ANTIOXIDANT PROPERTIES OF *Carica papaya* LEAVES EXTRACTS PREPARED UNDER DIFFERENT CONDITIONS AND INFUSED WITH "KELULUT" HONEY

THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

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IJAZAH: **MASTER OF SCIENCE (INDUSTRIAL CHEMISTRY)**

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DECLARATION

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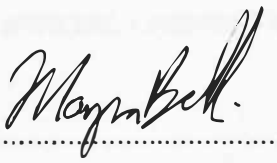

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ABSTRACT

Carica papaya is a tropical plant belonging to the *Caricaceae* family. Rich in phytochemical content, its leaves especially is renowned for its many health benefits especially for its anticancer properties as well as a supplement in treating dengue disease. This study thus aimed to optimise aqueous extraction and to determine the effect of honey infusion on the leaves extract which was analysed using TPC, TFC, FRAP and DPPH assays respectively. Based on analysis of 1 g of sample extracted with 100ml deionised water, the optimal extraction conditions for aqueous extraction were determined to be at 70^0C for 20 minutes where its TPC was 9.97±0.47 mg GAE/mL ($p<0.05$), TFC was totaled at 2.64±0.01 mg QUE/mL ($p<0.05$) while its FRAP assay was amounted to 16.84±1.10 mg TE/mL. Radical scavenging values using DPPH assay was recorded to be 87.53% with its IC_{50} at 492.54±2.45 mg TE/mL ($p<0.05$). Study on the infusion of "kelulut" honey with the leaves extract provides evidence that not only does it improve the taste of the bitter papaya extract; the positive synergy also increases the overall antioxidant activity. The aforementioned tests and assays recorded higher antioxidant activity with increasing honey dosage (max 4tbsp.), where its TPC was 21.66±0.54 mg GAE/mL ($p<0.05$), while antioxidant activity based on FRAP assay yielded 24.02±0.87 mg TE/mL ($p<0.05$). Accordingly, radical scavenging activity based on DPPH assay was 98.20% and lower IC_{50} value compared to infusion at lower dosage, amounting at 408.02±4.98 mg TE/mL. Subsequently, gallic acid and quercetin isolated from freeze dried *Carica papaya* leaves extract was higher compared to freshly prepared sample. However, conversely, gallic acid and quercetin yielded from fresh "kelulut" honey and *Carica papaya* leaves extract infused with "kelulut" honey is higher in fresh sample, suggesting that preservation effect using freeze dry method differ depending on sample type and method of preparation. Future study can therefore be done to improve and develop standardised preparation of infused papaya leaves with honey, and subsequently releasing it for commercial usage.

ABSTRAK

KEGIATAN ANTIOKSIDAN DAUN Carica papaya YANG DISEDIAKAN DALAM KEADAAN BERBEZA DAN HASIL INFUSINYA DENGAN MADU KELULUT

Carica papaya atau betik adalah tumbuhan tropika daripada keluarga Caricaceae. Kaya dengan kandungan fitokimia, daun betik terkenal kerana membawa banyak manfaat kesihatan seperti antikanser dan sebagai suplemen untuk merawat penyakit denggi. Kajian ini bertujuan untuk mengoptimumkan pengekstrakan daun betik menggunakan air sebagai pelarut dan mengkaji kesan infusi madu pada ekstrak daun yang dianalisis menggunakan ujian TPC, TFC dan esei FRAP dan DPPH. Berdasarkan analisis berat sampel 1 g yang diekstrak dengan 100 mL air nyah-ion, keadaan optimum untuk pengekstrakan daun betik ialah pada 70°C selama 20 minit di mana jumlah TPC dalam sampel adalah 9.97±0.47 mg GAE/mL ($p<0.05$), TFC berjumlah 2.64±0.01 mg QUE/mL ($p<0.05$) manakala jumlah antioksidan berdasarkan esei FRAP ialah 16.84±1.10 mg TE/mL. Selain itu, aktiviti pemerangkapan radikal bebas menggunakan ujian DPPH pula mencapai 87.53% dengan IC_{50} berjumlah 492.54±2.45 mg TE/mL ($p<0.05$). Kajian mengenai infusi madu kelulut dengan ekstrak daun betik membuktikan bahawa, selain memperbaiki rasa ekstrak betik yang pahit, sinergi positif antara kedua-dua sampel ini juga meningkatkan keseluruhan aktiviti antioksidannya. Ujian yang dinyatakan di atas mencatat nilai lebih tinggi sejajar dengan peningkatan dos madu (maksimum 4 tbsp.), di mana TPC berjumlah 21.66±0.54 mg GAE/mL ($p<0.05$) manakala ujian antioksidan FRAP berjumlah 24.02±0.87 mg TE/mL ($p<0.05$). Aktiviti pemerangkapan radikal bebas sampel infusi daun betik dan madu adalah 98.20% dengan nilai IC_{50} yang lebih rendah berbanding dengan dos infusi lain iaitu 408.02±4.98 mg TE/mL. Seterusnya, kuantiti galik asid dan kuarsetin untuk sampel esktrak daun betik yang melalui proses pengeringan beku adalah lebih tinggi berbanding sampel segar. Walaubagaimanapun, kandungan galik asid dan kuarsetin yang diperoleh daripada sampel madu kelulut segar dan sampel infusi daun betik dengan madu kelulut segar adalah lebih tinggi berbanding kedua-dua sampel sama yang melalui proses pengeringan beku. Justeru, keberkesanan kaedah pengeringan beku bergantung kepada jenis dan kaedah penyediaan sampel. Sehubungan itu, kajian masa depan harus dilakukan untuk memperbaiki dan memperkenalkan penyediaan standard infusi daun betik dengan madu, yang mana berpotensi untuk dikomersialkan.

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LIST OF ABBREVIATIONS

aq.	aqueous
cm	centimetre
CP	*Carica papaya*
FRAP	Ferric Reducing Ability of Plasma
DF	Dengue Fever
DHF	Dengue Haemorrhagic Fever
DPPH	2,2-diphenyl-1-picrylhydrazyl
kg	kilogram
m	metre
min	minute
mm	millimetre
RSA	Radical Scavenging Activity
RSD	Relative Standard Deviation
TAC	Total antioxidant activity
tbsp.	Tablespoon
TFA	Trifluoroacetic acid
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
sp.	Species

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Human has been recorded throughout the history to make use of various materials form nature to restore and improve their health (Lev and Amar, 2000). Ghorbani *et al.* (2006) also added that natural plants has been used by humans for thousands of years as a source of medicine, and it has also been an inspiration for creation of a substantial amount of modern drugs, which was possible through the isolation of natural sources used in traditional medicine. Consequently, a gradual worldwide mainstream acceptance of medicinal plants occurred during the latter part of the 20th century. The widespread acceptance is partly due to an awareness of the benefits of traditional and native remedies, and also because of an increased production of natural derivatives sources based pharmaceutical products (De Smet, 1997; Dukes, 1992; Winslow and Kroll, 1998). Furthermore, the significance of medicinal plant value is positively accrued throughout the years since there is an increasing demand for affordable health care. Successful research done also proves that natural based medicines are reputable and effectual compared to conventional drugs (Bateman *et al.*, 1998; Elvin-Lewis, 2001; Murphy, 1999).

In developing countries, the usage of traditional medicinal plants is part of an established form to maintain one's good health (UNESCO, 1996). A report by UNESCO (1998) also echoes the outcome of other studies, where it was determined that a heightened dependency in industrialised society towards utilisation of medicinal plants are influenced by effective herbal remedies normally used in rural areas, as well due to successful isolation and establishment of a number of chemotherapeutics and drugs from plants (UNESCO, 1998). Not only that, the alternative method of using herbal remedies to treat minor ailments is fast becoming popular since it is economically cheap compared to the conventional drug

treatment (Hoareau and DaSilva, 1999). One of such medicinal plants is *Carica papaya*.

Carica papaya tree are one of the many plants determined to be of very important values. Its leaves especially has been used traditionally for generation to treat all types of digestive and abdominal disorders, and has also been tested to have anticancer, anti-inflammation and antibiotic property (Aravind *et al.,*2013). Consequently, with the synergy between vitamins and bioactive compounds contained in the leaves, *Carica papaya* leaves are even used as a supplement to treat dengue disease, with positive result when subjected to clinical trials (Ahmad *et al.*, 2011).

Overall, natural based medicine such as *Carica papaya* leaves are worth it to consume since it brings extremely minimal to no side effect (Brahmachari, 2001). With prevention is better than cure mindset, consuming these plants arms mankind with robust protective nutrients, making mankind less susceptible to diseases that might show itself predominantly in the later age. It is not a stretch to say then that with these wonderful findings, it gives a better chance of fighting against diseases and also promotes natural healing with less strain on the body, which in turns provides mankind with an option of not having to develop a lifelong dependency on allopathic drugs.

1.2 Justification of study

Inevitably, it is interesting to be noted that there are currently no standardised preparation of *Carica papaya* leaves so much so that there are calls for it to be done (Ansari, 2016). Thus, through the optimisation of temperature and time of extract preparation, this paper aims to systematically explore the common household preparation of *Carica papaya* leaves extract (juicing and brewing) through their antioxidant properties.

Correspondingly, this study is a continuation of a previous study headed by Vuong *et al.*, (2013) which incorporates the usage of water as a solvent based extraction of *Carica papaya* leaves. Based on the chosen manipulating factor, the addition of this particular study is therefore hoped to add existing knowledge and providing supporting evidences on the optimisation of aqueous *Carica papaya* leaves extract.

Carica papaya leaves extract are also bitter in taste, which might proves to be inconvenient for consumption. Some clinical trials and home remedy therefore added honey to improve the taste of the aqueous extract (Ahmad *et al.*, 2011).

Subsequently, there is little research done on the effect of infusing honey to aqueous *Carica papaya* leaves to their antioxidant activity. Thus, this study also aims to study the effect of infusing honey into *Carica papaya* leaf aqueous extract through their antioxidants activity. The honey selected to be infused with the leaf extract in this study is 'kelulut' honey. To the best of our knowledge, this is the first report on antioxidants in papaya leaves extract infused with 'kelulut' honey.

1.4 Objectives

The objectives of this current study are as shown below:

1. To determine the antioxidant properties of *Carica papaya* leaves extract prepared under different temperature.
2. To determine the antioxidant properties of *Carica papaya* leaves extract prepared under different time duration.
3. To determine the antioxidant properties of *Carica papaya* leaves extract infused with "kelulut" honey.

1.5 Scope of study

This study was conducted on *Carica papaya* leaves self-grown in the vicinity of Analytical Chemistry Laboratory located in Faculty of Science and Natural Resources, Universiti Malaysia Sabah. The samples of young healthy *Carica papaya* leaves was freshly harvested from the third most uppermost shoot of a single mature papaya tree. Throughout the entire duration of study, sample was harvested from the same source to maintain data accuracy and reliability. Sample was taken in the morning within the range of 8:00-9:00 A.M. accordingly. The analysis of young *Carica papaya* leaves extract as well as its infusion with "kelulut" honey were analysed through TPC, TFC, FRAP and DPPH assays using microplate spectrophotometer. On the other hand, quantification of gallic acid and quercetin in all samples were performed using HPLC.

CHAPTER 2

LITERATURE REVIEW

2.1 Polyphenols

Due to its tremendous roles in human health, dietary polyphenols has long gained interests among food researchers, nutritionists as well as consumers. Research done early in the nineties has strongly agreed that polyphenol plays a crucial part in inhibiting degenerative disease, particularly cardiovascular diseases, cancers as well as neurodegenerative diseases (Milner, 1994; Duthie and Brown, 1994).

According to (Tsao, 2010), polyphenols are categorised as strong antioxidants that supplement and complement the antioxidant vitamins and enzymes to curb oxidative stress triggered by excess reactive oxygen species (ROS). Despite the proof of antioxidant activity exhibited by polyphenols are mostly available through *in vitro* studies, Tsao, (2010) added that there are more studies that supported the fact that polyphenols functions more than just an antioxidant *in vivo*. Correspondingly, all polyphenols are natural compounds that has phenolic structural features (Tsao, 2010).

2.1.1 Phenolic acids found in *Carica papaya* leaves

Dietary phenolics or polyphenols are one of the most abundant natural products in the plant kingdom. Being a widely distributed and diverse group, eventhough polyphenols are generally categorised as compounds having phenolic structural features, it can be further broken down to several sub-groups of phenolic compound. Examples of rich sources of polyphenols are different parts of plant, whole grain and other type of foods including wine, chocolate and tea (Tsao, 2010). Listed below are some examples of polyphenols commonly found in plant, and is abundantly found in *Carica papaya* leaves as well.

i. Phenolic acids

Phenolic acids, classified as non-flavonoid polyphenolic compounds are further characterised into two main types which are benzoic acid and cinnamic acids. These derivatives are structured based on C1–C6 and C3–C6 backbones (Figure 2.1).

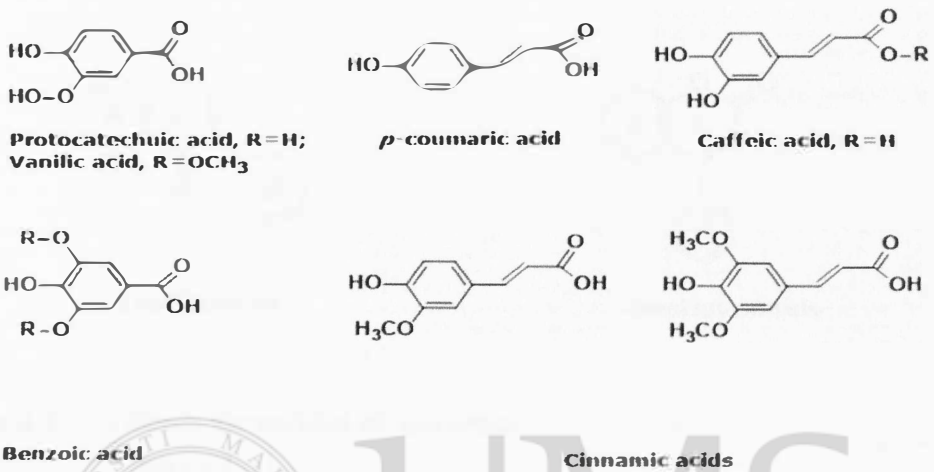
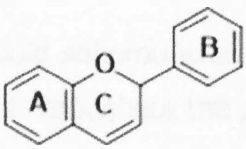


Figure 2.1 : **Some of phenolic acids found in *Carica papaya*.**
Source : Tsao, (2010)

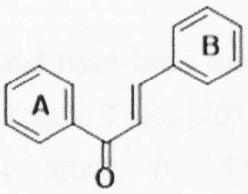
ii. Flavonoids

Any compounds with C6–C3–C6 general structural backbone, where two C6 units (Ring A and Ring B) are of phenolic nature are classified as flavonoids (Figure 2.2). However, due to the variations in the chromane ring (Ring C) and hydroxylation pattern, flavonoids are further dividable into separate sub-groups, anthocyanins, flavones, flavanones, flavonols and flavan-3-ols for example (Tsao, 2010).

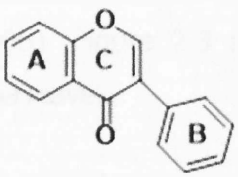
Although there are a large percentage of flavonoids with their Ring B attached to the C2 position of Ring C, there are compounds found in plants whose Ring B is connected at the C3 and C4 position of Ring C; namely isoflavones and neoflavonoids, respectively. Other compound listed in the flavonoid company is chalcones, eventhough it lacked the heterocyclic Ring C (Tsao, 2010).



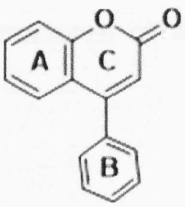
Flavonoid backbone



Chalcones



Isoflavones



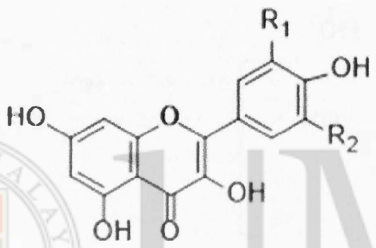
Neoflavonoids

Figure 2.2 : Basic flavonoid structures.
Source : Tsao, (2010)

In plants most of flavonoid compounds exist as glycosides. Tsao, (2010) hence appended that depending on both glycosylation patterns and structural difference of the compounds, its biological activities may differ from one another.

iii. Flavonols

These flavonoid subgroups are the most common and have been mostly identified to be present throughout the plant kingdom. According to Tsao, (2010), this is the largest subgroup among all polyphenols. A study by Tsao, (2009); Valant-Vetschera, (2006); Williams, (2006) reported that the usual flavonol aglycones, kaempferol has been identified to have at minimum 279 and 347 different glycosidic combinations, respectively (Tsao, 2009; Valant-Vetschera, 2006; Williams, 2006). Figure 2.3 shows the general structures of flavonols found in *Carica papaya* leaves.



Kaempferol R_1=H, R_2=H
Quercetin R_1=H, R_2=OH
Myricetin R_1=OH, R_2=OH

Figure 2.3 : Structures of flavonols found in *Carica papaya* leaves.
Source : Tsao, (2010)

iv. Proanthocyanidins

Proanthocyanidins are classified as oligomeric flavonoids, where most of them are polymers of catechin and epicatechin. Hence, catechin and epicatechin, both flavonols structure are also known as proanthocyanidins since according to (Tsao, 2010) the cleavage of the polymeric chains catalysed by acid will create anthocyanidins.

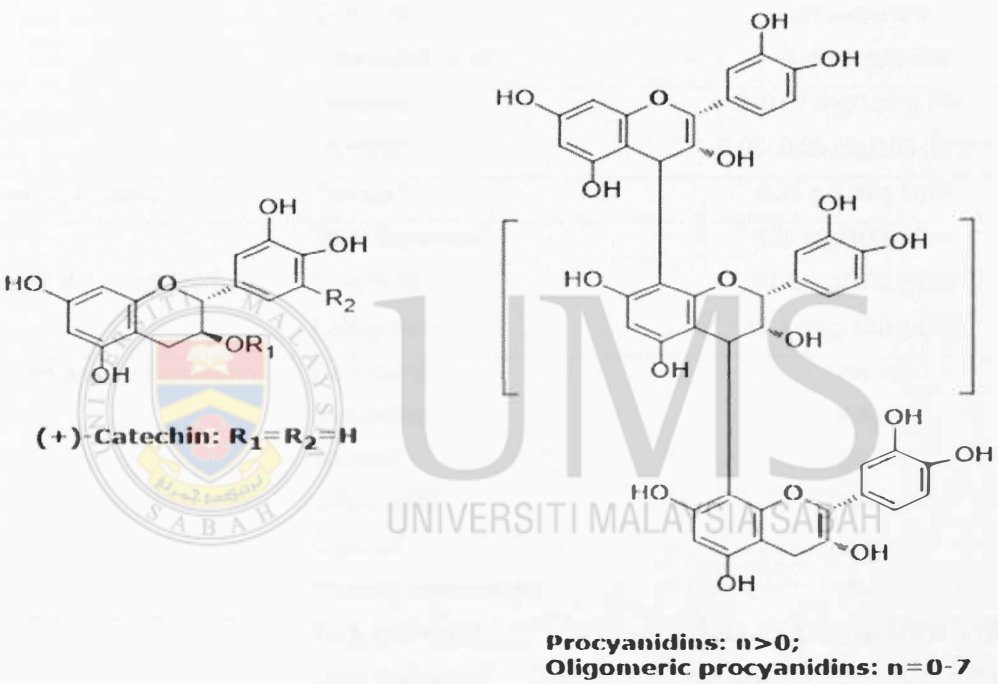


Figure 2.4 : Flavonols and Procyanidins.
Source : Tsao, (2010)

Accordingly, Table 2.1 below tabulates some of the phenolic acids found based on several studies in *Carica papaya* leaves.

Table 2.1: Phenolic compounds found in *Carica papaya* leaves

Reference	Analysed compound(s)	Concentration
Canini *et al.*, (2007)	Protocatechuic acid[b]	0.11 mg/g DW
	p-Coumaric acid[b]	0.33 mg/g DW
	5,7-Dimethoxycoumarin[b]	0.14 mg/g DW
	Caffeic acid[b]	0.25 mg/g DW
	Kaempferol[b]	0.03 mg/g DW
	Quercetin[b]	0.04 mg/g DW
	Chlorogenic acid[b]	<0.001 mg/g DW
Duke (2011)	Flavonols*	0–0.02 mg/100 g FW*
	Tannins*	0.05–0.06 mg/100 g FW*
Marfo *et al.*, (1986a)	Tannins[e]	6.35 g/100 g DW*
Miean and Mohamed (2001)	Total flavonoids[c]	126 mg/100 g DW
	Quercetin[c]	81.1 mg/100 g DW
	Kaempherol[c]	45.3 (mg/100 g DW)
Sagadevan and Jayaramjayaraj (2018)	Flavonoids[e]	NA
	Glycosides[e]	NA
	Tannins[e]	NA
	Terpenoids[e]	NA
	Saponin[e]	NA
	Phenolic compounds[e]	NA
Vuong *et al.*, (2013)	Total phenolics[a]	9.43–23.1 mg GAE/100 g FW
	Total flavonoids[d]	6.44–17.1 mg CE/100 g FW
	Saponins[d]	26.4–82.9 mg Aes/g FW
	Proanthocyanidins[d]	1.91–7.91 mg CE/100 g FW

DW=dry weight

FW=fresh weight.

[a] Folin-Ciocalteau method.

[b] GC-MS.

[c] HPLC.

[d] UV-Vis spectrophotometry.

[e] Paper chromatography.

GAE: gallic acid equivalents.

CE : catechin equivalents.

Aes : aescin equivalents.

N.A.: not available. * Method of analysis and/or unit is not available.