as well as against yeasts such as *Candida albicans* and *Saccharomyces cerevisiae*. The antibacterial properties of honey also possess wound healing activity (Boorn *et al.*, 2010).

Experiment on mice proved that the stingless bee honey can improve granulation tissue thickness and open wound size (Pasupuleti *et al.*, 2016). In addition, strong antioxidant activities of the honey proved that the stingless bee honey has potential chemopreventive agents (Beretta *et al.*, 2005). The stingless bee honey possesses moderate anticolon cancer activity due to caffeic acid ester that responsible as agent against colon cancer (Latifah *et al.*, 2016).

Although varies reports on the anticancer and antimicrobial effects of stingless bee honey are available, but the information on specific species of stingless bee honey such Itama and Laeviceps are lacking and further investigation is required.

1.2 Problem statement

As the cancer becomes one of the life threatening diseases throughout the world, there are interest to develop a new anticancer drugs, which are more specific and less side effects than chemotherapeutic drugs. Chemotherapeutic treatment can cause side effects in short-term and also for the long term (Katrin, 2012). Besides, long-term use of chemotherapy can lead to drug resistance via several different mechanisms, such as gene mutation, DNA methylation and histone modification (Genevieve *et al.*, 2014).

While in pharmacological industries, resistance to antibiotics drugs by microorganisms has increased (Gislene *et al*., 2000). The emergence of antibiotic-resistant bacteria has drawn major attention among healthcare and medical practitioners. According to Assegid *et al.*, (2003), the drug resistance of bacteria and side effects of some pharmaceutical products give rise to an aversion to synthetic drugs. However, synthetic drugs are very expensive and the doubt on the safety of the drug arose among community. Synthetic drugs possess many negative effects like anxiety, allergic reactions, heart palpitations, kidney and renal failure, or may increase the risk of fatal diseases.

Stingless bee honey is one of the natural bee products which have long been used as traditional medicine due its healing properties. Therefore, nature products like *Trigona spp*. honey will be the cheapest and safer alternative sources of anticancer and antimicrobial agents.

1.3 Significant of study

This study is focusing on the anti-proliferative and antimicrobial activity *Trigona spp*. honey. The result of this research will prove the honey contain with active compounds that lead to the anti-proliferative and antimicrobial properties of the honey.

Therefore, *Trigona spp*. honey may serve as natural product and further developed to be used in medical industries, cosmetic, household products, cleaning agents, sanitizers, dermatological product and other commercial values. In addition, *Trigona spp*. honey can be potential sources for developing new drugs and more effective anti-cancer and antimicrobial agents for future therapy. The new drugs based on natural product will be more save and reduce the risk of side effect. Besides that, the information that gained from this study is important to add more collection of database for future studies in Malaysia.

1.4 Objectives of study

1.4.1 General objective

To evaluate the anti-proliferative and antimicrobial activities of three types of *Trigona spp*. honey.

1.4.2 Specific objectives

a. To determine the anti-proliferative activity of three types of *Trigona spp*. honey on selected cancer cell lines.
b. To determine the minimal inhibitory concentration (MIC) of three types of *Trigona spp*. honey against the microorganisms.
c. To determine the time-related antimicrobial efficacy of three types of *Trigona spp.* honey**.**

CHAPTER 2

LITERATURE REVIEW

2.1 Stingless bees

Bees are well known insects for their roles in pollination. These insects have approximately about 20,000 known species of bees in seven recognized biological families. Stingless bees (Figure 2.1) are one of the species of bees other than honey bee and bumblebee (bombini). Stingless bees come from subfamily *Meliponinae* while the honey bees from subfamily *Apinae*, and both are grouped in the same family *Apidae* (Ewnetu *et al*., 2013). There are around 64 genera and 500 species of stingless bees that abundant in subtropical and tropical regions of the world such as Tropical America, Australia, Africa and Southeast Asia (*Araujo et al.*, 2004).

Figure 2.1: Stingless bee *Trigona spp.* (Jaymi, 2017).

Stingless bees are smaller in size than common honey bees. Besides, stingless bees are not harmful to humans as they have reduction of sting (Kyle *et al.*, 2015). However, the stingless bee will defend their hives aggressively by biting, irritating, and crawling into the eyes or ears of the intruder. Furthermore, the bees also can produce a caustic liquid from their mouth and releasing unpleasant odors to annoy the predator (Zweden *et al.*, 2011). Different with honey bees which make honey in vertical comb, the stingless bees hive (Figure 2.1.1) consists of thimble-sized oval shaped honey or pollen pots placed around the one cell thick brood area of the combs that are arranged horizontally (Assegid *et al.*, 2003).

Usually, the storage pots build of resinous cerumen in the ground or in the tree trunk (Yalemwork *et al.*, 2013). Cerumen is similar to propolis which produced by the secretion of the stingless bee during construction of the hive (Simone-Finstrom and Spivak, 2010). Uses of cerumen are important to store the honey and to avoid insect attack as well as to ensure the sterile environment in the hive (Mohd Azri *et al.*, 2016). Both groups make honey in perennial nests founded by a swarm of sterile workers and a queen, and colonies occasionally produce male bees.

Meliponinae stingle are divided into two tribes, which are *Trigona* and *Melipona* based on taxonomic characteristics (Maria *et al*., 2006). These two tribes of *Meliponinae* are used in stingless bee keeping because can produce large amounts of honey. The *Melipona* species are restricted to central and south America whereas *Trigona* species occur in all the tropical continental regions (Amano *et al.*, 2000).

There are nearly 150 species of *Trigona* bees in this world such as *Trigona apicalis* (*T. apicalis*), *Trigona drescheri* (*T. drescheri*), *Trigona fuscibasis* (*T. fuscibasis*), *Trigona fuscobalteata* (*T. fuscobalteata*), *Trigona incisa* (*T. incisa*), *Trigona itama* (*T. itama*), *Trigona laeviceps* (*T. laeviceps*), *Trigona melina* (*T. melina*), *Trigona terminate* (*T. terminate*) (Syafrizal *et al.*, 2012).

2.1.1 *Trigona spp*. honey

Honey is a natural sweet substance used in the world. The honeybees suck the honey from nectar of flowers and then transform it by combining with specific substances of their own before store and leave in the honeycomb to ripen and mature (Alimentarius, 2001). In some region especially in the tropics and subtropics, stingless bees honey is more popular than honey by typical honey bees. The stingless bee honey is primarily produced by *Tetragonisca*, *Melipona*, *Scaptotrigona*, *Plebeia* (in America), *Meliponula* (in Africa) and *Tetragonula* (in Asia) (Souza *et al.*, 2006).

In Malaysia, honey from these bees is known as Kelulut honey. This multifloral honey is stored in clusters of small resin pots near the extremities of their nests (Siok *et al.*, 2014). The nectar collected by the stingless bee will undergo three different transformation processes before turning into honey in the cerumen pots (Mohd Azri *et al.*, 2016). Firstly the physical of nectar is changed due to evaporation of water content in the nectar. Next, biological transformation occurs when fermentation process by yeast and bacteria which play a symbiotic role with the bee. Lastly, a chemical transformation take places by secretion the enzymes from the bee cephalic glands which hydrolyses the nectar's sucrose into fructose and glucose (Menezes *et al.*, 2013).

The honey that is produced by these stingless bees or *Trigona spp*. is viscous and darker than *Apis spp*. honey (Assegid *et al*., 2003). The dark colour of honey is impacted due to the abundant resin chemicals and hydrogen peroxide which makes the honey does not ferment in the nest. Most *Trigona spp*. honeys are oil-looking at the first glance and then turn to whitish paste after crystallization (Souza *et al.,* 2006). According to Biluca and other researchers (2014), *Trigona spp.* honey has a distinct taste and aroma, more fluid in texture, and undergoes slow crystallization. Besides, the *Trigona spp.* honey also has a stronger acidic flavor (Garedew *et al.,* 2013). Usually the pH value of the honey is around 3.2 to 4.9 (Assegid *et al.*, 2003).

Approximately one to two liters of honey can be yield from a colony of stingless bee (Assegid *et al.,* 2003). The honeys are collected using disposable syringes and suction pump for more efficiently (Souza *et al*., 2006). The productions of stingless bee honey are controlled by environmental changes and by inappropriate management during harvest the honey or transfer the hive to other location (Villanueva *et al.*, 2005).

Thus the market price of stingless bee honey is sold at higher price than *Apis* honey mainly due to its rarity and low production of the honey. In addition, the honey has special medicinal value and the production costs are high because of its harvesting process (Heard and Dollin, 2000).

2.1.2 Uses of *Trigona spp.* honey

Honey is a special gift from nature for human. Honey is highly valued as a food source and natural sweetener since the earliest times (Boorn *et al.,* 2009). Human used honey started as early as the Stone Age (Jose *et al.,* 2010). The ancient Chinese, Egyptians, Greeks, Assyrian and Romans make practical and effective use of different types of honey to treat diseases at that time (Pasupuleti *et al.,* 2016). Uses of honey are started as a drug and an ointment in 2100–2000 BC (Jose *et al*., 2010). Besides, the stingless bee honeys are used by Aboriginal people of northern Australia for cultural significance, playing a role in the social traditions and rituals of the people (Isaacs, 2000).

Stingless bee honey is more powerful than honey from common bees in folk medicine due to "natural" cure for treating variety type of diseases (Vit, 2001). Therefore, honey has been used as traditional medicines, alone or by adding with other substances. The honeys are widely applied as traditional medicine in Central and South America, and Africa (Cortopassi-Laurino *et al.* 2006). This honey has been administered both topically and orally (Samrudhi *et al.*, 2015). But some people do not eat the honey because of the strong sour taste (Assegid *et al.*, 2003).

Stingless bee honey which is also known as Kelulut honey is used traditionally by local people in Malaysia for anti-ageing, enhancing libido and immune system, killing bacteria, treatment of bronchial phlegm, and relieving sore throat, cough, and cold (Barakhbah, 2007). Usually the honey is taken twice daily, once in the morning and at night to heal sore throat, cough and cold. In Ethiopia, stingless honey also used traditionally to treat wounds, respiratory infections and diarrhea (Yalemwork *et al.*,

2013). In addition, many panaceas are produced from the honey to treat stomach disturbance, tonsillitis, stomach and intestinal ulcers, disease of the mouth and mucus membrane, and as wound dressing (Assegid *et al.*, 2003). These panaceas are applied by poor people in the rural area to treat these serious illnesses.

The stingless bee honey plays an important role in Ayurvedic medicine to cure eye diseases, hiccups, thirst, blood vomiting, leprosy, asthma, diabetes, worm infestation, diarrhoea, obesity and healing wounds (Samrudhi *et al.*, 2015). In addition, the *Trigona spp.* honey also applies as eyedropper to treat eyesight problems (Pasupuleti *et al.*, 2016). Other than being used as sweetener, the honey is also used in treating glaucoma and cataracts (Jose *et al.*, 2010).

2.1.3 Chemical constituents of *Trigona spp.* honey

Generally, the composition of honey is inconstant and variable based on the floral source where the bees collect raw material (Pasupuleti *et al.*, 2016). Besides, external factors also give effect to the composition of honey, such as seasonal and environmental factors and processing (Jose *et al.*, 2010). Furthermore, the composition of honey is influenced by processing, handling, and storage time (Bertoncelj *et al.*, 2007).

Table 2.1: Physico-chemical properties of stingless bee honey (Souza *et al.*, 2006).

Physico-chemical properties of stingless bee honey	
Appearance	Amber brown
Moisture content	25.02 %
pH	3.05–4.55
Total reducing sugars	55.00–86.00%
Glucose	8.20–30.98 %
Fructose	31.11–40.20 %
Sucrose	0.31- 1.26 %
Electrical conductivity (mS/cm)	0.49–8.77
HMF (mg/kg)	8.80–69.00
Ash content (g/100 g)	0.01–0.12

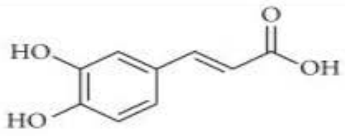
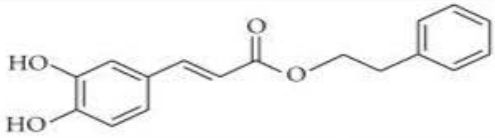
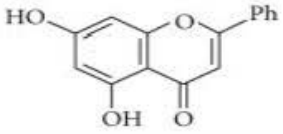
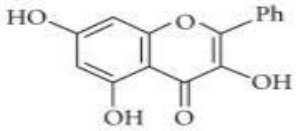
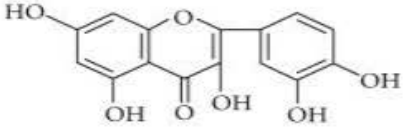
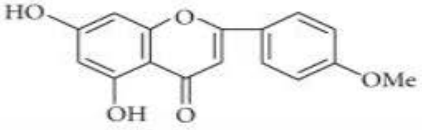
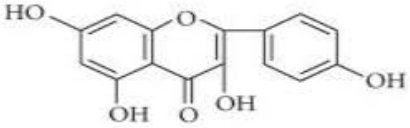
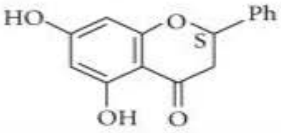
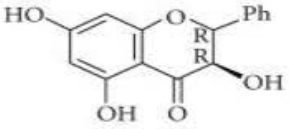
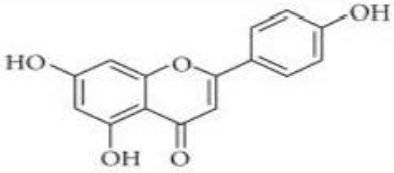
Approximately 200 compounds are discovered consist in honey such as vitamins, enzymes, amino acids and minerals (Ramanauskiene *et al.*, 2012). The major contents in honey are 15 % to 17 % of water and 80 % to 85 % of sugar (Bogdanov *et al.*, 2008). The intense sweetness of honey arises from the very high content of two monosaccharaides, glucose and fructose with addition to about 25 different oligosaccharides (Sohaimy *et al*., 2015). The honey also consist organic acids, minerals and trace elements such as calcium, potassium, sodium, magnesium, phosphorus, sulphur, iron, zinc, copper and manganese (Pasupuleti *et al.*, 2016).

Honey also rich with various vitamins, including ascorbic acid (VitaminC), thiamine (Vitamin B1), pantothenic acid (Vitamin B5), riboflavin(Vitamin B2), nicotinic acid (Vitamin B3), pyridoxine (Vitamin B6), biotin (Vitamin B8), folic acid (Vitamin B9) and cyanocobal-amin (Vitamin B12) (Ciulu *et al.*, 2011).

Additionally, honey is rich with other active compounds that exert several biological activities such as antibacterial, hepatoprotective, anti-inflammatory, hypoglycemic, antioxidant, anti-proliferative, antitumor, antimetastatic, anticancer and antihypertensive effects (Omotayo *et al.*, 2014). The antioxidant effects of stingless bee honey depend on the presence of flavonoids, phenolic acids, ascorbic acid, catalase, peroxidase, carotenoids, and the products of Maillard reactions (Khalil *et al.*, 2011). Based on Beretta *et al.* (2005), the total phenolic content in honey is strongly correlated with its antioxidant activity. Furthermore, flavonoids are the main component in ameliorating oxidative stress that play important role in antioxidant activity (Pasupuleti *et al.*, 2016).

Many studies have reported that there are a great variety of flavonoids and phenolic compounds that have been identified in honey including chrysin, kaempferol, quercetin, pinobanksin, pinocembrin, luteolin, apigenin, genistein, naringenin, hesperetin, *p*-coumaric acid, gallic acid, ellagic acid, ferulic acid, syringic acid, caffeic acid and vanillic acid (Meda *et al.*, 2005, Bertoncelj, *et al.*, 2007 and Kassim *et al.*, 2010). However, the total phenolic content of the honey are differs based on type of bee species, region, season and type of floral sources (Almeida da Silva *et al.*, 2013). Honey also contains two important enzymes that play a major role in its biological activities which are bee-origin glucose oxidase and floral-origin catalase (Zainol *et al.*, 2013). These enzymes are the key elements in regulating the level of peroxide activity in honey that is important in producing the antimicrobial activity of the honey (Kacaniova *et al.*, 2011).

Table 2.2: Molecular representation of Polyphenols found in the honey (Saravana and Mahitosh, 2009).

Polyphenols	Descriptive figures
Caffeic acid	
Caffeic acid phenyl ester	
Chrysin	
Galangin	
Quercetin	
Acacetin	
Kaempferol	
Pinocembrin	
Pinobanksin	
Apigenin	

Ph- phenyl; Me- methyl

2.1.4 Previous study of *Trigona spp.* honey

The majority of research has focused on honey of *Apis mellifera*, however attention has been paid to honey from stingless bees. Recently, the study according to pharmacological properties of the stingless bee honey from researcher all over the world has increased. Various pharmacological properties of the stingless bee honey have been reported and shared by researcher such as anti-inflammatory (Massaro *et al.*, 2011), antioxidant (Siok *et al.*, 2014; Bertoncelj *et al.*, 2007), anticancer (Choudhari *et al.*, 2013), anti-diabetic (Adesoji and Oluwakemi, 2008), anti-ageing (Sabir *et al*., 2005), and antibacterial properties (Temaru *et al*., 2007, Boorn *et al.*, 2009, Zainol *et al.*, 2013).

Numerous studies report on anticancer activity of stingless bee propolis but still lacking on stingless bee honey (Pasupuleti *et al.*, 2016). Stingless bees honey from *Trigona* species showed strong antioxidant activities that related to the potential of anticancer activity of the honey (Latifah *et al*., 2016). The honey produce from stingless bee *Tetragonula carbonaria* have higher total antioxidant activity than that of the European floral honey (Oddo *et al.*, 2008).

From the study done by Yazan *et al.* (2016), stingless bee honey have potential as chemo-preventive agent by reducing the total number of aberrant crypts (AC), crypt multiplicity and aberrant crypt foci (AFC) in *Sprague Dawley* rats which induced with colorectal cancer. Furthermore, stingless bee honey showed a cytotoxicity effect on many types of cell lines (Kuatiawan *et al*., 2014). The cytotoxic of the honey on the cells are act as anti-proliferative or apoptotic (Porcza *et al.* 2016). Patricia and other researchers (2013) discovered the anti-proliferative action of pot honey

from *Frieseomelitta*, *Melipona*, *Scaptotrigona*, and *Tetragonula* on human ovarian cancer cell lines.

Commonly, the antimicrobial activity of stingless bee honey is investigated by using the Kirby-Bauer antibiotic test, the agar dilution test, broth microdilution, and time-kill viability assays (Temaru *et al*., 2007, Boorn *et al.*, 2009, Zainol *et al.*, 2013). Kelulut honey has quantitatively poor but gave large zones of inhibition on agar diffusion assay, especially against *S. aureus*, indicating high antibacterial activity (Zainol *et al*., 2013). Honey of the stingless bees also produced the highest mean inhibition compared to white honey and yellow honey on all the standard and resistant strains (Yalemwork *et al.*, 2013).

In addition, *Trigona laeviceps* honey show antimicrobial activity against bacteria strain *E. coli* and *S. aureus* and the fungal strain *Aspergillus niger*, as well as two types of yeasts which are *Auriobasidium pullulans* and *C. albicans* (Chanchao, 2009). Data from both the agar and broth dilution methods indicated that stingless bee honey has broad-spectrum antibacterial activity but limited activity against *Candida* (Boorn *et al.*, 2009). The antibacterial effect of stingless bee honey is influenced by non-peroxide activity such as high sugar content and the acidity of the honey (Temaru *et al*., 2007).

2.2 Cancer

Cancer can arise from a single cell which multiplies and divides clonally in an uncontrolled manner and made up of billion more cell cancers (Trosko, 2001). The abnormal proliferative of cells at any part of body can cause cancer cell at the tissues and organs eventually spreading throughout the body (Cooper, 2000).

A group or lump of cells that grow abnormally is known as tumor and divided into two classes which are benign and malignant based on their different cellular characteristics (Paula *et al.*, 2007). Usually benign tumor remains and grows slowly in its original location and does not invade other surrounding tissues (Cooper, 2000). Thus, this tumor is noncancerous and can be removed by surgery, but probably to recur again. However, malignant tumor can cause more changes in cell biology (Paula *et al.*, 2007). This tumor can lead to uncontrolled and overgrowth of cells which cause damage to the cells and tissues of the body.

Besides, the tumor cells can grow rapidly and easily spread around the body through circulatory or lymphatic systems, and attacked the surrounding cells (Cooper, 2000). The movement of malignant tumor cells from the initial site to colonize other distant organs is known as metastasis which leads to cancer and difficult to treat (Patricia, 2016). Furthermore, the cancer cells do not die because the cells do not undergo programmed cell death which is known as apoptosis unlike normal cell (Graham, 2015). Thus, the cancer cells keep growing uncontrollably and invade neighbouring tissues.

2.2.1 Cancer statistics

Nowadays there are more than 100 different types of cancers that invade almost any part of human body. Cancer is known by the type of cell that is initially affected (Graham, 2015). According to Paula *et al.* (2007), cancer was the leading cause of death amongst the global population after cardiovascular diseases. In 2013, about 14.1 million new cancer cases and 8.2 million deaths recorded worldwide (Linsey *et al.*, 2015). In United State, one in 4 deaths is caused by cancer (Rebecca *et al.*, 2013).

In addition, rates of cancer of prostate, colorectal, female breast, and lung are multiple times higher in more developed regions than less developed countries. In less developed countries, common cancers that cause deaths are liver, stomach, and cervical cancers which are related to infection and they are detected at the last stage (Linsey *et al.*, 2015).

In Malaysia, cancer is also known as one of major health concerns due to the number of diseases that arise from cancer keep growing yearly (Gerard, 2002). It is estimated that the annual incidence of cancer is 30 000 and expected to increase follow the growing of aging and lifestyle of the population. The common cancer types that cause death in Malaysia population are lung, liver, breast, stomach, leukemia, colon, nasopharynx, cervix, lymphoid tissue and ovary (Gerard, 2002).

Cancer is terrifying disease that affecting everyone regardless to gender and age. According to Gerard (2002), the most invasive cancers for males are lung, nasopharynx, stomach, urinary bladder, rectum, non-Hodgkin's lymphoma, larynx, liver, colon and

esophagus. On the other hand, common cancers among female are including cervix, breast, ovary, lung, nasopharynx, esophagus, thyroid, colon, rectum and non-Hodgkin's lymphoma.

In 2009, almost half of the cancer cases were diagnosed in adults aged more than sixty five years (Robert, 2014). Based on growing numbers of older adults, the total number of cancers is expected to increase by 45 % from 2010 to 2030 (Smith *et al.*, 2009). However, the cancer does not only attack at older age but also can be found in children and also infant. Worldwide, approximately 150 in 1 million children are affected with cancer annually (Merenu *et al.*, 2016). The estimated rate of pediatric malignancies is 77.4 per million children in Malaysia, whereby the range of age is less than fifteen years (Gerard, 2002).

Usually, the cancer symptoms in children are unrealized at earlier stage (Roberta and Lesley, 2003). The leading cancer types among children are leukemia, tumors of the brain and spinal cord, lymphomas, neuroblastoma, gonadal and germ cell tumors, kidney tumors, soft tissue sarcomas and retinoblastomas (Gerard, 2002). Therefore, cancer is emerging as the fourth leading cause of death for children especially in Asia, Central and Middle East (Merenu *et al*., 2016).

2.2.2 Breast cancer

Breast cancer is recorded as the major public health problem in women. Each year, approximately more than 40 000 women are dead caused by this malignant disease (Muhammad Al-hajj *et al.*, 2003). This cancer can cause death because it can metastasize only within 3 years from the initial detection of the primary tumor to the lymph nodes and other distant organ (Britta *et al*., 2005). In 2008, about 1.38 million of breast cancer cases are estimated worldwide and 458 400 cases ended with deaths (Ahmedin *et al*., 2011). However, the number of the cases recorded all around the world is increased to 1.7 million cases and 521,900 deaths in 2012 (Lindsey *et al.*, 2015). Usually, about half of the breast cancer cases and deaths are highly recorded in developing countries (Ahmedin *et al.*, 2011).

According to Zarghan and other researcher (2017), an individual has higher potential to get breast cancer if any family member has history of breast cancer which is caused by gene mutations in the BRCA1 and BRCA2 genes. Besides, one of risks to have breast cancer is coming from reproductive factors such as a long menstrual history, a woman who have never gave birth, use of hormone therapy or oral contraceptives, and have the first child at late age (Hulka and Moorman, 2001). Other potential risk factors include overweight or obese, weight gain after 18 years old, physical inactivity, and alcohol consumption (Lindsey *et al.*, 2015).

Therefore, early diagnosis by mammographic screening and the implementation of systemic adjuvant therapy is important to prevent metastasis of the breast cancer (Britta *et al.*, 2005). Furthermore, early signs and symptoms of the breast cancer can be found through clinical breast examination (Anderson *et al.*, 2010). Chemotherapy can help to increase fifteen years of survival rate by 10 % for women who are younger than 50 years old (Britta *et al.*, 2005).

2.2.3 Cervical cancer

Cervical cancer is another silent killer disease among women although the cancer develops very slowly. Cervical cancers mostly are squamous cell carcinomas that grow in the squamous (flattened) epithelial cells that line the cervix (Gunjan and Keyur, 2012). The common symptoms of cervical cancer like abnormal vaginal bleeding after sexual contact and continuous vaginal discharge (Ashlesha and Neha, 2016). In year 2012, approximately 527,600 new cases of cervical cancer and 265,700 deaths are estimated worldwide (Lindsey *et al.*, 2015).

Thus, this cancer is stated as one of the most common diagnosed cancer and the fourth leading cause of cancer death in females worldwide (Ahmaden *et al.*, 2011). Usually this cancer cases are highest recorded in sub-Saharan Africa, Latin America and the Caribbean, and Melanesia but lowest in Western Asia, Australia, New Zealand, and Northern America (Lindsey *et al.*, 2015). In Malaysia, about 12.9 % of female with cancers are diagnosed with cervical cancer (Zaridah, 2014).

About 90 % of cervical cancer is caused by Human papillomavirus (HPV) infection that is easily spread through sexual intercourse (Gunjan and Keyur, 2012). Besides, other common risk factors include smoking, tough framework for women, intake of conception prevention pills, having sex at a youthful age, and numerous sexual accomplices. Apart from that, women with numerous pregnancies also risk with higher potential of cervical cancer (Dooley, 2016).

Therefore, HPV vaccine is important to prevent the cervical cancer and it is recommended for girls start from age eleven to twelve years old (Zaridah, 2014). In addition, early detection of cervical cancer is also important by using pap smears screen, colposcopy, and cone biopsy (Gunjan and Keyur, 2012). Normally, the cervical cancer still can be treated with cisplatin in chemotherapy, trachelectomy and also hysterectomy (Ashlesha and Neha, 2016). The treatment of the cancer is determined based on the stage of the cancer, size and shape of the tumor, age of the patient and also desire to have children in the future (Smith, 2010).

2.2.4 Causes of cancer

Based on Paula and other researchers (2007), development of cancer is classified by two factors which are exogenous and endogenous. Exogenous factor of cancer development includes nutritional habits, socio-economic status, lifestyle, physical agents, chemical compounds and biological agents. Endogenous factors of the cancers mean it is caused by immune system damage and inflammation, uncertain aetiology, genetic makeup, age, endocrine balance and physiological condition.

Unhealthy lifestyle of an individual can turn to be the major factor that leads to the cancer. In 2015, approximately 25 % of 1,658,370 cancer cases are attributed from poor nutrition which based on the food preparation and preservative, physical inactivity, overweight, and obesity (Stacey *et al.*, 2015). According to Giovanni and Franco (2013), obesity and overweight can cause cancer of endometrial, esophageal adenocarcinoma, colorectal, postmenopausal breast, prostate, and kidney.

Besides, excess alcohol consumption and smoking of tobacco are also responsible in result of cancer (Weisburger, 1999). Estimated 171,000 deaths of cancer patients are caused just by tobacco smoking alone (Stacey *et al.*, 2015). Commonly, smoking and drinking alcohol heavily will be factor for cancer of lung, oral cavity, esophagus, larynx, pharynx, esophagus, stomach, rectum, liver, and breast (Stacey *et al.*, 2015).

Some cancers are developed from infection of biological agents such as *Helicobacter pylori*, Epstein Barr virus, human T lymphotropic viruses I and II, human papilloma virus and the hepatitis B virus (Paula *et al.*, 2007). For example, bacterium *H. pylori* causes stomach cancer and gastric lymphoma, hepatitis C virus (HCV) can causes cirrhosis and liver cancer, while human papillomavirus (HPV) can lead to cervical cancer (Stacey *et al.*, 2015).

In addition, cancer also can be induced by other carcinogenic agents including chemical, ultraviolet radiation exposure, exposed to ionizing radiation in the form of gamma rays from outer space and from global distribution of manmade gamma and X-radiation from nuclear weapons testing and nuclear reactor accidents (Stacey *et al.*,