Phytochemical and Toxicity Screenings in Different Parts of *Eleiodoxa conferta*

By

Nishalani A/P Thivakar

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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Student

Name: NISHALANI A/P THIVAKAR

Date:

I certify that the report of this final year project entitled "**Phytochemical and Toxicity Screenings in Different Parts of *Eleiodoxa conferta***" by Nishalani A/P Thivakar, matric number F15A0285 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Product Development Technology) with Honours, Faculty of Agro Based Industry, Universiti Malaysia Kelantan.

Approved by:

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Supervisor

Name: Dr. Liew Jeng Young

Date:

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ABSTRACT

The potential of much of the indigenous fruits are still undiscovered. Recently, researchers are giving attention to discover the locally available fruits that may have potential to use for therapeutic remedies. One of the underutilized fruits which has been consumed by the rural communities in Malaysia is *Eleiodoxa conferta* fruit which is locally known as 'Buah Kelubi'. This project was conducted to screen phytoconstituent and toxicity activities of different parts of *E. conferta* by using different solvents extraction. Types of solvent used to extract the different parts of these fruit were 100% ethanol, 50% ethanol and water. The total phenolic content (TPC) and total flavonoid content (TFC) were studied by using Folin-Ciocalteu method and Aluminium chloride colorimetric method. Besides, phytochemical sreening was done to screen the bioactive compounds which can be further studied for development of medicinal remedies. The toxic activity of different parts of *E. conferta* was tested by using Brine Shrimp Lethality Assay (BSLA). The mortality percentage of a simple zoological organism-brine shrimp (*Artemia salina*) was tested to identify the toxicity of different parts of fruits. All data were analysed using Minitab software version 18.0. The results revealed that the content of TPC and TFC were significantly different in different parts of *E. conferta.* The ethanol (50% v/v) was found to be the best extraction solvent for extraction yield, TPC, and TFC. The peel extract showed the highest yield at 34.77% whereas the lowest yield was shown in seed extract at 4.89%. Besides, the highest content of TPC and TFC were found in the peel part of *E. conferta* fruit. Moreover, the presence of bioactive compounds and level of toxic activity have found to be significantly different in different parts of *E. conferta*. Therefore, it was concluded that *E. conferta* is beneficial for human health and it should be further studied to use it in the development of drugs.

Keywords: *Eleiodoxa conferta*, total phenolic content, total flavonoid content, phytochemicals, toxicity, *Artemia salina*

Penyaringan fitokimia dan toksisiti di bahagian berbeza untuk *Eleiodoxa conferta*

ABSTRAK

Potensi kebanyakan buah-buahan asli masih belum ditemui. Baru-baru ini, penyelidik memberi perhatian untuk menemukan buah-buahan tempatan yang tersedia ada yang mungkin berpotensi untuk digunakan bagi rawatan terapeutik. Salah satu buah yang dimakan oleh komuniti luar bandar di Malaysia adalah buah *Eleiodoxa conferta* yang dikenali sebagai 'Buah Kelubi'. Projek ini dijalankan untuk menyaring fitokimia dan toksisiti di bahagian yang berbeza untuk *E. conferta* dengan menggunakan pelarut pengekstrakan yang berlainan. Jenis pelarut yang digunakan untuk mengekstrak bahagian berbeza untuk buah ini adalah etanol 100%, etanol 50% dan air. Jumlah kandungan fenolik (TPC) dan jumlah kandungan flavonoid (TFC) dikaji dengan menggunakan kaedah Folin-Ciocalteu dan kaedah kolorimetri Aluminium klorida. Selain itu, penyaringan fitokimia dilakukan untuk menyaring sebatian yang boleh dikaji selanjutnya untuk perkembangan ubat-ubatan. Aktiviti toksisiti di bahagian yang berbeza untuk *E. conferta* diuji dengan menggunakan ujian kelebihan udang air garam (BSLA). Peratusan kematian organisma zoologi-udang air garam (*Artemia salina*) telah diuji untuk mengenalpasti ketoksikan bahagian buah yang berbeza. Semua data dianalisis dengan menggunakan perisian Minitab versi 18.0. Hasil penyelidikan menunjukkan bahawa kandungan TPC dan TFC adalah berbeza secara bererti di bahagian *E. conferta* yang berbeza. Etanol (50% v/v) didapati sebagai pelarut pengekstrakan terbaik untuk hasil pengekstrakan, TPC, dan TFC. Ekstrak kulit menunjukkan hasil tertinggi pada 34.77% manakala hasil paling terendah ditunjukkan dalam ekstrak biji pada 4.89%. Selain itu, kandungan TPC dan TFC tertinggi didapati di bahagian kulit buah *E. conferta*. Selain itu, kehadiran sebatian bioaktif dan tahap aktiviti toksik didapati berbeza secara bererti di bahagian *E. conferta* yang berbeza. Oleh itu, adalah disimpulkan bahawa buah *E. conferta* adalah bermanfaat untuk kesihatan manusia dan ia perlu dikaji selanjutnya untuk digunakan dalam pembangunan produk ubat.

Kata kunci: *Eleiodoxa conferta*, jumlah kandungan fenolik, jumlah kandungan flavonoid,fitokimia, toksisiti, *Artemia salina*

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LIST OF ABBREVIATIONS AND SYMBOLS

G	Grams
nm	Nanometre
M	Molar
µL	Microlitre
Cm	Centimetre
mL	Millilitre
mg/mL	microgram per millilitre
mg/10g	milligram per 10 grams of sample
TPC	total phenolic content
GAE	gallic acid equivalent
TFC	total flavonoid content
QE	quercetin equivalent
et al	and others
°C	degree Celsius
%	Percentage
v/v	Volume/volume %
GLM	General Linear Model
N	Normality

CHAPTER 1

INTRODUCTION

1.1 Research Background

Mangrove is known as a tideland zone formed by an interaction between animals and plants which multiply abundantly in the coastal region throughout the low lying tropical and sub-tropical regions. This collective of plants that develops along the coastline is imperative to the ecological diversity since these plants shield the coastline from destruction as well as play a predominant part in the improvement of advanced herbal remedies. These therapeutic plants have an extremely noteworthy part in the health wellness of countryside people. This is because the larger part of medicine systems active against pathogens are in fact, derived from herbs (Cho-Ngwa et al., 2016). Reports have shown that plant-inferred volatile oils apply preferred helpful movement over disengaged significant compounds (Sheel, Nisha, & Jainendra Kumar, 2014).

Thereupon botanical plants are considered as a vital origin of potentially valuable structures for the change of remedial treatment. Therapeutic plants can be formed around the world. Notwithstanding, they are most plentiful in tropical areas. The classification of therapeutic plants deliberately is vital with a specific end goal to comprehend and examine them broadly and effectively. Medicinal plants include the underutilized fruits that are not commercially developed but rather give a remarkable source of occupation bolster for some urban communities. Other than their significance for their nutritious esteem and even as wellspring of family income, this fruit decent

variety likewise has a cultural and social value and adds to the stability of environments. The foremost reason of being underutilized is the absence of consciousness of their potential and low unstable bearing attitude for these fruit plant. In addition, some portion of these fruits are not satisfactory in the market in crisp frame because of their acidic nature and astringent taste. An intense consideration is basic for enhancing and adopting the developing and usage of these underutilized fruits as a therapeutic plant. Consequently, there is a need to focus on research endeavours in enhancement and advancement of such underutilized fruit crops.

Malaysia is known for wide variety of underutilized fruits that grow in large scale in the locale of Peninsular Malaysia, Sabah, and Sarawak. *Eleiodoxa conferta* which is locally known as “Buah Kelubi” is one of the underutilized fruits grown from the ground that has drawn much attention among researchers for explore purposes. A wide range of their skin, pulp and seed may have potential advantages to human well-being. Hence, it is crucial to include these fruits in daily dietary consumption. These fruits can possibly be utilized and prepared according to own preferences. These seasonal local fruits have potential to give defensive impacts to human health because it is rich in primary and secondary metabolites.

Phytochemicals are the bioactive compounds derived from various parts of the plants. The presence of bioactive compounds found in *E. conferta* fruit can be determined by biological and phytochemical screenings of their concentrates or concentrates from established procedure utilized in modern medicinal system (Arifah, Idiawati, & Wibowo, 2017). Effective systems for examining this preparation include the determination of crude extracts depend on ethnopharmacology and every day healer's practices. Example of bioactive constituents of these plants are steroids, terpenoids,

carotenoids, flavanoids, alkaloids, tannins, and glycosides. These compounds have different activities, for example, antimicrobial and antibacterial some have been accounted for revealing haemolytic and frothing movement. There are some ecological factors, for example, atmosphere, elevation, precipitation and different conditions that may influence the development of plants which thus influence the nature of herbal plant and produce major variations in the bioactive compounds present in the plants even when it is produced in the same country. A subjective phytochemical screening will comprehend an assortment on different types of chemical components produced by plants and measurement of those metabolites will extricate, cleanse and distinguish the bioactive compounds for valuable perspectives such as herbal medicines to human beings.

It has been viewed as that if a drug is compelling, it will have side effects. Subsequently, natural remedies as medications either have symptoms or are incapable. Nonetheless, herbal medicines are by and large thought to be sheltered and successful agents. As a result, individuals turn to natural prescription since they trust therapeutic medicines are free from harmful side effects. Insignificant to no lethality is a basic improvement of a pharmaceutical product. Hence, toxicity of herbal medicines needs to be considered to avoid adverse events. Toxicity screening is a valuable test in deciding the potential harmfulness of bioactive compounds segregated from plants. This test is crucial for the advancement of new medications and for the extension of the therapeutic potential of existing molecules. Hence, this plant's bioactive constituents are recognized and its toxicity level for the successful future standardization of the plant as restorative plant is also determined.

1.2 Problem Statement

Some undiscovered plants play an important role in therapeutic remedies. *E. conferta* fruit is one of the cultivations where its potential is less utilized. Studies had shown that *E. conferta* has an undiscovered potential. The *E. conferta* is less utilized by the local community, in term of its use where only the ripen fruit is sometimes used as a substitute for sour fruit for vegetable spices. At the same time, it is found that *E. conferta* fruits rich in secondary metabolites. A study focused on antioxidant activity in the peel, flesh and seed of the fruit but considering other chemical contents of *E. conferta* has yet to be emerged in the literature. In addition, bioactive compounds and toxic activity of *E. conferta* extracts have also not yet been reported before. By considering long term use of the local communities, it is necessary to evaluate the quality of *E. conferta* as an important source for developing new drug molecules. Toxicity screening was also considered in this research to determine the toxic activity of the *E. conferta* that has effect on the brine shrimp. Hence, this research was carried out based on combinations of phytochemical and toxicity screening of *E. conferta* extracts in 100% ethanol, 50% ethanol, and water.

1.3 Hypotheses

H_0: There are no significant difference of bioactive compounds and toxic activity in different parts (peel, flesh and seed) of *E. conferta* using different extraction solvents.

H_1: There are significant difference of bioactive compounds and toxic activity in different parts (peel, flesh, and seed) of *E. conferta* using different extraction solvents.

1.4 Objectives

The objectives of this study were to:

1) screen bioactive compounds in different parts (peel, flesh, and seed) of *E. conferta* using different extraction solvents.
2) determine the toxic activity in different parts (peel, flesh, and seed) of *E. conferta* using different extraction solvents.

1.5 Scope of Study

This study aimed on screening the bioactive compounds and potential toxic activity contain in the different parts of *E. conferta* fruits. The chemical compounds found in the fruits may be therapeutically active. Hence, the phytochemical research approach is considered effective in discovering bioactive profile of this plant of beneficial importance. The peel, flesh and seed were extracted using three different solvents, namely, 100% ethanol, 50% ethanol and distilled water. The samples were subjected to qualitative and quantitative phytochemical screenings to test the presence of various phytochemical compounds by adopting standard procedures. Bioactive compounds such as alkaloids, saponins, phenols, flavonoids and terpenoids were observed to be present in this investigated plant. The toxic activity was determined by using the brine shrimp lethality assay (BSLA). Ethanolic extracts of *E. conferta* should show the presence of the phytochemicals compared to that of the other extracts.

1.6 Significance of Study

The importance of this research was to identify the chemical compounds and toxic activity present in the fruit. *E. conferta* has some advantageous properties that can be further used for studies to well utilise the fruit. This study had evaluated different parts of *E. conferta* to screen the bioactive compounds and identified the toxic activity by using standard procedures. The bioactive compounds could contain biological activities which can give beneficial impact to health. For instance, alkaloids can be associated with medicinal uses and one of their biological activities is toxicity. Hence, this study has helped to identify the potential of underutilized fruits to be sources of development of drug molecules. Future studies can be done to find out different utilities of *E. conferta* fruits. Thus, utilisation of this fruit can reveal its unknown potential and this increases the economic value of the fruit.

CHAPTER 2

LITERATURE REVIEW

2.1 Underutilized Fruits

Malaysia is honoured with exceedingly rich widely varied vegetation in its tropical rainforests. Malaysia has a rich decent variety tropical fruits which incorporates common, decorative, unusual, wild and highland fruits, but yet the vast majority of the indigenous fruits are viewed as underutilized. Those indigenous fruits are less prominent and once in a while developed locally for utilization and restorative purposes. Underutilised fruit species are the remarkable and palatable species, which are not economically developed. They are as often as underestimate in buyer inclination compared to presented, fascinating fruits and vegetables. In addition, the species are underexploited and understudied in research and development (R&D) (Ikram et al., 2009). The distinctive underutilized fruits accessible in Malaysia are ‘asam’, ‘bacang’, ‘bambangan’, ‘cerapu’, ‘durian’, ‘jambu’, ‘kuini’, ‘pulasan’ and ‘salak’.

Majority of the underutilised fruits possess have great flavour, charmingly sweet, and high in dietary benefits. Underutilised fruits have likewise been appeared to contain noteworthy amounts of bioactive phytochemicals, which may give desirable medicinal advantages and play a vital part in the prevention of chronic diseases, for example, malignant growth, cardiovascular disease and diabetes. Based on epidemiological studies, eating five bits of these fruits day by day can decrease the danger of these diseases (Voon & Kueh, 1999). Ethnobotanical studies have demonstrated that underutilised fruits are imperative wellsprings of sustenance and

medication for local people, particularly to the natives. These plant species likewise give extra income to the households as they are developed in home gardens and plantations and sold in niche markets or 'pasar tani' (Raziah et al., 2008). Further investigations are required to utilize their dietary conformation, cancer prevention agent ability and therapeutic properties.

Moreover, the significance and capability of these plant species ought to be deliberate to upgrade the use of the plant. A few parameters determined to recognize dietary benefits of underutilised fruits are phenolic compounds, antioxidants, vitamin C and mineral substances. It is notable to focus on the exploration and exploitation of these new origin of fruits to increase economic esteem and to ensure an ideal utilization of assorted variety of local resources. Production of these fruits can be popularized to build the salary and expectation for living standard of cultivators. The estimation of these unusual fruits species which have not been utilized can give numerous financial advantages to the cultivator's sustenance (Melisa & Mardesci, 2018).

2.2 *Eleiodoxa conferta*

E. conferta is a monotypic genus, dioecious, lowland forest freshwater swamp-dwelling plant, native to South East Asia (Thailand, Malaysia, Borneo, and Sumatra) where the fruits are highly gregarious and often forms large colonies. *E. conferta* belongs to the Arecaceae family. The common names of *E. conferta* fruits are Asam paya, Asam kelubi, Kelubi, Kelumi, Salak hutan, Asam paya, Kuwai-kuwai, and Kelumbi. These fruits are especially abundant in peat swamp forest with some water movement. *Eleiodoxa conferta* fruits grown on palm trees (Liang, 2015). It remains at the underground swamp forest where the trunks of these palms are clustering and form dense thickets.

As one of the few hapaxanthic in the family, individual trunks are determinate and die after flowering. A mature leaf reaches 3.5 m in length on 3 m petioles which are armed with whorls of 5 – 7 cm long spines. The green to deep green pinnae is regularly arranged along the rachis, 1.5 m in length, and toothed along the margins. The inflorescence emerges at ground level, bearing either male or female flowers, in the latter forming scaly, red fruit with one or occasionally two seeds. *E. conferta* fruits is shown in the Figure 2.1

At the point when this palm is developed, it requires liberal water, and rich, acidic soil and shade or filtered light for a decent development. Palm additionally to form thick groves. Leaves are expansive and pinnate, with a curving appearance when it transmits from the underground stem. Rachis and petiole are secured with spines (about 5 – 7 cm), exhibit in whorls along their entire length. Inflorescence are stretched and emerged from the ground. Fruits are pear formed and particularly flaky, abandoning green to rosy dark coloured when developed (Intan Mokhtar & Ain Abd Aziz, 2015). Singular trunks of the palms develop for a few years previously they blossom, however die after blooming.

This species is generally utilized by local people throughout its range, providing sustenance and materials. The fruit is intensely sourish, subsequently it is utilized to savour curries or is boiled to make sweetmeats. It can be used as a substitute for tamarind. A decoction of the fruit divider is utilized as a treatment against cough. The leaves are utilized as thatched-roof and are woven into mats (Voon & Kueh, 1999). The apical bud of the palm can be cooked and eaten as vegetable. In spite of the fact that collecting the bud will prompt the death of its trunk since it can't make side shoots, the plant produces new trunks from the underground stem and thus it is conceivable to

reasonably harvest the buds whenever picked with some restraints. “Kelubi fruits” are generally prepared as desserts, sour mix vegetables, mixed salad, sauce or raw materials (Intan Mokhtar & Ain Abd Aziz, 2015).

Figure 2.1: *Eleiodoxa conferta* fruit

Source: http://www.palmpedia.net/wiki/File:Ecfruit.jpg

2.3 Extraction method

Extraction is the partition of medicinally active portions of plant using selective solvents through standard procedures. Extraction usually use two immiscible phases to separate the substances from one phase to another. There are different kinds of technique have used for the extraction of chemical compounds desired from a plant source material. Some of them are solvent extraction, supercritical fluid extraction, solid phase extraction, hot continuous extraction, microwave-assisted extraction, and chromatographic techniques. The basis of all extractions is to separate the soluble plant metabolites, leaving behind the insoluble residue. The initial crude extracts using specific extraction methods contain complex mixture of many plant metabolites, such

as alkaloids, glycosides, phenolics, terpenoids and flavonoids (Wadood et al., 2013). Some of the initially obtained extracts may be ready for use as medicinal agents in the form of tinctures and fluid extracts but some need further processing.

2.3.1 Maceration

Maceration is a technique used in wine making and has been embraced and generally used in medicinal plants research. Maceration includes soaking powdered plant materials in a closed system with a solvent and permitted to stand at room temperature for a period of minimum 3 days with regular agitation until the point that the soluble matter has broken down (Sonam, Singh, & Pooja, 2017). The mixture then is strained, the marc which is known as the moist solid material is squeezed, and the consolidate fluids are cleared up by filtration or decantation subsequent to standing.

The procedure proposed to diminish and break the plant's cell wall to discharge dissolvable phytochemicals. After 3 days, the mixture is squeezed or stressed by filtration. In this conventional technique, heat is transferred through convection and conduction. The selection of solvents will determine the kind of compounds extracted from the samples. This technique is the easiest and simple method. However, organic waste come into an issue as large volume of solvents is used and appropriate management of the waste is required. Other than that, adjustment in the temperature and selection of solvents upgrades the extraction procedure (Trusheva, Trunkova, & Bankova, 2007). When these alterations are not objectionable, the volume required for extraction can be decreased and introduced in the maceration method. In this technique, solvents used in the soaking procedure play a critical role. For instance, ethanolic and hydroalcohol extracts result in most noteworthy extraction yield with

maximum presence of phytoconstituents such as alkaloids, saponins, carbohydrates, tannins and flavonoids compared to the other solvents such as petroleum ether, chloroform and water (Sheel et al., 2014).

2.3.2 Solvent Extraction

Solvent extraction, also called liquid-liquid partition (LLP) is a technique to separate the bioactive compounds based on their relative solubility in two different immiscible liquids. Immiscible liquids such as water and organic solvents are ones that cannot get mixed up together and separate into layers when shaken together. The most common use of the distribution principle in the extraction of substances by solvents, which are often employed in a laboratory or in large scale manufacturing (Vijayameena, Subhashini, Loganayagi, & Ramesh, 2013).

Selection of the extraction solvent depends on the specific nature of the bioactive compound being targeted. The extraction yield and biological activity of extracts can be strongly affected by the solvent applied. Different solvents, including organic or aqueous solutions use extraction of bioactive compound. Recent studies by Siddiqui, Rauf, Latif, & Mahmood, (2017) showed that the amount of TPC extracted from flaxseeds was affected by the solvent polarity, for example, solvents with low polarity, such as ethyl acetate, are less efficient than that of the more polar solvents. Water and ethanol are often recommended for extract preparation because of their differences in polarity. The use of organic solvents for industrial extractions has several disadvantages such as solvent residue in the product, worker exposure, and disposal of waste solvents. Several studies have shown that ethanol and boiling water are effective for polyphenol extraction (Chauhan, Nagar, Bala, & Sharma, 2016).

Ethanol has been known as a good solvent for polyphenol extraction and is safe for human consumption. Methanol is also a solvent used for extraction has been generally found to be more efficient in extraction of lower molecular weight polyphenols, whereas aqueous acetone is good for extraction of higher molecular weight flavanols. For instance, the maximum TPC was obtained from barley flour by extraction using a mixture of ethanol and acetone (Lay, Karsani, Mohajer, & Abd Malek, 2014).

2.4 Rotary Evaporator

Most rotary evaporators have four noteworthy segments which are heat bath, rotor, condenser, and solvent trap. Additionally, an aspirator or vacuum siphon should be attached, as well as a bump trap and round bottom flask containing the sample to be concentrated. The essential usage of a rotary evaporator is to dry and purify samples for downstream applications. Its speed and capacity to deal with huge volume of solvent make rotary evaporation a favoured technique of solvent removal in numerous research centres, especially in cases involving low boiling point solvents. Rotary evaporation is the process of reducing the volume of a solvent by conveying it as a thin film over the inside of a vessel at elevated temperature and reduced pressure (Ikram et al., 2009). Roto-evaporation requires mechanical rotation of a flask under vacuum. The rotation of the flask increases the surface area of the solvent to be removed, increasing the rate of evaporation, and reducing the risk of "bumping". Bumping occurs when a large pocket of solvent vapor forms rapidly and displaces the surrounding liquid. The vacuum decreases the boiling point of the solvent, as well as providing a means to separate the solvent from the compound of interest. This promotes the rapid removal of excess solvent from less volatile samples.

The inertia and frictional forces between the liquid sample and the vapour cause the liquid sample spreads over the surface of the vial and forms a liquid film. The rotation of the sample produced by the force generated by the liquid film, effectively suppresses the sample boiling. The characteristics and convenience of the modern rotary evaporator enable it to use for distillation quickly and gently. Figure 2.2 shows the rotary evaporator used to evaporate the solvents and collect the crude extracts. The biggest drawback of rotary evaporator applications is the boiling of certain samples, such as ethanol and water evaporator, which will cause the researcher to collect the loss of the sample (Noreen, Semmar, Farman, & McCullagh, 2017). During the operation, it is usually possible to prevent boiling by carefully adjusting the working strength of the vacuum pump or the temperature of the heating pan during the mixing phase of the distillation process. Alternatively, the anti-boiling particles may be added to the sample. For samples that are particularly difficult to distil, including samples that are prone to foaming, special condensing tubes can be configured for the rotary evaporator.

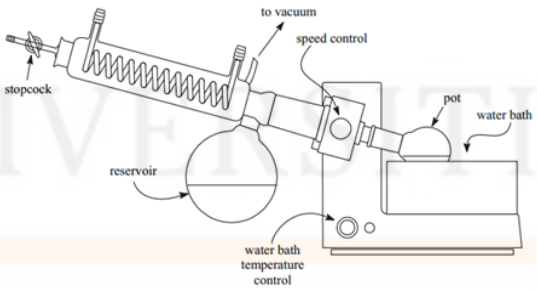

Figure 2.2: Rotary evaporator

Source: http://www.umich.edu/~chemh215/W13HTML/SSG2/ssg2.1/rotovap.png

2.5 Total Phenolic Content (TPC)

Phenolic compounds are secondary metabolites, which are produced in the shikimic acid of plants and pentose phosphate through phenylpropanoid metabolization. They contain benzene rings, with one or more hydroxyl substituents, and range from simple phenolic molecules to highly polymerized compounds (Abdulkadir, Zawawi, & Jahan, 2016). Phenolic compounds include simple phenols and phenolic acids, hydroxycinnamic acid derivatives and flavonoids are bioactive compounds that occur commonly in plants. Many of them are good sources of natural antioxidants. The enzymatic reaction of phenolic compounds will cause the formation of undesirable colour, flavour and loss of nutrient in fruits (Ozcan, Akpinar Bayizit, Yilmaz Ersan, & Delikanli, 2014).

Phenolic metabolites play an important part in other processes, for instance incorporating attractive substances to accelerate pollination, colouring for camouflage and defence against herbivores, as well as antibacterial and antifungal activities. Phenolic compounds, including stress-linked phytochemicals, have been related to favourable impacts, which are caused by the consumption of fruits and vegetables, particularly due to their antioxidant activities. Balasundram et al, (2006) reviewed the antioxidant activity, occurrence and latent uses of phenolic compounds in plants and agri-industrial by-products. Under this report, fruits, vegetables and beverages are the principle sources of phenolic compounds in the human diet. Plant polyphenols as dietary antioxidants in human health and disease might offer some protection against oxidative damage. As natural antioxidants, phenolic compounds are found abundantly in plant food and beverages, which play vital parts in pabulum and healthcare. Some researchers have indicated that phenolic compounds are the most affluent in ordinary human diets among the dietary antioxidants (Rajesh, Vasantha, Rajesh, & Panneerselvam, 2014).

Many studies have reported the advantages of phenolic compounds, such as anti-aging, anti-inflammatory, antioxidant and antiproliferative agents (Wadood et al., 2013). In addition to the adjustment of the above, there are relevant antioxidant enzymes to counter oxidants. Polyphenols, especially flavonoids, phenolic acids and tannins, have the important property of inhibiting α-glucosidase and α-amylase, which are key enzymes and responsible for the digestion of dietary carbohydrates to glucose. Dietary plant polyphenols and polyphenol-rich products modulate carbohydrate and lipid metabolism, attenuate hyperglycaemia, dyslipidaemia and insulin resistance, improve β-cell function, stimulate insulin secretion, improve adipose tissue metabolism and alleviate oxidative stress, stress-sensitive signalling pathways and inflammatory processes. Polyphenolic compounds can also prevent the development of long-term diabetes complications, including cardiovascular disease, neuropathy, nephropathy and retinopathy (Chauhan et al., 2016). Phenol structure is displayed in Figure 2.3.

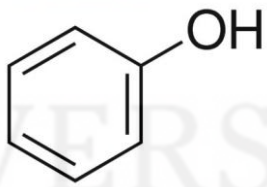

Figure 2.3: Phenol structure

Source: https://study.com/cimages/multimages/16/phenol.png

2.6 Total Flavonoids Content (TFC)

Flavonoids comprise of a large group of polyphenolic compounds. These compounds can be synthesized by phenylpropanoid pathway. The secondary metabolites of phenolic nature including flavonoids are in charge for the diversification of medicinal action (Bacanli, Aydin, Basaran & Basaran, 2017). The chemical nature of flavonoids depends on their structural class, hydroxylation degree, other substitutions and conjugations, and polymerization degree. Flavonoids have capacity to enhance human defensive systems. There are some impacts of flavonoids against many contagious and infectious diseases such as heart diseases, malignancies, and other age-related diseases. In addition, flavonoids act as a secondary antioxidant defence system in plant tissues exposed to different abiotic and biotic tensions (Lay, Karsani, Mohajer, & Abd Malek, 2014).

Flavonoids are potent antioxidants and have aroused considerable interest recently because of their potential beneficial effects on human health in fighting diseases. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. For instance, quercetin is the most abundant dietary flavanol, is a potent antioxidant because it has the structural features for free radical scavenging activity (Linn, 2013). Flavonoids occur as aglycones, glycosides, and methylated derivatives. Tests such as ferric chloride test, shinoda test, zinc hydrochloric acid reduction test are used to identify the presence of flavonoids in a plant. Figure 2.4 shows the structure of flavonoid compound.

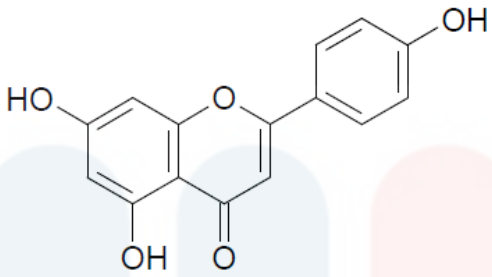

Figure 2.4: Flavonoids structure

Source: http://www.tuscany-diet.net/wp-content/uploads/2014/01/Flavonoid-skeleton1.gif

2.7 Phytochemical

Phytochemical refers to an assortment of plant-derived compounds with remedial activities such as anticarcinogenic, antimutagenic, anti-inflammatory, and antioxidant properties (Yadav, Chatterji, Gupta, & Watal, 2014). "Phyto" originates from the Greek word "phuton" signifying "plants", consequently phytochemicals are the substance supplements found in plants. It refers to non-nutritive compounds exhibit in a plant-based that provides defensive or disease-preventing effects. Phytochemicals give colour to plants and a variety of flavours both charming and upsetting when consumed. Phytochemicals in plants assume essential parts in their development and advancement. They shield plants from dangerous agents for example, bugs and microorganisms and also unpleasant situation, for example, bright (UV) light and maximum temperatures (Bacanli, Aydin, Basaran, & Basaran, 2017).

Phytochemicals also give medical advantages when consumed. They comprise of fundamental nutrients and different chemicals for ideal well- being such as proteins, vitamins, minerals, phenolic acids, and flavonoids. Some of these phytochemicals are perceived as bioactive compounds in traditional herbal medicines (Tona, Kambu,

Ngimbi, Cimanga, & Vlietinck, 1998). Plants are able to produce a large number of diverse bioactive compounds. High concentrations of phytochemicals, which may protect against free radical damage, accumulate in fruits and vegetables. Plants containing beneficial phytochemicals may supplement the needs of the human body by acting as natural antioxidants (Rajesh et al., 2014). Various studies have shown that many plants are rich source of antioxidants. For instance, vitamins A, C, E, and phenolic compounds such as flavonoids, tannins, and lignin, found in plants, all act as antioxidants. The consumption of fruits and vegetables has been linked with several health benefits, a result of medicinal properties and high nutritional value.

Antioxidants control and reduce the oxidative damage in food by delaying or inhibiting oxidation caused by reactive oxygen species, ultimately increasing the shelf-life and quality of these food. Beta carotene, ascorbic acid, and many phenolics play dynamic roles in delaying aging, reducing inflammation, and preventing certain cancers. Increasing the consumption of fruits and vegetables has been recommended by many agencies and health care systems throughout the world (Amzad Hossain & Shah, 2015). Novel chemical compounds synthesis from the plant active constituents, which are of potential use in medicine and other useful applications. Herbal plants having many pharmacologically active compounds like flavonoids, alkaloids, tannin, steroids, glycosides, phenols, and fixed oils. These compounds are stored in their specific parts of leaves, bark, flowers, seed, fruits, root having different pharmacological activities such as dengue, anticancer, anti-inflammatory, chemoprotective, antidiabetic and wound healing activities of their different parts.

2.7.1 Alkaloid

Alkaloid is a natural nitrogen-containing bases. Alkaloids are one of the secondary metabolites of plants. Alkaloids are discovered principally in plants and are particularly regular in specific groups of flowering plants. They can be found in various parts of the plant like leaves, stem, bark, and roots. Examples of alkoloids are morphine, strychnine, quinine, ephedrine, and nicotine. The structure of alkaloid is given in Figure 2.5. The concentration of alkaloids in plants only present before seed development and afterward drops off when the seed is ready to ripe. Alkaloids may also shield some plants from destruction by certain insect species. Alkaloids have different and essential physiological impacts for people. The usage of alkaloids depends on their impacts on the body (Lay et al., 2014).

Excess dosage will be certainly toxic and unsafe to man. By taking in account their particular impacts on body, they are utilized as a part of research and logical examination. For instance, atropine is an alkaloid can cause pupil dilation. Each type of alkaloid will have an alternate kind of symptoms, hence there are no conception can be made. Alkaloids are delivered by numerous plants as barrier chemicals basically against herbivores as well as against microbial pathogens (Melisa & Mardesci, 2018). A few alkaloids can be utilized as a part of drug to treat diseases, health issue, and much tumour.

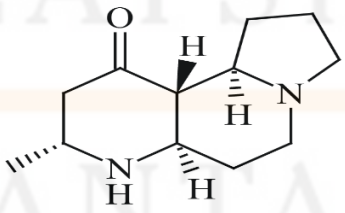

Figure 2.5: Alkaloid structure

Source: https://study.com/cimages/multimages/16/alkoloid.png

2.7.2 Organic Acid found in the fruits

Organic acids are great importance in plants. Recently, researchers have discovered that acids are contributed in growth, development and organic acids in some fruits can hinder development of some senescence. A striking component of plant tissues is that the aggregate substance of organic acids is higher than in different living beings. The arrangement of natural acids that collect differs relying on the species, age of the plant and type of the tissue. The high content of organic acids in plant tissues is most likely because of their essential characteristic as photosynthetic intermediates. Nevertheless, organic acids have a potential role as metabolically active solutes for the osmotic change and the equalization of cation overabundance. Additionally, organic acids take part as key segments in the mechanisms that a few plants use to adapt to supplement inadequacies, metal resistance and plant. (Mokhtar & Aziz, 2015) examined the organic acid in tropical fruit such as longkong *(Aglaia dookoo*) at ripe stage and demonstrated that citric acid and malic acid were available at the level of 0.22 % w/w and 0.15% w/w. Researchers have discovered that organic acids in some fruits can repress development of a few microorganisms and parasites (Rangkadilok et al., 2012). It is likewise discovered that organic acids, for example, malic, citric, lactic, and tartaric acid had antibacterial activity with explicit pH conditions.

2.7.3 Saponin

Saponins are phytochemicals which can be found in many natural products, vegetables, beans and herbs. Saponin is a glucoside with frothing attributes. The frothing capacity of saponin is caused by the blend of a hydrophobic (fat-solvent) sapogenin and a hydrophilic (water-dissolvable) sugar part. Saponin have an unpleasant taste. A few saponin are lethal and are known as sapotoxin.

Facts state that saponin are beneficial compounds that boost health and protect from specific medical problem (Munari et al., 2012). Studies have delineated the helpful impacts on blood cholesterol levels, disease, bone wellbeing and incitement of the resistant framework. Most logical examinations explore the impact of saponin from particular plant sources and the outcomes cannot be connected to different saponin. Saponin additionally causes a decrease of blood cholesterol by keeping its re-assimilation.

Studies have demonstrated that saponin have antitumor and hostile to mutagenic exercises and can bring down the danger of human tumours, by keeping growth cells from developing (Wadood et al., 2013). Plants deliver saponin to battle contaminations by parasites. At the point when ingested by people, saponin additionally appear to help insusceptible framework and to secure against infections and microscopic organisms.

2.7.4 Tannin

Tannins regularly known as tannic acid are compounds that tend to collaborate with aqueous solutions of proteins and other organic macromolecules to form insoluble precipitates. High tannin concentrations are found in about all part of many plants such as in the bark, wood, leaves, fruit, roots, plant galls, and seed. In stem tissue, tannins are frequently found in the growth zones of trees, for example, the secondary phloem and xylem and the layer between the cortex and epidermis. Frequently, a high tannin production can be related with some infection of the plant. Consequently, it is expected that the biological role in the plant of numerous types of tannins is identified to provision of protection against microbial disease, insect or animal activity (Sindhu, Vasudeva, & Sharma, 2010). Condensed tannins are combined and stored inside tannosomes, a chlorophyllous organelle encased inside tonoplasts in the vacuoles. This compound do not interfere with plant digestion and don't communicate with proteins. The cell breakdown and death can able to act and incite certain metabolic impacts as herbivore hindrances because of their acid taste and the property of precipitating proteins. There are a lot of epidemiological information which recommend that tannins are helpful in the treatment of skin irritation and wounds, and the admission of tannins may keep the beginning of perpetual illnesses**.** Tannins may apply biological impacts in two diverse ways which are not absorbales, these are typically unpredictable structures with binding properties which may create local effects in the gastrointestinal tract (antioxidant, radical scavenging, antimicrobial, antiviral, antimutagenic, and antinutrient effects), or as absorbable, these are usually low molecular weight structures which are easily absorbed, and produce systemic effects in various organs (Vaghasiya, Dave, & Chanda, 2011).

2.7.5 Terpenoid

Terpenoids establish a group of chemical compounds present in all living organisms. However, green plants and flowering plants display an abnormally high number of terpenoids, both per species and altogether, compared with other living organisms. Different distribution has evaluated that the quantity of particular terpenoid compounds. The heredity explicit terpenoids, which have emerged all through the development of green plants, have for the most part been hypothesized to assume an activity in the biological associations of plants with biotic and abiotic parts of their condition. Such activities have included barrier against herbivores and pathogens and advantages to gain life forms, for example, pollinators and mycorrhiza. Terpenoid shades, for example, bixin, lycopene and astaxanthin, are intensely utilized in the nourishment development (Zwenger & Basu, 2008) .

2.7.6 Quinone

Quinones is a type of plant derived secondary metabolites and can be mainly divided into four types, namely, benzoquinone, naphthoquinone, phenanthrenequinone, and anthraquinone, according to the number of benzene rings in the structural skeleton. Quinones are widely distributed in the plant kingdom and mainly exist in higher plants, such as those from the *Polygonaceae, Rubiaceae, Leguminosae, Rhamnaceae, Labiatae, and Boraginaceae* families and plants widely distributed in tropic and subtropics regions (Zwenger & Basu, 2008).

These compounds are involved in defence mechanisms by plants against predators. Among the most important biological activities reported on quinones are

cytotoxic, antioxidant, anti-inflammatory, antimalarial, antimycobacterial, and antifungal. Quinones are more likely to be lipid-soluble. Some of the classical techniques reported for extracting slightly polar quinones involve maceration, ultrasonic waves extraction and soxhlet extraction as reported (Baba & Malik, 2015). This principle is used to identify quinones, which turn red and purple in the presence of basic solutions. During these reactions, the solubility properties of slightly polar quinones changes and it is possible to dissolved these molecules in polar solvents as aqueous solutions.

2.8 Spray Reagent

The discovery of chromatographic zones on the thin layer plate usually depends on the retention or outflow of electromagnetic radiation in the ultraviolet range. To develop the TLC plate, appropriate spraying or dipping reagent is required whereby there are some compounds that are visibly coloured or exhibit fluorescence when it is viewed under UV or visible light. For an example, reagents such as sulphuric acid, and hydrochloric acid are used to detect the separation of compounds and such reagents are included with nitric acid or iodine vapour as the universal reagent that can be used to identify a wide range of chemical compounds. Meanwhile, some reagents are made specifically to detect groups of compounds such as aldehydes, ketones, alcohols, esters or acids (Ayoola et al., 2008).

2.9 Brine Shrimp Lethality Assay (BSLA)

Toxicity screening is a useful process to determine the toxic activity of a test sample, which includes plant extract and bio-active compounds isolated from plants. It can indicate the antitumor and anti-carcinogenic potentials, which have always been a gateway for a development of novel anticancer drugs. Negligible to no toxicity is fundamental for the good development of a pharmaceutical or cosmetic preparation. This is because toxicity is almost by default an unwanted characteristic in drug discovery and development since toxic compounds could have serious adverse effects (Nasri & Shirzad, 2013). Fortunately, toxicity can be studied by several *in vitro* methods based on measuring cell viability or cell death (Pisutthanan, Plianbangchang, Pisutthanan, Ruanruay, & Muanrita, 2004).

BSLA is one of the simple and effective screening assay of chemical compounds to determine the toxic activity of test sample. It depends on the ability to kill of test compounds on a simple zoological organism known as brine shrimp (*Artemia Salina*). One of the crucial parts of this assay is the type of solvent used in this test may provide false positive significant due to its toxicity (Roslen, Alewi, Ahamada, & Rasad, 2014). Probit analysis can be used to calculate the mortality percentage LC50 (lethal concentration for 50% of the population) and a graph of sample extract's concentration against percentage of mortality should be plotted. Moreover, organic chemicals are generally used as carriers in this screening assay. The BSLA represents as an effective and low cost bioassay that used to examine the plant extract bioactivity which correlates reasonably well with toxicity and anti-tumour properties (McGaw, Elgorashi, & Eloff, 2014).

CHAPTER 3

METHODOLOGY

3.1 Apparatus and Equipment

The apparatus and equipment used in this study were oven, beakers (100 mL, 250 mL, 500 mL), conical flask (1000 mL), measuring cylinder (10 mL, 50 mL), round bottom flask 29/32 (500 mL and 1000 mL), microcentrifuge tube (2 mL), glass rod, dropper, filter funnel, rubber gloves, hot plate, weighing balance, pipette (1000 mL), micropipette tubes, test tubes, and boiling tubes.

3.2 Chemicals and Reagents

The chemicals and reagent used in this study were ethanol, folin-ciocalteu reagent, sodium bicarbonate, gallic acid, dimethyl-sulphoxide (DMSO), quarcetin, bismuth nitrate, glacial acetic acid, sulphuric acid, hydrochloric acid, ferric chloride, potassium iodide, distilled water, potassium iodide, sodium nitrate, aluminium chloride, sodium hydroxide, and zoological organism-brine shrimp (*Artemia salina*). These chemicals and reagents can be obtained from Faculty of Agro Based Industry (FIAT), Universiti Malaysia Kelantan (UMK), Jeli Campus.

3.3 Methods

3.3.1 Sample Preparation

The selected *E. conferta* fruits were de-branched and cleaned with tap water. The fruits were then separated into peel, flesh, and seed using a knife. The different parts of fruit were cut into small pieces and dried separately in an oven at a temperature of not more than 50°C. It was then grinded into a fine powder. The dried and finely powdered samples were stored in an airtight container.

3.3.2 Extraction

The powdered different parts of *E. conferta* 200 g were extracted by three different solvents which were 100% ethanol, 50% ethanol, and water. The process takes place by placing the fine powder with the solvent in a closed system at room temperature (25°C) for three days with frequent agitation until the soluble matter had dissolved. The macerates were then filtered twice and the marc (the damp solid material) was pressed. Later, the filtrate was subjected into rotary evaporator to obtain the crude extracts. Lower temperature was used to evaporate the solvent where ethanol solvent had been evaporated at a temperature of 45°C and water at 100°C. The vacuum was adjusted as needed. The crude extract that was collected in the round bottom flask was then transferred into a beaker. The crude extracts were then kept for further bioactivity assay.

3.3.3 Determination of Total Phenolic Content (TPC)

The TPC was assayed based on Folin-Ciocalteu method as described by Noreen, Semmar, Farman, & McCullagh (2017) with slight modification. A total of 200 µL of extract solution (1 mg/mL) and 500 µL of Folin-Ciocalteu reagent were added and the contents were mixed thoroughly. After 3 minutes, 2 mL of 20% Na_2CO_3 was added, and then the mixture was allowed to stand for 2 hours at room temperature in dark. The absorbance was measured at 765 nm using a (Thermo Fisher Scientific, model 4001/4) spectrophotometer. The concentration of the TPC was calculated as mg of gallic acid equivalent by using a linear equation obtained from gallic acid calibration curve. The determination of total phenolic compounds in the fractions was carried out in triplicate and the results were averaged. The gallic acid calibration standard was prepared as described by Noreen, Semmar, Farman, & McCullagh (2017) with slight modification. A total of 0.01 g of gallic acid was dissolved in 100 mL of 100% ethanol (100 mg/mL). Gallic acid standard of 5.0, 10.0, 25.0, 50.0, 75.0, and 100.0 mg/mL concentrations was prepared with serial dilution. The gallic acid solution was then analysed with Folin-Ciocalteu method. A calibration curve was plotted for calculating the TPC of the samples.

3.3.4 Determination of Total Flavonoid Content (TFC)

Aluminium chloride colorimetric method was used to determine the flavonoids into the defined extracts of peel, pulp, and seed of *E. conferta* fruit. The first step was about 1.5 mL methanol, and 0.1 mL aluminium chloride (10%) were added into a test tube containing 0.5 mL of extracts (1 mg/mL), followed by 0.1 mL of potassium acetate solution (1M) and 2.8 mL of distilled water and then mixed well. After incubation at room temperature (25°C) for 30 minutes, the absorbance was measured at 415 nm using a Spectro (ThermoFisher Scientific, model 4001/4) spectrophotometer.

Quercetin (6.25 to 100 µg/mL) was used to make the standard calibration curve. The calculation of total flavonoids in the extracts was carried out in triplicate and the results were averaged. A standard calibration plot was constructed to determine the concentration of flavonoids in the extract. The standard test was carried out in triplicate and the concentration of flavonoid in the extracts were calculated from the calibration plot and it was expressed in mg quercetin Equivalent/g of extract (Firdouse & Alam, 2011).

3.2.5 Phytochemical Screening

3.2.5.1 Test for Acidic compounds

About one pinch of sodium bicarbonate powder was added to 300 µL of each extract in separate test tubes. The formation of effervescence indicates the presence of acidic compounds.

3.2.5.2 Test for Alkaloids

Dragendoff test: A solution of 0.85 g of bismuth nitrate was dissolved in 10 mL of glacial acetic acid and 40 mL of distilled water was prepared. Meanwhile, another solution of potassium iodide was dissolved in 30 mL of distilled water. Stock solution was prepared by mixing both the solution with the ratio 1:1. About 1g of each extract was placed in separate test tubes and a few drops of dragendoff reagent was added to the test tubes. Brick red precipitate is then formed and it indicates the presence of alkaloids (Abdulkadir et al., 2016).

3.2.5.3 Test for Betaocyanin

Sodium Hydroxide test: The first step in this test was to weigh about 0.2 mg of plant extract in separate test tubes. Next, about 1 mL of 2N sodium hydroxide solution was added into each test tubes respectively and heat it for about 5 minutes at 100 ± 2°C. The formation of yellow colour indicates the presence of betacyanin (Amalia & Afnani, 2013).

3.2.5.4 Test for Flavonoid

Lead acetate test: A few drops of 10% lead acetate solution was added to 300 µL of each extract in different test tubes. Formation of white precipitate indicated the presence of phenolic compounds (Rajesh et al., 2014).

3.2.5.5 Test for Saponin

Foam test: About 1 mL solution of extract was diluted with distilled water to 20 mL and the solution was shaken in a graduated cylinder for 15 minutes. The formation of stable foam indicates the presence of saponin. Moreover, a persistent froth that last for about 15 minutes would indicate the presence of saponin (Vijayameena, Subhashini, Loganayagi, & Ramesh, 2013).

3.2.5.6 Test for Terpenoids

Salkowski test: A total of 300 µL extract was mixed with 1 mL of chloroform and few drops of concentrated sulphuric acid is carefully added to form a layer. A reddish-brown colouration of the interface was formed to show positive result of the presence of terpenoids (Chauhan et al., 2016).

3.2.5.7 Test for Phenol and Tannin

Ferric Chloride Test: About two to three drops of 10% of ferric chloride solution was added to 300 µL of extract in a test tube gives blue colour indicate the presence of phenol and tannin (Obouayeba, Diarrassouba, Soumahin, & Kouakou, 2015).

3.2.5.8 Test for Quinone

About 1 mL of each extract was taken in separate test tubes. Then, about 1 mL of concentrated sulphuric acid was added to each test tubes. The formation of red colour indicates the presence of quinones (Sonam et al., 2017).

3.2.6 Toxicity Screening

BSLA was used to test the toxicity of different parts of *E. conferta*. About 34.6 g of red sea salt was dissolved in 1000 mL of distilled water and then filtered with filter paper. A hatching chamber was filled with 300 mL of brine. An air pump was started and then poured a brine shrimp cyst. The cyst was allowed to hatch for 48 hours into larval (nauplii). First of all, three sample of vials containing brine shrimp *nauplii* with 500 µg/mL and prepare a concentration of 500 µg/mL and 1000 µg/mL extracts of

E. conferta in sample vials A, B, and C respectively. After 24 hours, a number of dead and alive nauplii in tube were counted. The vials are observed by using a magnifying glass to count the nauplii and the number of survivors in each microcentrifuge tube was recorded. A control sample which contains only *nauplii* is also prepared in order to ensure the mortality effect of plant extracts. From this data, the percentage of mortality of the *nauplii* in different concentration were calculated (Chowdhury et al., 2017). A graph between the concentration of plant extracts versus the percentage of mortality was plotted. The percentage of mortality was calculated by using the equation 3.1.

$$\text{Percentage of mortality (\%)} = \frac{\text{Number of dead nauplii}}{\text{Total nauplii}} \times 100\% \qquad (3.1)$$

3.2.7 Statistical Analysis

The results were compared using two-way ANOVA. Minitab version 18.0 was used to conduct the statistical analysis of data. The data were expressed as mean ± standard deviation of triplicate measurements. Significant differences at $p < 0.05$ among means from triplicates samples were determined by Tukey's multiple comparison test.

CHAPTER 4

RESULTS

4.1 Extraction Yields

The extracts from the dried peel, flesh, and seed of the *E. conferta* fruits were macerated using different solvents including ethanol, 50% ethanol, and water for three consecutive days. The extracted aqueous extracts were evaporated using the rotary evaporator. After obtaining the extracts in the crude form, the percentage yield of the extracts was calculated and tabulated in the Table 4.1. Results showed that maximum percentage yield was obtained in 50% ethanolic extract of each sample while water extract resulted minimum percentage yield.

Table 4.1: Percentage yield of the extracts made from different parts of *E. conferta* fruit using different extraction solvents.

Samples	Extraction yield (%)
Peel ethanolic extract	18.3033 ± 0.01^{E}
Peel 50% ethanolic extract	34.7667 ± 0.06^{B}
Peel water extract	9.57000 ± 0.01^{H}
Flesh ethanolic extract	23.4667 ± 0.06^{D}
Flesh 50% ethanolic extract	39.2467 ± 0.01^{A}
Flesh water extract	16.3670 ± 0.21^{F}
Seed ethanolic extract	11.4867 ± 0.01^{G}
Seed 50% ethanolic extract	26.9667 ± 0.06^{C}
Seed water extract	4.8900 ± 0.01^{I}

Data represented by mean ± standard deviation (n=3). Means that do not share a letter are significantly different at $p < 0.05$ using Tukey's test.

4.2 Total Phenolic Content (TPC)

Total phenolic content (TPC) was measured using the Folin-Ciocalteu method. Table 4.2 shows the TPC values of the extracts. The calibration curve of gallic acid standard showed a linearity in range of 5.00 to 100 mg/L concentration according to the Figure 4.1 below. The absorbance readings were measured at 765 nm. TPC values were obtained from the calibration curve $y = 0.008x + 0.2631$ with $R^2 = 0.9986$, where x is the absorbance and y is the concentration of gallic acid solution (µg/mL) expressed as mg GAE/10g.

The TPC values of the extracts decrease in the order of 50% ethanolic peel > 50% ethanolic flesh > ethanolic peel > 50% ethanolic seed > 50% ethanolic flesh > ethanolic flesh > ethanolic seed > peel water extract > flesh water extract > seed water extract. The TPC of the 50% ethanol extract was significantly higher than that of the 100% ethanol extract whereas the TPC of the water extract was significantly less than that of other solvents. It was also found that the TPC value of the peel extracts was higher than that of the flesh and seed extracts. Multiple comparison test between different parts of sample and the extraction solvents had done by using Tukey's test as shown in Appendix C. The results had shown significant difference at $p < 0.05$ between all the sample.

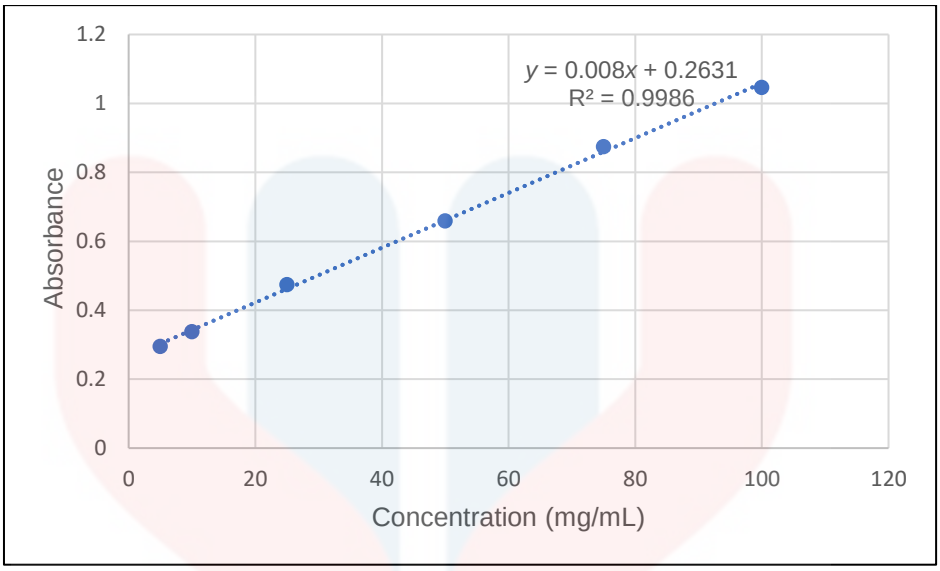

Figure 4.2: Standard Gallic Acid Calibration Curve.

Table 4.2.1: TPC in different parts of *E. conferta* fruits by using different extraction solvents.

	Total Phenolic Content (GAE mg/g)		
	Ethanol	**50% ethanol**	**Water**
Peel	69.787 ± 0.006^{A}	80.653 ± 0.0751^{D}	46.7567 ± 0.029^{G}
Flesh	56.570 ± 0.069^{B}	78.780 ± 0.069^{E}	34.4467 ± 0.075^{H}
Seed	47.820 ± 0.069^{C}	68.320 ± 0.069^{F}	17.99 ± 0.029^{I}

Results are represented by mean ± standard deviation, means are significants different at $p < 0.05$ (Tukey's test). Means that do not share a letter are significantly different at $p < 0.05$ using Tukey's test. Data expressed as mg of gallic acid equivalent (GAE) per 10 mg of the sample.

4.3 Total Flavonoid Content (TFC)

TFC values of the extracts (Table 4.3) were measured using quercetin standard method. The absorbance readings were measured at 415 nm. The calibration curve of quarcetin had shown a linearity in the range of 6.25 to 100.00 mg/mL as seen in the Table C.1 (Appendix C). TFC values were obtained from the calibration curve $y = 0.0019x + 0.5081$ with the coefficient $R^2 = 0.9993$, where x is the absorbance and y is the concentration of quarcetin acid (µg/mL) expressed as mg QE/10g. This equation is shown in the Figure 4.2 below.

The TFC values decreased in the order of 50% ethanolic peel > 50% ethanolic flesh > ethanolic peel > ethanolic flesh > 50% ethanolic seed > peel water extract > ethanolic seed > water extract of flesh > water extract of seed. Almost a similar trend was observed as seen in the TPC values. It was observed that the effect of solvents on TFC is similar to that on TPC. The highest TFC was obtained in the 50% ethanol extract, followed by the 100% ethanol and water extract. As the concentration of water in ethanol increases, the TFC in the extract decreases. Besides, the highest TFC value was found in the peel sample while the lowest TFC value was found in the seed sample.

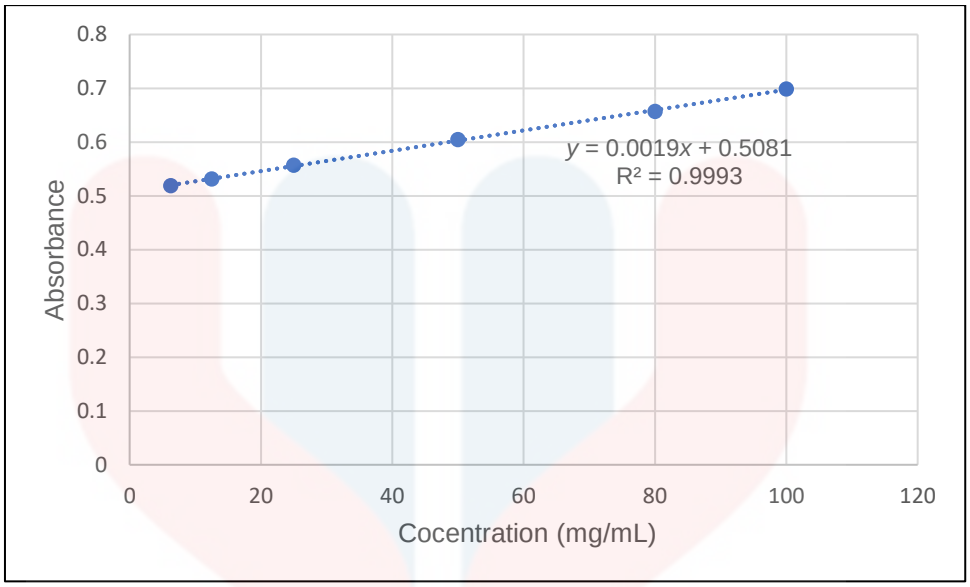

Figure 4.3: Standard Quarcetin Calibration Curve.

Table 4.3.1: TFC different parts of *E. conferta* fruits by using different extraction solvents.

	Total Flavonoid Content (QE mg/g)		
	Ethanol	**50% ethanol**	**Water**
Peel	69.140 ± 0.520^{A}	91.00 ± 0.300^{C}	47.667 ± 1.518^{E}
Flesh	69.067 ± 0.306^{B}	88.017 ± 0.306^{C}	30.123 ± 0.300^{G}
Seed	36.857 ± 0.029^{D}	62.227 ± 0.306^{F}	7.950 ± 0.277^{H}

Results are represented by mean ± standard deviation, means are significant difference at $p < 0.05$ (Tukey's test). Means that do not share a letter are significantly different at $p < 0.05$ using Tukey's test. Data expressed as mg of quercetin equivalent (QE) per 10 mg of the sample.

4.4 Phytochemical Screening

The study revealed that different parts of *E. conferta* fruit extracted using various solvent shows the presence of phytochemical compounds such as acidic compounds, alkaloids, flavonoids, phenols, tannins, terpenoids, quinones but saponins were not present in the crude extract as shown in Table 4.4. Some of the phytochemical compounds were not present in the water extracts of the samples.

Table 4.4: Phytochemical screenings of different parts of *E. conferta* fruits using various solvent.

	Types of samples								
	PE	P50E	PW	FE	F50E	FW	SE	S50E	SW
Acidic compounds	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	–	+	+	–	+	–	–
Betacyanin	+	+	+	+	+	+	+	–	–
Flavonoids	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	–	+	+	–	+	+	–
Saponin	–	–	–	–	–	–	–	–	–
Quinones	+	+	–	+	+	–	+	+	–

Ethanolic peel extract (PE), 50% ethanolic peel extract (P50E), peel water extract (PW), ethanolic flesh extract (FE), 50% ethanolic flesh extract (F50E), flesh water extract (FW), ethanolic seed extract (SE), 50% ethanolic seed extract (S50E), flesh seed extract (SW).

(+) indicates the presence of phytochemical compounds
(–) indicates the absence of phytochemical compounds

4.5 Toxicity Screening

BSLA is a simple bioassay test to study the toxicity of a chemical compound in *Artemia salina* Leach. Toxicity of different parts of fruits against *A. salina* (brine shrimp) was tested at concentration of 500 ppm and 1000 ppm. The mortality rate was calculated as percentage of ratio between number of death brine shrimp after 24 hours of incubation and the total number of total brine shrimp transferred. The result was analysed by plotting average percentage mortality against different samples of different concentration (ppm).

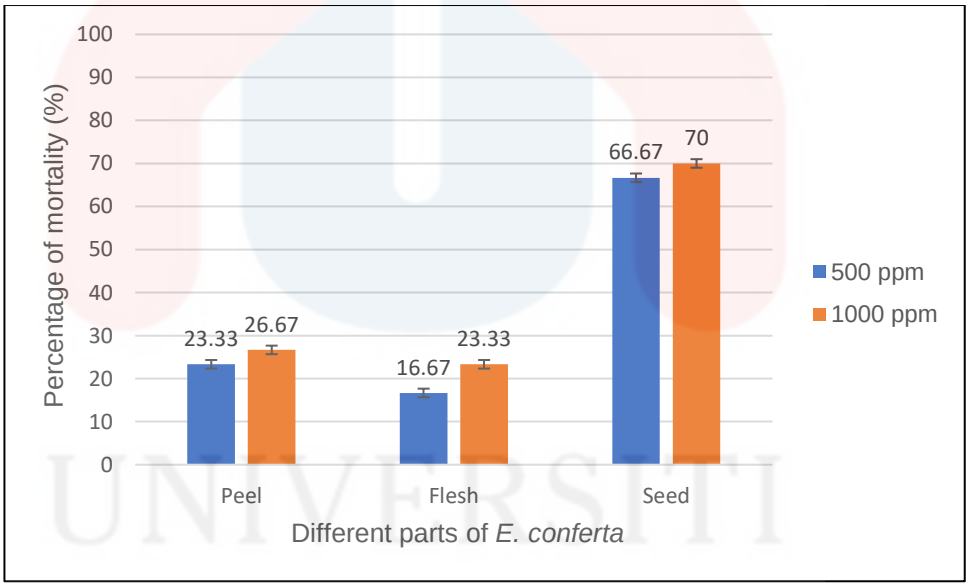

Figure 4.5: The percentage of brine shrimp lethality after treated with ethanolic extracts of different parts of *E. conferta* using different concentration (ppm) for 24 hours.

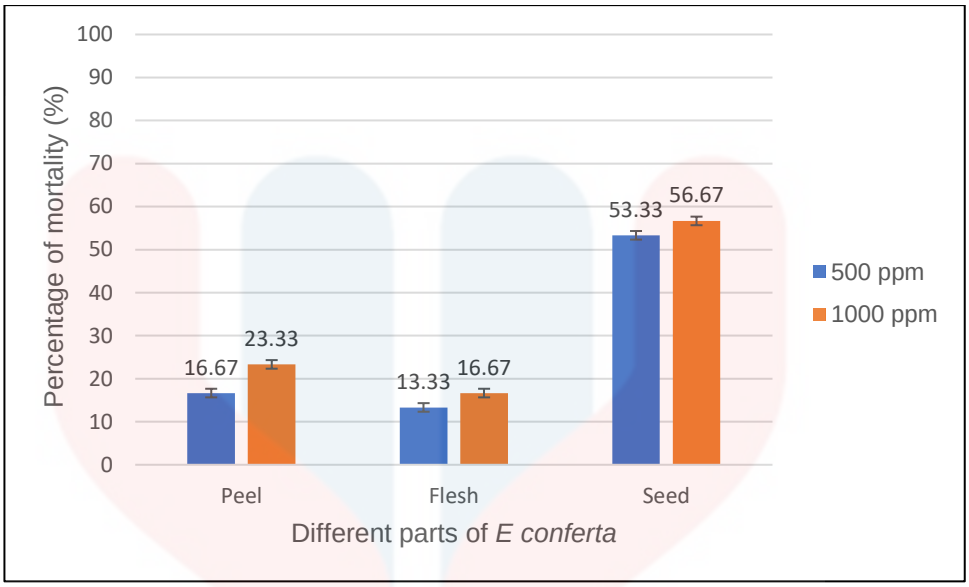

Figure 4.6: The percentage of brine shrimp lethality after treated with 50% ethanolic extracts of different parts of *E. conferta* using different concentration (ppm) for 24 hours.

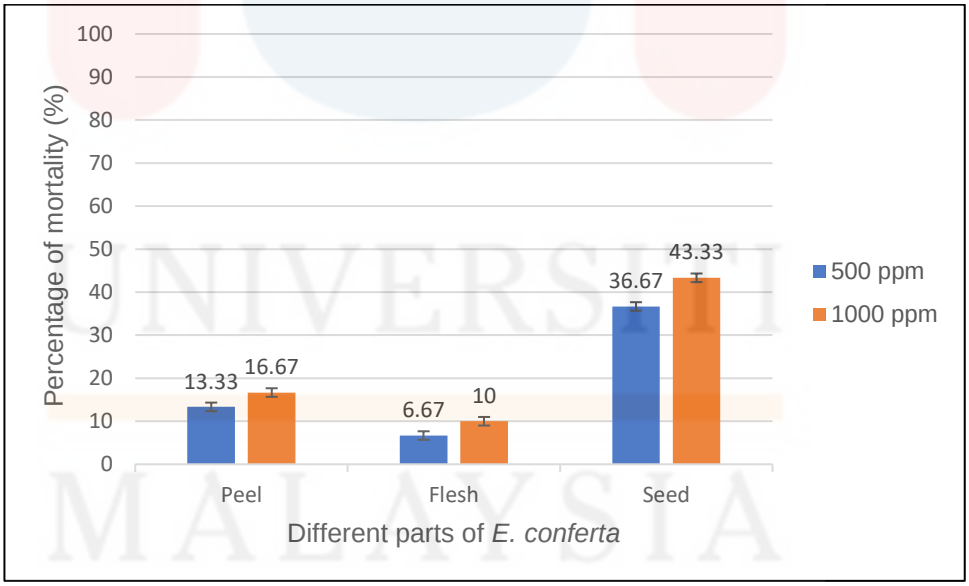

Figure 4.7: The percentage of brine shrimp mortality after treated with water extracts of different parts of *E. conferta* using different concentration (ppm) for 24 hours.

CHAPTER 5

DISCUSSION

5.1 Extraction Yields

There are various methods to examine phytochemicals from plant such as milling, grinding, homogenization, and extraction. Among these methods, extraction is the mostly used method for recovering and isolating phytochemicals from plant materials. Extraction efficiency is affected by the chemical nature of phytochemicals, the extraction method used, sample particle size, the solvent used, as well as the presence of interfering substances. The yield of extraction depends on the solvent with varying polarity, pH, temperature, extraction time, and composition of the sample. Under the same extraction time and temperature, solvent and composition of sample are known as the most important parameters.

In this study, different parts of *E. conferta* extracts were obtained by using different concentrations of ethanol (50% and 100%) and water. The highest extraction yield was found in the flesh sample ranged from 16.37% for 100% ethanol extract to 39.25% for 50% ethanol extract whereas the lowest extraction yield was found in the seed sample ranged from 4.89% for water extract to 26.97% for 50% ethanolic extracts. The 50% ethanolic solvent gives the highest yield while water gives the lowest extraction yield.

According to Sharma & Janmeda (2017), there are influence of different parts of fruit residues on extraction yield and observed that the type of residue was more influential than the solvent system on extraction yield. However, extraction yield not only depend on the sample but also the solvent used for the extraction. Polar solvents are mostly used for recovering polyphenols from plant residues. The polarity of the solvent for the different parts of the fruit affects the efficiency of the extraction and the activity of the obtained extracts (Ngo, Scarlett, Bowyer, Ngo, & Vuong, 2017). The most suitable solvents are aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate. Nevertheless, samples extracted by 50% ethanol has the highest extraction yield. This is because increasing the water concentration in the organic solvent enhances extraction yield (Vaghasiya et al., 2011). Compounds other than phenolics may have been extracted and contribute to the higher yield. Addition of water, which has the polarity index of 9.0 enhanced the solubility of both the methoxylated and hydroxylated compounds and hence improved the overall extraction yield. Therefore, the combined use of water and organic solvent may facilitate the extraction of chemicals that are soluble in water or organic solvent.

An extraction solvent is that which is able to obtain extracts with high yield and with minimal changes to the functional properties of the extracts required. Several studies have reported variations in the biological activities of extracts prepared using different extraction techniques. Therefore, it is necessary to select the suitable extraction method as well as solvent based on sample matrix properties, chemical properties of the analytes, matrix- analyte interaction, efficiency and desired properties. Several studies have reported variations in the biological activities of extracts prepared using different extraction techniques (Swami Handa, Singh Khanuja, Longo, & Dutt Rakesh, 2008).

5.2 Total Phenolic Content (TPC)

The TPC of the crude extracts as determined by the established method are reported as gallic acid equivalents. The highest TPC was taken in the ethanolic peel extract (80.65 mg/mL) whereas the lowest TPC value was taken in the water extract of seed (17.99 mg/mL). Among the three types of extraction solvents, 50% ethanol extracts contained the highest TPC followed by the order of ethanol > water. Besides, the peel sample have the highest TPC while seed sample showed a low content of TPC. A general comparison among samples, the TPC value decreases in the range of peel > flesh > seed with significant difference of $p < 0.05$.

The results showed that phenolic compounds are abundantly present in the different parts of *E. conferta* fruits. The *E. conferta* fruits can be categorised as moderately high amount of TPC as mentioned in the (Babbar, Oberoi, Sandhu, & Bhargav, 2014). The chemical constituents in the crude extracts of the fruit are known to be biologically active ingredients. Some chemical constituents such as phenolic compounds are considered as secondary metabolites components. Presence of these compounds in food enhances food quality as they provide various benefits to human health. Hence, it is important to evaluate and quantify effective phytochemical principles of medicinally or economically viable plant such as *E. conferta* (Ngo et al., 2017).

Besides, the recovery, and yield of phenolics in an extract are influenced by the type and polarity of extracting solvents as well as physical characteristic of the samples (Abarca-Vargas, Peña Malacara, & Petricevich, 2016). The different parts of the fruits may have varying physical characteristics due to different chemical composition. Furthermore, it is found that ethanol has been extensively used to extract bioactive

compounds from various plants and plant-based foods like fruits, vegetables and so on but considering water extract, phenolic compounds have less solubility in water. Apart from that, according to (Babbar et al., 2014) the maximum phenolic compounds were obtained from the 50% ethanol solvent due to its high extraction efficiency.

This may be attributable to the content of more non-phenol compounds such as carbohydrate and terpene in water extracts than in other extracts. It may also be caused by the possible complex formation of some phenolic compounds in the extract that are soluble in ethanol. These phenolic compounds may possess more phenol groups or have higher molecular weights than the phenolics in the water extract. Apparently, the result in this study showed that 50% aqueous solvent gave a better extraction of TPC compared to those of pure solvent. This is due to the difference in polarities of which affects the solubility of chemical constituents in the sample.

Many studies have focused on the biological activities of phenolics which are potent antioxidants and free radical scavengers (Amzad Hossain & Shah, 2015). The antioxidant activity of phenolics is mainly due to their redox properties, which allows them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. The interest in phenolic compounds derived from vegetables and their roles in nutrition are therefore increasing. Phenolic compounds are also known to play an important role in stabilizing lipids against peroxidation and inhibiting various types of oxidizing enzymes (Ozcan et al., 2014).

5.3 Total Flavonoid Content (TFC)

The TFC in different parts of the *E. conferta* fruit was determined by the aluminium chloride colorimetric method. Among different part of the samples, the highest amount of flavonoid content was found in the peel followed by flesh, and seed. Similar to phenolic content, 50% ethanolic peel extract had higher flavonoid content compared to that of the other parts of the fruits (91.00 QE/10g) whereas the water extract of seed sample had shown the lowest TFC value (7.95 QE/10g). It can be indicating that peel extract has more potential effective compounds. A significant difference at ($p < 0.05$) was shown in the Tukey's test as the different parts of the samples contain varying amount of TFC. The mixture of solvents was more effective than one solvent extraction to extract bioactive compounds.

Flavonoids are the largest group of naturally occurring phenolic compounds, which occurs in different plant parts both in free state and as glycosides. They are found to have many biological activities including antimicrobial, mitochondrial adhesion inhibition, antiulcer, antiarthritic, antiangiogenic, anticancer, protein kinase inhibition (Yao et al., 2004). Hence, there are possible pharmacological effects present in different parts of the *E. conferta* but the intensity of the reaction would be varying due to different concentration of TFC. Flavonoids are particularly beneficial which acting as antioxidants and giving protection against cardiovascular disease, certain forms of cancer and age-related degeneration of cell components. Their polyphenolic nature enables them to scavenge injurious free radicals such as super oxide and hydroxyl radicals (Yao et al., 2004). Hence, there is beneficial use of the different parts of the *E. conferta* fruit.

It is found that flavonoids are widely distributed group of plant phenolic compounds which is occurring virtually in all parts of the fruit. Flavonoids possess many

biochemical properties that contribute to the human and animal diet (Baba & Malik, 2015). It cannot be synthesized by the humans and animals. Thus, flavonoids found in animals are of plant origin rather than being biosynthesized in situ. These bioactive compounds in food are generally responsible for colour, taste, prevention of fat oxidation, and protection of vitamins and enzymes. The estimation of the dietary intake of flavonoids is difficult due to wide varieties of available flavonoids, the extensive distribution in various plants, and also the diverse consumption in humans (Linn, 2013).

5.4 Phytochemical Screening

Qualitative phytochemical screenings had been carried out to detect the presence of different phytochemicals on the different parts of *E. conferta* fruit extract formed by using different solvents such as 100% ethanol, 50% ethanol and water. Table 4.3 shows the presence of identified compounds that was expected to be found during the study. All the extracts of the different solvents showed the positive result for the presence of flavonoids detected by the appearance of the white precipitate in all while it shows the appearance of brick red precipitate colour in the test tubes which confirms the presence of alkaloids in the plant extracts. The appearance of blue colour by ferric chloride test confirms the presence of phenol and tannin. The all the extracts were diluted with the distilled water and the test tubes were shaken for 15 minutes by hand but there were no any changes in the reaction. Previous study have reported there are presence of the saponin in the *E. conferta* fruit (Vaghasiya et al., 2011). Thus, there are some technical error that had been occurred which have destroyed or evaporated during extraction or the drying process due to the absence of the compound.

The ethanolic extracts of peel, flesh and seed had shown the presence of acidic compound, alkaloids, anthocyanin, flavonoids, phenols, tannin and terpenoid. Saponin was not found in all the extracts whereas quinone present in the ethanolic flesh and seed extract and 50% ethanolic flesh extract. The efficacy of ethanolic extracts showing the presence of more phytochemicals in is high compared to that of the water extracts of the different parts of the plants. This could be due to poor solubility of these phytochemicals in water. Thus, it concludes water is not eligible to be used as phytochemical extraction solvent from different parts of *E. conferta* fruit.

E. conferta fruits known to have health benefits against many diseases. These health benefits are mainly accounted to the presence of many active phytochemicals in various parts of this plant. They are directly responsible for different activity such as antioxidant, antimicrobial, antifungal and anticancer. All these secondary metabolites components showed antioxidant and antimicrobial properties through different mechanism (Trusheva et al., 2007). Thus, *E. conferta* fruits are the best sources for chemical ingredients, antimicrobial and antioxidant agents for cure of different diseases.

5.5 Toxicity Screening

Toxicity of different parts *E. conferta* against *A. salina* (brine shrimp) was tested at concentration of 500 ppm and 1000 ppm. Among the different parts of the *E. conferta*, the lowest and highest percentage of mortality was found in the flesh and seed samples respectively. Besides, the ethanolic extracts had the highest toxic compared to that of the 50% ethanolic and water extracts as shown in the Table E.1 of Appendix E. Although the extraction solvent used are different the toxicity level showed

low percentage of mortality for low concentration (500 ppm) and high percentage of mortality for high level of concentration (1000 ppm) except for the seed sample. As the concentration of seed sample increases, the percentage of mortality decreases for different extraction solvents. Hence, the test results showed that the different part of the samples by using different extraction solvent showed that at different concentration levels will have an impact on mortality and larval toxicity.

From the result, it can be concluded that the flesh sample has less toxicity compared to that of the peel and seed samples. The flesh sample is safe to consume and also for other uses due to less toxic. The ethanolic extract has a high toxicity level followed by 50% ethanolic extract and water. This may be due to the content of ethanol in the extract since the probability of survival of the brine shrimp is less in the organic solvent. Consequently, brine shrimp has a high probability of survival in the water extracts. The chemical constituents which might be acidic could also contribute to the toxic content in the fruit. This statement is in agreement with the previous studies conducted by Intan Mokhtar & Ain Abd Aziz (2015) which reported that the *E. conferta* fruit extracts at different maturity stages were exhibit to contain three types of organic acids which were ascorbic acid, mallic acid, and oxalic acids.

CHAPTER 6

CONCLUSION

E. conferta is established for its therapeutic properties. This study has been investigated with intention to determine phytochemicals from different parts of the plants and to examine the different levels of toxicity of each part of the samples. The results from this research showed that using mixture of solvent such as 50% ethanol as extraction solvent results in the maximum extraction of yield, TFC, and TPC. The water results in the least extraction of these various contents due to its low solubility of the compounds in water. The peel extract showed the highest yield whereas the lowest yield showed in seed extract. Besides, the highest TPC and TFC were found in the peel part of *E. conferta* fruit. Moreover, phytochemical screening revealed that the presence of bioactive compounds is significantly different in different parts of *E. conferta*. Nevertheless, the toxicity screening ensured that the toxicity level is high in the seed part of *E. conferta* while low toxicity shown in the flesh of the fruit.

As a recommendation for the future research, other extraction methods can be used instead of using the maceration method to reveal the bioactive compounds. For instance, microwave-assisted extraction, ultrasound-assisted extraction to mention few which are more efficient than the maceration method. In this study, the saponin compound does not revealed by the phytochemical screening. Hence, further research on identifying the saponin compound can aid in the studies of the fruit. Tests such as thin layer chromatography or uv spectroscopy can be conducted to identify the compound. It is also recommended to further study on the toxicity analysis on the fruit

by determining the LC50 value. This is because the scientific researches can help to produce unexplored medicines with no lethality which will be utilized for the improvement of health status of people. After this research was done, people will take initiative in the cultivation, collection and conversion of this species in use of the benefits gained from the fruits. In a nutshell, the bioactive compounds found in the fruit are known to be biologically active and therefore may provide medicinal advantages beyond basic nutrients.

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APPENDICES

APPENDIX A

Table A.1: Method of ANOVA for percentage of yield for all the extracts.

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Table A.2: Factor information for percentage of yield for all the extracts.

Factor	Levels	Values
Factor	9	PE, P50E, PW, FE, F50E, FW, SE, S50E, SW

Table A.3: Analysis of variance for percentage of yield for all the extracts.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	8	3215.54	401.942	67448.35	0.000
Error	18	0.11	0.006		
Total	26	3215.64			

Table A.4: Model summary for percentage of yield for all the extracts.

S	R^2	R^2 (adj)	R-sq(pred)
0.0771962	100.00%	100.00%	99.99%

Table A.5: Mean values for percentage of yield for all the extracts.

Factor	N	Mean	StDev	95% CI
PE	3	18.3033	0.0058	(18.2097, 18.3970)
P50E	3	34.7667	0.0577	(34.6730, 34.8603)
PW	3	9.57000	0.01000	(9.47636, 9.66364)
FE	3	23.4667	0.0577	(23.3730, 23.5603)
F50E	3	39.2467	0.0058	(39.1530, 39.3403)
FW	3	16.367	0.208	(16.273, 16.460)
SE	3	11.4867	0.0058	(11.3930, 11.5803)
S50E	3	26.9667	0.0577	(26.8730, 27.0603)
SW	3	4.89000	0.01000	(4.79636, 4.98364)

APPENDIX B

Table B.1: Absorbance of different concentration of gallic acid standard.

Concentration of gallic acid (mg/mL)	abs 1	abs 2	abs 3	Average
5	0.295	0.295	0.294	0.294667
10	0.338	0.338	0.337	0.337667
25	0.475	0.475	0.474	0.474667
50	0.659	0.658	0.659	0.658667
75	0.875	0.875	0.874	0.874667
100	1.046	1.046	1.046	1.046

Table B.2: Method of Total Phenolic Content (TPC) versus sample and solvent.

Factor coding	(-1, 0, +1)

Table B.3: Factor Information of TPC.

Factor	Type	Levels	Values
Sample	Fixed	3	F, P, S
Solvent	Fixed	3	50E, E, W

Table B.4: Analysis of Variance for TPC.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	2	2000.1	1000.05	287247.23	0.000
Solvent	2	8340.2	4170.11	1197796.26	0.000
Sample*Solvent	4	248.6	62.15	17851.35	0.000
Error	18	0.1	0.00		
Total	26	10589.0			

Table B.5: Model Summary of TPC.

S	R^2	R^2 (adj)	R^2 (pred)
0.0590041	100.00%	100.00%	100.00%

Table B.6: Tukey Pairwise Comparisons of TPC of different parts of *E. conferta* using different solvents.

Sample*Solvent	N	Mean	Grouping								
P E	3	80.6533	A								
F E	3	78.7800		B							
P 50E	3	69.7867			C						
S E	3	68.3200				D					
F 50E	3	56.5700					E				
S 50E	3	47.8200						F			
P W	3	46.7567							G		
F W	3	34.4467								H	
S W	3	17.9900									I

Means that do not share a letter are significantly different.

APPENDIX C

Table C.1: Absorbance of different concentration of quarcetin standard.

Concentration of quarcetin (mg/mL)	abs 1	abs 2	abs 3	Average
6.25	0.519	0.518	0.519	0.518667
12.5	0.531	0.531	0.532	0.531333
25	0.557	0.557	0.557	0.557
50	0.605	0.604	0.605	0.604667
80	0.657	0.657	0.656	0.656667
100	0.698	0.698	0.699	0.698333

Table C.2: Method of GLM for Total Flavonoid Content (TFC) versus sample and solvent.

Factor coding	(-1, 0, +1)

Table C.3: Factor Information of TFC.

Factor	Type	Levels	Values
Sample	Fixed	3	F, P, S
Solvent	Fixed	3	50E, E, W

Table C.4: Analysis of Variance for TFC.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	2	5669.1	2834.56	8435.37	0.000
Solvent	2	12179.9	6089.96	18123.07	0.000
Sample*Solvent	4	289.3	72.34	215.26	0.000
Error	18	6.0	0.34		
Total	26	18144.4			

Table C.5: Model Summary of TFC.

S	R^2	R^2 (adj)	R^2 (pred)
0.579684	99.97%	99.95%	99.92%

Table C.6: Tukey Pairwise Comparisons of TPC of different parts of *E. conferta* using different solvents.

Sample*Solvent	N	Mean	Grouping							
P E	3	91.0000	A							
F E	3	88.0167		B						
P 50E	3	69.1400			C					
F 50E	3	69.0667			C					
S E	3	62.2267				D				
P W	3	47.6667					E			
S 50E	3	36.8567						F		
F W	3	30.1233							G	
S W	3	7.9500								H

Means that do not share a letter are significantly different.

APPENDIX D

Table D.1: Method of One Way Anova for percentage of mortality after treated with different part of *E. conferta* extracted by different solvent at different concentration.

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Table D.2: Factor information for percentage of mortality after treated with different part of *E. conferta* extracted by different solvent at different concentration.

Factor	Levels	Values
Factor	18	PE_500, FE_500, SE_500, P50E_500, F50E_500, S50E_500, PW_500, FW_500, SW_500, PE_1000, FE_1000, SE_1000, P50E_1000, F50E_1000, S50E_1000, PW_1000, FW_1000, SW_1000

Table D.3: Analysis of Variance for percentage of mortality after treated with different part of *E. conferta* extracted by different solvent at different concentration.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	17	20326	1195.64	23.06	0.000
Error	36	1867	51.85		
Total	53	22193			

Table D.4: Model summary for percentage of mortality after treated with different part of *E. conferta* extracted by different solvent at different concentration.

S	R^2	R^2 (adj)	R^2 (pred)
7.20082	91.59%	87.62%	81.07%

Table D.5: Means of the extracts for toxicity screening.

Factor	N	Mean	StDev	95% CI
PE_500	3	23.33	5.77	(14.90, 31.76)
FE_500	3	16.67	5.77	(8.24, 25.10)
SE_500	3	70.00	10.00	(61.57, 78.43)
P50E_500	3	16.67	15.28	(8.24, 25.10)
F50E_500	3	13.33	5.77	(4.90, 21.76)
S50E_500	3	53.33	5.77	(44.90, 61.76)
PW_500	3	13.33	5.77	(4.90, 21.76)
FW_500	3	6.67	5.77	(-1.76, 15.10)
SW_500	3	43.33	5.77	(34.90, 51.76)
PE_1000	3	26.67	5.77	(18.24, 35.10)
FE_1000	3	23.33	5.77	(14.90, 31.76)
SE_1000	3	66.67	5.77	(58.24, 75.10)
P50E_1000	3	23.33	5.77	(14.90, 31.76)
F50E_1000	3	16.67	5.77	(8.24, 25.10)
S50E_1000	3	56.67	5.77	(48.24, 65.10)
PW_1000	3	16.67	5.77	(8.24, 25.10)
FW_1000	3	10.00	10.00	(1.57, 18.43)
SW_1000	3	36.67	5.77	(28.24, 45.10)

Table D.6: Tukey Pairwise Comparisons Grouping Information Using the Tukey's Method and 95% Confidence.

Factor	N	Mean	Grouping				
SE_500	3	70.00	A				
SE_1000	3	66.67	A				
S50E_1000	3	56.67	A	B			
S50E_500	3	53.33	A	B			
SW_500	3	43.33		B	C		
SW_1000	3	36.67		B	C	D	
PE_1000	3	26.67			C	D	E
P50E_1000	3	23.33			C	D	E
FE_1000	3	23.33			C	D	E
PE_500	3	23.33			C	D	E
PW_1000	3	16.67				D	E
F50E_1000	3	16.67				D	E
P50E_500	3	16.67				D	E
FE_500	3	16.67				D	E
PW_500	3	13.33					E
F50E_500	3	13.33					E
FW_1000	3	10.00					E
FW_500	3	6.67					E

Means that do not share a letter are significantly different.

APPENDIX E

Table E.1: Phytochemical screenings of different parts of *E. conferta.*

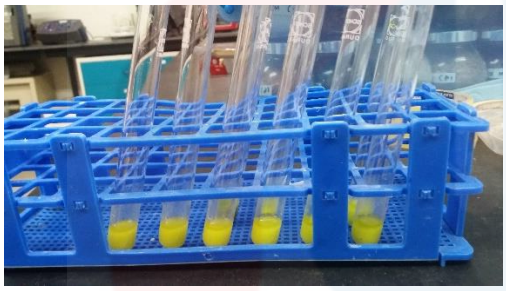
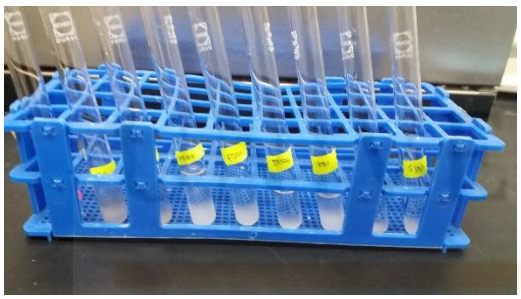
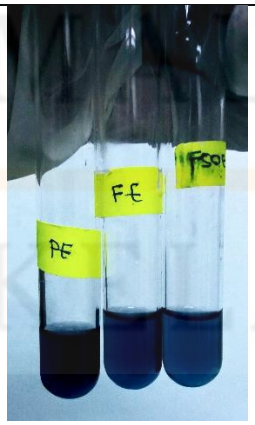
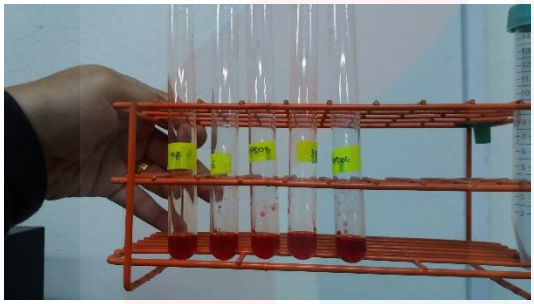
Details	Description
	The brick-red precipitate formed indicates the presence of alkaloids.
	The presence of yellow colour indicates the presence of betacyanin.
	The white precipitate formed indicates the presence of flavonoids.
	The blue colouration formed indicates the presence of phenols and tannin.

Table E.1: Phytochemical screenings of different parts of *E. conferta* (Cont.).

	The reddish-brown ring formed indicates the presence of terpenoid.
	The red colouration indicates the presence of quinone.