PHYTOCHEMICAL ANALYSIS AND POTENTIAL ANTICANCER ACTIVITY OF PROPOLIS EXTRACT DERIVED FROM MALAYSIAN TRIGONA APICALIS IN HUMAN CERVICAL CELLS

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by

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LIST OF ABBREVIATION

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
BHT	Butylated hydroxytoluene
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
DPPH	1,1- Diphenyl-2- picryl- hydrazyl
EAP	Extract aqueous propolis
EEP	Extract ethanolic propolis
FRAP	Ferric ion reducing antioxidant power
FTC	Ferric thiocyanate
HeLa	Henrietta lack cell
L929	Fibroblast cell normal
MCF-7	Michigan Cancer Foundation- 7 cell line
mM	milli Molar
ORAC	Oxygen radical absorbance capacity
TPC	Total phenolic content
TFC	Total Flavonoid content
µM	micro Molar
µL	micro Litre

ANALISIS FITOKIMIA DAN POTENSI AKTIVITI ANTIKANSER KE ATAS EKSTRAK PROPOLIS *TRIGONA APICALIS* DARI MALAYSIA DALAM SEL SERVIK MANUSIA

ABSTRAK

Propolis adalah sejenis bahan resin produk sarang lebah yang diperbuat daripada pelbagai sumber tumbuhan. Propolis berwarna kuning ataupun perang bergantung kepada umur dan bahan yang terlibat dalam pembentukannya. Propolis terkenal dengan sifat-sifat terapeutik dan mempunyai pelbagai aktiviti biologi seperti anti-oksida dan anti-kanser. Dalam kajian ini propolis telah dilarutkan dengan pelarut yang mempunyai polariti berbeza dan seterusnya diuji untuk kandungan anti-oksida di dalam setiap ekstrak. Ekstrak etanol didapati mempunyai hasil tertinggi; (28.6%). Kecekapan pelarut untuk pengekstrakan adalah mengikut turutan etanol> akueus> diklorometan> petroleum eter. Julat asid fenolik ditemui dalam lingkungan 32.45-85 µg asid galic acid/g manakala julat flavonoid yang ditemui ialah sekitar 13- 147.44 µg quercetin/g. Ekstrak etanol mempunyai kandungan fenolik yang paling tinggi dan merupakan pengurai radikal bebas yang paling berkesan terhadap ujian DPPH dan ABTS. Sementara itu ekstrak akueus mempunyai nilai FRAP tertinggi, yang berkesinambungan dengan komposisi flavonoid tertinggi. Kolerasi positing wujud dalam kandungan fenolik dan flavonoid dengan aktiviti-aktiviti antioksidan propolis Trigona apicalis. Sehubungan itu, kedua-dua ekstrak polar diteruskan untuk kajian potensi sifat- sifat antikanser pada sel HeLa. Keputusan yang diperolehi menunjukkan ekstrak etanol dan akueus menunjukkan agen anti-kanser yang berpotensi dengan perencatan juga merangsang mekanisma apoptosis dengan membunuh sel-sel > 50 % dan ~ 30 % untuk ekstrak akueus pada 50 % pada 31.25 **µ**g/mL dan 120 **µ**g/mL kepekatan dos.

Kedua- dua ekstrak propolis juga merangsang mekanisma apoptosis dengan membunuh sel-sel > 50 % dan ~ 30 % untuk ekstrak akueus pada kepekatan dos maksimum. Selain itu, apabila diuji untuk perencatan kitaran sel kanser, ekstrak etanol merencatkan penangkapan sel pada fasa G0/G1. Sementara itu bagi ekstrak propolis akueus mencadangkan perencatan sel pada fasa G2/M. Kompoun analisis oleh GC-MS mengesan beberapa potensi sebatian dalam potensi ekstrak kutub dinamakan; 1,6-Cyclodecadine, 1-metil-5-metilena-8-(1-metiletil) -, s- (E, E), 4- (1,3,3- trimetil-bicyclo (4.1 0) hept-. 2-YL) tetapi 3-en-2- 1, Lanosterol dan (+) - (z)- Longipinan, Aromadendrin oksida-(2), Androsta- 1, 4- dien-3-1, 17-hidroksi-17-metil-, (17.Alpha)- dan 9-Isopropil-1 metil- 2- metilen-5- oksatricyclo (5.4.0.0 (3, 8)) undeken. Oleh itu, popolis berpotensi disyorkn sebagai salah satu alternatif untuk merawat kanser.

PHYTOCHEMICAL ANALYSIS AND POTENTIAL ANTICANCER ACTIVITY OF PROPOLIS EXTRACT DERIVED FROM MALAYSIAN *TRIGONA APICALIS* IN HUMAN CERVICAL CELLS

ABSTRACT

Propolis is a resinous substance of beehive which is made from various sources of a plant. It exists in yellow to dark brown in colour depending on its age and sources. Propolis is well known for its therapeutic properties. It possesses a wide range of biological activities such as antioxidant and anticancer properties. The impact of different solvents of various degree of polarities, on the extraction of antioxidant compounds of Malaysian stingless bee propolis, *Trigona apicalis,* were studied. Ethanolic extract was found to have the highest yield (28.6 %). The efficiency of solvents for extraction of propolis was found to be in the order of Ethanol > Aqueous> Dichloromethane > Petroleum ether. The phenolic acid ranged from 32.45 to 85 µg gallic acid equivalents/g while the flavanoid range from 13 to 147.44 µg quercetin equivalents/g. Ethanolic extract of propolis had the highest phenolic content and was the most potent scavenger of DPPH and ABTS. Meanwhile, the aqueous extract had the highest FRAP value, which correlates with the highest flavanoid composition. A positive correlation existed in regards to the phenolic and flavanoid content with the antioxidant activities of *Trigona apicalis* propolis. In this study, both polar extracts were examined for the anticancer properties on HeLa cell line. The result obtained shows that ethanolic and aqueous propolis extracts exhibited potential anticancer agent with inhibition of 50% at 31.25 and 120 µg/mL respectively.The extracts killed the HeLa cells via apoptosis mechanism by killing the cells more than 50% for ethanolic extract and about 30% for aqueous extract at maximum dose concentration; 62.5 and 160 µg/mL,

respectively. Beside when tested for cell cycle arrest, ethanolic extract caused cell arrest at G0/G1 phase. Meanwhile, aqueous propolis extract caused cell arrest at G2/M phase. Compound analysis by GC-MS detected some potential compounds in polar extract named 1,6-Cyclodecadine,1-methyl-5-methylene-8-(1-methylethyl)-, s- (E, E), 4-(1,3,3- Trimethyl-bicyclo (4.1. 0) hept-2-yl)-but 3-en-2-one, Lanosterol and (+)- (z)-Longipinane, Aromadendrine oxide- (2), Androsta- 1, 4-dien-3-one,17-hydroxy-17-methyl-, (17. Alpha.)- and 9-Isopropyl-1 methyl- 2-methylene- 5- oxatricyclo (5.4.0.0 (3, 8)) undecane. Based on the current study, propolis has antiproliferative activity against HeLa cell lines and has the potential to be developed into anticancer agent in the near future.

CHAPTER 1

INTRODUCTION

Stingless bees or also known as *Trigona* or *Meliponine* species are distributed in the tropical region; the habitats like forest, agricultural, riparian or littoral regions are also taken into account. Brazil became among the pioneer countries in stingless bee-keeping industry. The devoted commitment such as initiation and advanced courses in species management marked a significant improvement in stingless bee history that lead to first congress in Natal (2004) and second Brazilian Congress of Meliponiculture in Aracaju (2006).

The number of stingless bee species of *Meliponine* is estimated over 600 species within 56 named genera, which have been recorded in the tropical and subtropical regions of the world. Out of these, 400 known species exist in the Neotropical regions and at least 45 species are described in Southeast Asia. Recently in Indonesia, 9 stingless bee species have been recorded by researchers from University of Mularwan Botanical Garden, Samarinda. These species are *Trigona apicalis, Trigona drescheri, Trigona fuscibasis, Trigona fuscobalteata, Trigona incise, Trigona itama, Trigona laeviceps, Trigona melina and Trigona terminate*. 39 plant species from 13 families have been claimed as their source of pollen and 22 of them are forest plants that act as their natural resources of nectar.

It is believed that stingless bees are able to produce quality products which can be applied into many aspects like food industries and integrative medicine for management of health and diseases. In fact, the utilization of honey produced by stingless bee has started long time ago. The products of stingless bee species consist of honey (15.4%), beebread (20.9%), and propolis (63.7%). The interesting part of stingless bee products is their biological properties may vary greatly, depending on their geographical areas, seasons and food preferences, which may result in different chemical compositions, even for the same species of same region. As reported by Sawaya et al. (2009), the distribution of stingless bee species and their food preferences play a vital role as a major contribution to their characteristics that varied according to their geographical area.

A few studies carried out abroad have proven the effectiveness of propolis derived from *Apis* spp. as an antitumor agent. However, there is still lack of knowledge of propolis derived from local stingless bees towards anticancer activity. Thus, the focus of present study is to investigate the anticancer activity of propolis extract produced by one of the Malaysian stingless bee species, *Trigona apicalis*, and its correlation with the major phytochemical contents. This approach was chosen in order to establish the quality standardization data and reference guidelines towards the development of propolis as potential anticancer agent in the near future.

In conjunction to the preliminary data compiled from all over the world, the local propolis extract of *Trigona apicalis* was hypothesized to possess anti-cancer activities due to the presence of flavonoids that act through induction of apoptosis and inhibiting proliferation of cancer cells. Despite all the above studies, there is yet any study carried out on propolis derived from local stingless bee, specifically looking at *T. apicalis* species. As this species is widely available and commercially viable colony it would be beneficial to explore the potential roles of propolis gathered from such species on the anticancer activity. Therefore, this study was conducted to fulfill the following objectives:

1. To evaluate the antioxidant properties of different propolis extracts of local *T. apicalis* produced by different solvent extraction methods
2. To quantify the total phenolic and flavonoid contents of different propolis extracts from *T. apicalis*
3. To assess the correlation between antioxidant properties of propolis extracts and the total phenolic and flavonoid contents
4. To evaluate the anticancer properties of local propolis extracts derived from *T. apicalis* through apoptosis, cell cycle and proliferation assays

CHAPTER 2

LITERATURE REVIEW

2.1 Identification of *Trigona apicalis*

Stingless bees in Malaysia can be divided into 3 major species according to their distinct physical characteristics and the shape of its hive entrance namely *Trigona itama, Trigona apicalis and Trigona thoracica. T. apicalis* has split coloured wings while *T. itama* has uniform, sepia- tinged wing which set them apart from one another (r). *T. thoracica* has mounted- shape entrance with the widest size of entrance compare to other species (Roubik, 2006). **Table 2.1** shows the scientific classification of *Trigona apicalis.*

Table 2.1. Scientific classification of *Trigona apicalis*

Main taxonomic ranks	**Classifications**
Kingdom	*Animalia*
Division	*Arthropoda*
Class	*Insecta*
Order	*Hymenoptera*
Family	*Apidae*
Genus	*Trigona*
Species	*apicalis*

Trigona apicalis is one of the known species of stingless bee family. It has been differentiated from other species by the shape and structure of the wood channel for their beehives and a few distinct physical characteristics. The cytogenetic study of 31 species of genus *trigona* reported that females had 2n = 34 chromosomes and males had n = 17 chromosomes. The C-banding patterns showed that the karyotypes of these species consisted mainly of acrocentric and pseudoacrocentric chromosomes (Kleber et al., 2004).

This insect has black head covered with a cinereous pile as well as black thorax with a cinerous pile at the disk part. The clypeus and lower parts of the face are yellowish testaceous. The outer margin is thickly fringed with black pubescence and the apex of the abdomen is palerufo-testaceous (Wallace and Smith, 1857). **Figure 2.1** shows the physical appearance of *Trigona apicalis*.

Figure 2.1. Physical appearance of *Trigona apicalis* (Smith, 1857)

The Japanese are now appreciating the value of *Trigona* over the *Apis* species because of the worry of a sting by the *Apis* species. *Trigona sp.* has a very low tendency to swarm due to the presence of more than two queens per colony. Thus, they rarely abscond and have a longer working life of up to 60 days per worker. Stingless worker bees forage up to a 500-meter radius which means they are capable to perform intensive pollination of crops near the hive (Amano, 2005).

Their short tongue makes stingless bees capable to collect more nectar and pollen compare to other species. Stingless bees are highly tolerant to pests and diseases because of their smaller size and extensive use of propolis which serves as germicidal and pest repellent. They are highly tolerant of heat because of their propolis canopy. These characteristics lead stingless bees as the best pollinator in horticultural crops (Amano, 2005).

In Malaysia, about 32 different species of stingless bee has been recorded. Some commonly known species of stingless bees include *T. thoracica, T. itama, T. terminata, T. scintillans and T. laeviceps.* Among all *T. itama* is highly preferred and it contributes 83.2% of the total colonies in the stingless bees farm (Kelly et al., 2014).

2.2 Propolis

Propolis is a resinous substance of beehive which is made from various sources of a plant with an aromatic odor. It exists in yellow to dark brown in colour depending on its age and sources.

2.2.1 Chemical composition of propolis

Over the time, propolis is well known for its therapeutic properties and become a high economic value. It has been utilized and popularly distinguished as the traditional medicine for many centuries dated back to at least 300 BC (Ghisalberti, 1979). It consists of a sticky plant substances brought and collected by bees and formulated with beeswax and other secretion. The secretion varies and depends upon the vegetation of the collection area, which results in slightly different in composition of propolis. Nevertheless, there have been reports previously that both propolis from temperate zones and South America continent possessed the same biological characteristics (Banskota et al., 2002).

Although the propolis from temperate zones consists of predominantly phenolic compounds including flavonoids and cinnamic acid derivatives (Banskota et al., 2002), the South America continent has reported the presence of diterpenes and prenylated compounds in their propolis together with lignans, flavonoids and other classes of compounds (Bankova et al., 2000).

From the previous study on honey bee propolis, 31 different constituents from Brazilian propolis have been notified, which 3 of them were new and 15 were distinguished for the first time from propolis together with either their antiproliferative activity or hepatoprotective activity. Study on Netherland propolis has revealed 17 compounds with two new glycerol derivatives, compound which are 2- acetyl-1,3-dicoumaroylglycerol and 2- acetyl-1- coumaroyl-3-feruloyglycerol (Banskota et al., 2002) whereas in study of Myanmar propolis of methanolic extract after subjected to a series of chromatographic separation has resulted in isolation of two new cycloartene-type- triterpenes; (22Z,24E)-3-oxocycloart-22,24-dien-26-oic acid and (24E)-3-oxo-27, 28-dihydroxycycloart-24-en-26-oic acid (Li et al., 2009). **Table 2.2** indicated the chemical composition of propolis.

Table 2.2. Chemical compositon of propolis reported by Sawicka et al., 2012

Chemical compositions (%)	**Details**
Fatty and aliphatic acid (24- 26)	Butanedioic acid, propanoic acid, Decanoic acid, Undecanoic acid, Malic acid, D- Arabinoic acid, Tartaric acid, Gluconic acid, a- D- Glucoa- D- GLucopyranuronic acid, Octadecanoic acid (Stearic acid), Hexadecanoic acid, b- D- Glucopyranuronic acid, 9,12- Octadecadienoic acid, Tetradecanoic acid, Pentanedioic acid, Glutamic acid, 2,3,4- trihydroxy butyric acid, Phophoric acid and Isoferulic acid
Flavonoids (18- 20)	Astaxanthin, Apigenin, Chrysin, Tetrochrysin, Pinobanksin, Squalene, Pinostrobin chalcone, Pinocembrin, Genkwanin, Galangin, Pilloin, Acacetin, Kaemferide, Rhamnocitrin, 7, 4'- dimethoxyflavone, 5,7- dihydoxy-3, 4' dihydroxyflavone, 3,5- dihydroxy-7,4'- dimethoxyflavone.
Microelements (0.5- 2.0)	Aluminium, Copper, Magnesium, Zinc, Silicon, Iron, Manganese, Tin, Nickel, Chrome.
Aromatic acids (5- 10)	Benzoic acid, Caffeic acid, Ferulic acid, Cinnamic acid
Vitamins (2- 4)	A, B1, B2, E, C, PP
Sugars (15 – 18)	Sorbopyranose, D- Ertythrotetrofuranose, D- Altrose, D- Glucose, Arabinopyranose, d- Arabinose, a-D- Galactopyranose, Maltose, a- D- Glucopyranoside, D-

	Fructose.
Esters (2-6)	Caffeic acid phenethyl ester, 4,3- Acetyloxycaffeate, Cinnamic acid, 3,4 dimethoxy- trimethylsilyl ester, 3-Methoxy-4 cinnamate, Cinnamic acid 4 methoxy 3 TMS ester, 2- propenoic acid methyl ester.
Others (21-27)	Cyclohexanone, 3-methyl, antitricyclo undec-3-en 10-one, Cyclohexane, Cyclopentene, 5-n- propyl-1,3 dihydroxybenzene, Butane, 2 (3H)- Furanone, L-Proline, 2- Furanacetaldehyde, 2,5-is-3-phenyl-7-pyrazolopyrimidine, Cliogoinol methyl derivative
Alcohol and terpens (2-3.3)	Glycerol, Erythritol, a- Cedrol, Xylitol, Germanicol, Stigmast-22-en-3-ol, Farmesol, Pentitol, Ribitol Vanilethanediol and Bicyclohept-3-en-2-ol

2.2.2 Medicinal value of propolis

Since ancient time, propolis has been proven to have medicinal value. As reported by the previous study, propolis possessed beneficial health properties such as antibacterial, antiviral and antifungal (Kujumjgev et al., 1999), anticancer (Kimoto et al., 2001), anti-inflammatory (Strehl, Volpert, & Elstner, 1994), hepatoprotective, local anesthetic (Burdock, 1998), immunostimulatory, and antimutagenic (Kim et al., 2008).

The ongoing research towards propolis is intensely being carried worldwide in order to evaluate the significance of propolis development in terms of therapeutic value. A research on Brazilian species of stingless bees, *Tetragonisca fiebrigi*, exhibited that the ethanolic extract of propolis (EEP) of this

species affects the microorganisms positively. The antimicrobial activity was accessed in gram-positive and gram-negative bacteria and it was concluded that, the propolis extract presented greater activity against the gram-positive bacteria than against gram-negative bacteria (Campos et al., 2015).

In addition, propolis has been used in the prevention of inflammatory diseases. Inflammation is a biological response against tissue injuriessuch as irritants, damaged cells and pathogens. It has been reported that propolis worked out for both *in vivo* and *in vitro* inflammatory activity by modulating key inflammatory mediators, raise the production level of anti-inflammatory cytokines and blocking the activation of nuclear factor-(NF-)B (Machado et al., 2012).

Another study on propolis of *Tetragonisca fiebrigi* reported that EEP inhibited hyaluronidase enzyme in a concentration- dependent manner; with the highest concentration of 75 mg/mL was able to inhibit 43.06 ± 3.06% of enzymatic activity. The measurement of hyaluronidase enzyme is an indirect way to show anti-inflammatory activity with reference to hyaluronic acid as the main component of articular cartilage. It is also important for tissues renovation as it may cause bone loss, inflammation and pain due to degradation of this acid (Campos et al., 2015).

2.3 Phytochemistry study of natural products

Research on the herbal medicinal plant has increased dramatically worldwide, and given huge impact in the development of traditional and complementary medicine as well as integrative medicine. *Pimpinella barbata*, an annual plant of *Apiaceae* family has been studied in an aspect of antioxidant capacity in different extracts. The antioxidant capacity of the extracts when tested with DPPH scavenging assay ranged from the highest to lowest potency is as followed; methanolic> dichloromethane> n-hexane. In contrast, in FRAP assay, dichloromethane extract shows the highest activity. These results imply that different tests do not confirm another (Namjooyan et al., 2010).

2.3.1 Different solvent extraction approach

In one particular plant sample, it can be hundreds of undefined compounds based on the polarity. The presence of compounds in samples is highly dependent on the extraction method. Different extraction techniques have been developed to elute particular target compounds such as partitioned, soxhlet extraction and supercritical fluid extraction. They are widely used in research to obtain the potential compounds ranging from polar to non- polar.

The application of different polarity of solvents, such as polar solvents like aqueous, methanol and ethanol, and non-polar solvents like chloroform, dichloromethane, hexane, n-butanol and petroleum ether are very significant in order to achieve the target compounds. For example, a research on *Clausena excavata*

Leaves extract utilized petroleum ether, chloroform, ethyl acetate and methanol, by which the yields produced were 1.56, 2.57, 0.38, and 0.94% respectively. Subsequently, each extract underwent screening for antioxidant activity to determine the most potential solvent extract (Albaayit et al., 2014).

In general, the activities of extracts produced by different solvents are compared to value the most suitable solvent used that can contribute the highest activity. A study looked into the effects of various solvents used in the extraction of leaves, seeds, veins and skins of *Tamarindus Indica L*., and indicated that the efficiency of extraction was in the order of methanol> ethyl acetate> hexane (Razali et al., 2012).

In order to find out the safe therapeutic molecules from *Aphanamixis polystachya,* a famous medicinal Indian natural drug that has been used as an astringent, the polar and non- polar extracts of this plant were evaluated in a series of *in vitro* assay involving free radical and reactive oxygen species. The methanolic extract was concluded as the most effective solvent to extract the bioactive antioxidant compounds followed by chloroform extract, assessed by the metal chelation assay (Narayan and Rai, 2014).

2.3.2 High Performance Liquid Chromatography

Discovery of corresponding active compounds in a crude sample is an excellent benchmark in food research technology. Due to this, High Performance Liquid Chromatography (HPLC) has been widely used as an important separation tool of a pure compound from its complex mixture. This tool is considered as compulsory in pharmaceutical product development to maintain the quality and consistency, as well as establish standardization of the product (Hassan, 2012).

Chromatography has been developed in the early 20^{th} century by the Russian botanist, Tswet. The concept is based on the partitioning of solutes between two phases related to simple liquid-liquid extraction (Fornstedt et al., 2015). Besides, HPLC is also widely used in an application of clinical test related to urine analysis and antibiotic analysis in blood, and detection of endogenous neuropeptides in the extracellular fluid of brain (Strand, 2000). In addition, HPLC is applicable in forensic study for quantification of drug in biological sample, identification of steroids in blood and urine, forensic analysis of textile dyes and also determination of cocaine and other drugs of abuse in blood (Kamberi et al., 1999).

As far as pharmaceutical research is concerned, separation machine has been recognized as compulsory in leading new drug discovery. Some example of successful research is a determination of berberine in raw herb; *Berberis aristata*. This compound was estimated in three different extracts from different sources, and the outcome turned out to be in the range of 95.98-98.02% recovery with the validated HPLC-UV method at calibration curve of r^2=0.9942.

For the method to be confirmed, it has to be validated stage by stage for its linearity, specificity, accuracy, precision, intermediate precision and resistance under various adverse conditions (Pasrija et al., 2010)

Undoubtedly, HPLC has facilitated the development of traditional Chinese medicine when comparing with traditional plant chemical "purification-identification" research mode. HPLC promotes efficient separation peak, analysis of non- volatile substances especially in micro and trace amount 30% of the literature reported the successful story of HPLC in the analysis of metabolites of Chinese herbal medicinal ingredients which involved identification of metabolites, analysis of the metabolic pathways of TCMs and metabolic processes based on the metabolites (Li et al., 2011).

2.3.3 Gas Chromatography -Mass Spectrometry

The inspiration of Sir J. J. Thomson, who studied on electrical discharges in gases led to the discovery of the electron in 1897. Since then it has initiated the innovation of first mass spectrometer (MS), which was previously referred as parabola spectrograph; which is used in quantification of mass-to-charge ratios of ions. Throughout 100 years, a scientific breakthrough made possible by MS have included the discovery of isotopes, exact determination of atomic weight, characterization of a new element, quantitative gas analysis, stable isotopes labeling and the characterization of molecular structure (Borman, 1998).

Similar to HPLC, the contribution of Gas Chromatography-Mass Spectrometry (GC-MS) in herb research is undeniable. Qualitative analyses of different crude extracts of *Thymus vulgaris L;* an aromatic and medicinal plant native to Oman, was made possible by GC-MS. It showed that some biologically active molecules identified include thymol (40.868%), o-thymol (46.661%), linalyl anthranilate (1.064%) and α-terpineol (0.761%). The suitable solvent used for *Thymus vulgaris* extraction method were determined further based on the GC-MS analysis (Al Hashmi et al., 2013).

2.4 Antioxidant potential of medicinal plants

Food containing antioxidant compound becomes essential for the public to consume as their source of a nutrient. This has been scientifically approved since the antioxidant compound was found to play a crucial role as the health- protecting factor. Most of the antioxidant compounds in daily diet are available from plant sources and belong to various classes of compound with a wide range of physical and chemical properties.

Free radicals are present in our biological system attributed to external factors such as irradiation and xenobiotics. They are considered as one of the major contributors in the pathogenesis of several human diseases like cancer, cataract, immune system weakness and brain problem (Sies et al., 1992) as well as may induce nutritional and medical deteroriation (Maryadele et al., 2001).

Free radicals are capable of oxidizing materials like nucleic acid, proteins, lipids or DNA, which consequently may lead to many degenerative diseases. However, the productions of free radicals could be controlled by the antioxidant system by significantly reducing the oxidation rate (Jacob, 1995). The peak process of antioxidant is the ability to trap and demolish these free radicals by scavenging free radicals such as peroxide and lipid peroxyl.

Antioxidant compounds may exist in water-soluble, lipid-soluble, insoluble form or bound to cell walls. Thus, extraction method is a significant step in order to determine the efficiency to extract the compound that will ascribe to the antioxidant activity (Delfanian et al., 2015). Plant as a primary source of food in the food chain brings a lot of benefits, yet little is done to reveal and discover its potentials. It has been eminent through many scientific studies that, medicinal plants have the potential as the source of antioxidants with less adverse effects compared to the conventional medicine (Lobo et al., 2010).

2.4.1 Antioxidant activity of propolis

Propolis has a wide range of bioactivity, which is attributed mainly to the habitat of bee species and their source of food that varies between different regions. The composition of propolis fluctuates, based on the preference of bees for food, resulting in different antioxidant capacities. Indian stingless bee was observed for its antioxidant properties by using ABTS radical scavenging assay. It was found that the IC_{50} of EEP was approximately 5 times higher than the synthetic antioxidant BHT, used as control (Jacquieline et al., 2015).

According to a research on Brazilian propolis which was conducted to compare the antioxidant capacity between ethanol and petroleum ether's extract, it showed that both ethanolic extracts of propolis show higher activity than petroleum ether in DPPH and ferric thiocyanate (FTC) assays. Subsequently, phytochemical analysis of propolis marked the high content of flavonoids, which may explain the potent antioxidant capacity of propolis (Sheng et al., 2007).

Furthermore, a research on Brazilian propolis measured the antioxidant activity by using lipid peroxidation model system. Each propolis sample displays high superoxide-scavenging activity and it is directly proportional to the concentration of the sample. In fact, samples at 50 and 100 mg/mL completely inhibited the superoxide production similar to propolis reaction when in hydrooxyl radical scavenging activity (Nagai et al., 2003).

2.4.2 Total Phenolic (TPC) and Flavanoid Contents (TFC)

Generally, phenolic compounds like tocopherols, flavanoids and phenolic acids are examples of natural antioxidants. The potential of some phenolic compounds to act as antioxidant agents has been discussed in the literature for example; flavonoids provide beneficial effects to fight diseases in human. The capacity of flavonoids to act as antioxidants and free radical scavenger are believed to be associated with the position of hydroxyl groups and other features in the chemical structure of flavonoids (Velioglu et al., 1998).

Other than that, phenolics possess a wide spectrum of biological activities such as anti-mutagenic and anti-carcinogenic, as well as ability to modify the gene expression. Numerous epidemiological studies have confirmed the significant relationship between high dietary intake of flavonoids and the reduction of cardiovascular and carcinogenic risk. The formulation of preventive and healthy nutrition requires information about phenolic and flavonoid compositions in foods and their influence on various biological properties (Borkataky et al., 2013).

Research in enology showed a difference in phenolic profiles of wines which were attributed to the variable antioxidant and vasodilation activities. HPLC methods were later used for the analysis of major phenolics content in red wine, and it was found that the TPC was strongly correlated with the antioxidant potentials. Besides, phenolics were also beneficial in viticulture and enology fields which provide benefits including UV protection, disease resistance, pollination color and defense against predators (Burns et al., 2000).

A study on *Eclipta alba (L.) Hassk*, a small branched of an annual herbaceous plant with a long history of traditional medicinal uses in many countries, found that ethanolic extract has the highest TPC (8.933 ± 0.231) followed by ethyl acetate, petroleum ether, and aqueous extract (1.467 ± 0.231). Meanwhile, TFC for *E. alba* extracts varied from 0.5 mg RE/ g in the aqueous extract to 30.667 ±1.155 in ethanol extract. Similar to phenolics, flavonoids are also well-known for their antioxidant constituents and therefore have a wide spectrum of biological activities including radical scavenging activity (Borkataky et al., 2013).

2.5 Nature of cancer

Cancer can be explained as undesirable transformation, dysregulation of apoptosis, proliferation, invasion, angiogenesis and metastasis of certain cells. It is believed that the imbalance of immune system plays a subtle role in developing cancer. Cancer patients are known to be immuno-deficient and unable to control the tumor progression. However, the way that immune system is compromised by the tumor presence may be varied and the most aggressive tumors proved to have evolved, in terms of multiple mechanisms of escape from the host immune system (Gupta et al., 2010).

Throughout the years, multiple chemotherapy drugs have been developed to activate anti-tumor immune responses in cancer patients either via delivery of biologic response modifiers, genetic modification of immune cells or their adoptive transfers. These are aimed at providing the host with missing activation signals. Yet, these therapeutic strategies proved to be ineffective, and research has shown that immune-therapy alone is not sufficient to overcome tumor escape (Whiteside, 2006).

Worldwide, the compilation of adverse effects of cancer treatment has been recorded. More than 500 adverse effects have been related to modern cancer therapy, which range of severity started from minor, asymptomatic changes noted on physical examination to life- threatening injuries (Trotti et al., 2003).

A review on cancer–related inflammation has discussed the theory of chronic inflammation which inclined to the development of different forms of cancer. The utilization of non-steroid, anti-inflammatory agents that provide protection against various tumors is another proof that inflammation act as a risk factor for certain cancers. Some of the cancer-related inflammation (CRI) include the infiltration of white blood cells, prominently tumor-associated macrophages (TAMs); the presence of polypeptide messengers of inflammation (cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, chemokine such as CCL2) and the phenomenon of tissue remodeling and angiogenesis (Colotta et al., 2009).

2.5.1 Treatment of cancer and development of integrative medicine

The complexity of cancer, as it is multi-factorial disease, renders the process of patient prognosis. Most of cancer case remains unresolved making the cancer therapy an extremely difficult process with less percentage of survival rates. Many markers and established methods have been described to evaluate the prognosis of cancer patient yet very few could be translated into clinical practice (Angell and Galon, 2013).

Over the years, traditional medicines retain its popularity as one of the preferences for disease management among public all over the world. As a matter of fact, traditional medicine cannot be fully established due to many disputable issues such as quality assurance and quality control. Despite these, ongoing research about benefits of herbal plants is given intense attention Thousands of unknown biochemical compounds found in the herbal medicinal plants have been proven to possess significant bioactivity in regards to management and prevention of most diseases, as well as to maintain the general health (Lee, 2000).

The awareness of practicing integrative medicine among cancer patient has gone subtly over the period of years. The usefulness of medicinal herb has been compiled by folks in details with a description of ingredients and recipes for future used to maintain health, revitalize energy and disease prevention. Particularly in Malaysia, it is a significantly high record of traditional medicine use among the population (almost 70%). Approximately, 80% of the population in African and Asian countries nominated the complementary medicine as the main source of treating diseases (Hamidah et al., 2009).

2.5.2 Anticancer properties derived from herbs

Malaysia is a multiracial country with a wide ethnic diversity of its population and therefore, the influences of traditional medicine use among the people have been strong for many decades. Some of the traditional medicines originated from Malay medicine, Iban medicine and Kadazan medicine (Borneo), including Traditional Chinese and Indian Medicine (Chen, 1981).

Herbal therapies play important roles in primary prevention of cancer; prevention of a recurrence of cancer, enhancing the immune system, reducing side effects resulting from chemotherapy and as alternative options to conventional therapies. The concept of herbal medicine is way different than conventional chemical drugs because it does not involve DNA mutation in surviving cell (Tavakoli et al., 2012).

People are longing for natural and herbal products as sources of treatment for cancer. One of the successful researches on medicinal herb stated the *Paris polypholla* which commonly used for tumor patients in China to have predominant inhibitory effect for it's ethanolic crude extract ranging from 10 to 30 μg/mL against six human digestive tumor cell lines; human liver carcinoma cell lines (HepG-2 and SMMC – 7721), human gastric cancer cell line (BGC-823), human colon adenocarcinoma cell lines (LoVo and SW-116) and esophagus adenocarcinoma cell line (CaEs-17). Yet, the mechanism needs further study (Sun et al., 2007).

Herbal medicine assists in terms of strengthening the immune system in order to prevent the spread of cancer cells through inhibition of angiogenesis or growth of new blood vessels feeding the cancer cells. Additionally, it detoxifies the body and prevents further toxic build-up in the body, quenches free radicals that cause mutational changes leading to cancer formation, and supports all targeted organs, especially those affected directly by the cancer. Furthermore, it helps in creating the unfavorable environment for the survival of cancer cells, which include a high level of oxygen and temperature including increased metabolic rate, low sugar level and a high alkaline space in the body (Lam, 2003).