ANTI-OXIDANT CONTENT AND INHIBITORY EFFECTS OF LEAF EXTRACT OF *Piper sarmentosum* TOWARD COLLAGENASE

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ABSTRACT

Piper sarmentosum leaves possesses various types of phytochemical activity that yet to be discovered. This study aims to determine the total flavonoid content (TFC), radical scavenging and collagenase activity in crude leaf extract of *P. sarmentosum.* Aluminum chloride colorimetric method was employed to determine TFC in crude leaf extract of *P. sarmentosum.* DPPH assay was carried out to determine radical scavenging activity between crude leaf extract, QU standard and ascorbic acid. The binding affinity of collagenase was determined using various compounds (EDTA, QU standard, crude leaf extract and gelatin) and their K_m was determined from Lineweaver-Burk plot. TFC. TFC in crude leaf extract was measured as 52.0 ± 0.79 mg of QUE per gram of tissue whereas QU contents in crude leaf extract found to possess 7.0 ± 0.58 mg QUE per gram of tissues. Ascorbic acid has significantly highest radical scavenging activity as 89.4% compared to QU standard (65.3%) and crude leaf extract (49.4%). QU standard, crude leaf extract, gelatin and EDTA were ranked from lower to high according to their K_m as 0.14 mM, 0.25 mM, 0.50 mM and 2.00 mM. The binding affinity of each compound was directly proportional with to K_m. Crude leaf extract has shown higher affinity toward collagenase compared to gelatin and EDTA. Crude leaf extract bind to the active site of collagenase by competing with gelatin. Crude leaf extract and gelatin have no significant different in term of binding affinity by binding randomly to the active site collagenase. The study revealed that crude leaf extract may act as potential anti-aging source by scavenging radicals and inhibiting collagenase.

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LIST OF ABBREVIATIONS

%	Percentage
$AlCl_3$	Aluminium chloride
ANOVA	One-way analysis of variance
CE	Catechin equivalent
cm	Centimetre
DPPH	2,2-DIPHENYL-1-1PICRYLHYDRAZYL
EC	Enzyme commission
EDTA	Ethylenediaminetetraacetic acid
FALGPA	N-[3-(2-Furyl)acryloyl]-Leu-Gly-Pro-Ala
FR	Free radical
g	Gram
GAE	Gallic acid equivalent
hr	Hour
K_i	Dissociation constant for free enzyme
K_m	Michaelis-Menten constant
L	Litre
mg	Milligram
mg/mL	Milligram per millilitre
min	Minute
mL	Millilitre
mM	Millimolar
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide
ng	Nanogram
nm	Nanometre

°C	Degree Celsius
QU	Quercetin
QUE	Quercetin equivalent
R_f	Retention factors
ROS	Reactive oxygen species
SPSS	Statistical Package for the Social Sciences.
TFC	Total flavonoid content
units/mg	Units per milligram
units/mL	Units per millilitre
UV	Ultraviolet
v/v	Volume per volume
V_{max}	Maximal velocity
w/v	Weight per volume
x	Time

CHAPTER 1

INTRODUCTION

Piper sarmentosum is a fast growing plant known as wild betel that mostly found in Southeast Asia (Raman, Galal & Khan, 2012). Its leaf is bright green with a pungent taste. It can be cooked or consumed raw as 'ulam' (Chan & Wong, 2014). Traditionally, it was used to treat malaria fever, cough, tooth-ache and dermatitis (Atiax, Ahmad, Sirat & Arbain, 2011). To date, there are several studies carried out to demonstrate biological activity of *P. sarmentosum* extract. Aqueous extract of the whole plant of *P. sarmentosum* found to have hypoglycemic properties to be used in the treatment for diabetes mellitus (Pan et al., 2010). Leaf extract also found to possess anti-bacterial and anti-inflammatory activities (Estai, Suhaimi, Shuid, Das & Soelaiman, 2011). Phytochemical such as alkaloids, polyphenols and flavonoids have been isolated from *P. sarmentosum* leaves (Seyyedan et al., 2013). According to Seyyedan et al. (2013), flavonoids from *P. sarmentosum* contained natural anti-oxidant with high free radical scavenging activity. Due to these properties, *P. sarmentosum* is ready to be explored for its potential use in anti-aging research.

Wrinkles are the sign of aging caused by the loss of strength and elasticity in skin (Chattuwatthana & Okello, 2015). In term of physiological activity, formation of oxidants such as free radicals (FRs) and reactive oxygen species (ROS) increased when exposed to exogenous agents (Sariri, Sabbaghzadeh & Poumohamad, 2011). Generally, the imbalance contents of anti-oxidants and oxidants have led to oxidative stress that further induced the impairment of cellular function which indirectly promoted aging, especially the activation of collagenase (Saleh Mahdi et al., 2011).

Nowadays, anti-aging products have become dominant in cosmetic industry. There is an increasing trend of using natural materials in the development of skin care products because natural materials are easier to absorb by skin layer and reduce skin allergy problem (Wahab, Rahman, Ismail, Mustafa & Hashim, 2014). Plants have been widely used in the cosmetic industry. *P. sarmentosum* is not an exception as it was found to have strong anti-oxidant properties (Lee et al., 2014; Johnsen et al., 2011;

Seyyedan et al., 2013; Wang et al., 2016). Although there were studies on anti-oxidant activities of *P. sarmentosum* but anti-collagenase activity of *P. sarmentosum* has yet to be accessed.

Hence, the objectives of this study were:

1. to determine total content of flavonoids in the water-extract of *P. sarmentosum* leaves
2. to examine the scavenging activity towards DPPH by *P. sarmentosum* crude leaf extract
3. to determine the binding affinity K_m of *P. sarmentosum* crude leaf extract toward collagenase
4. to determine the inhibitory mode of *P. sarmentosum* crude leaf extract toward collagenase

CHAPTER 2

LITERATURE REVIEW

2.1 OVERVIEW OF *Piper Sarmentosum*

P. sarmentosum is a terrestrial herb with vertical, slim branchlets at about 30 cm tall which belongs to the family Piperaceae (Seyyedan et al., 2013). *P. sarmentosum* is known as 'Kaduk' in Malay word and categorised as dioecious plant. Its green leaves are alternate and heart-shaped with a waxy surface as shown in Figure 2.1(a). It produces large procumbent fruits that give sweet taste at maturity and small white flowers as shown in Figures 2.1(b) and 2.1(c) respectively. It is widely distributed in the tropical forests of Philippines, Vietnam, Cambodia, India, Indonesia, China, and Malaysia (Raman, Galal & Khan, 2012). It is a four seasonal plant with fruiting season falls between October to December (Seyyedan et al., 2013). It can be commonly found in humid area, clear riverbanks, shade place with warmth sunlight and damp soil. It is easy to be propagated via vegetative cuttings.

(a) (b) (c)

Figure 2.1 The leaves (a), fruits (b), and flowers (c) of *P. sarmentosum* (Seyyedan et al., 2013).

It contains various active components such as aristolactams, amides, lignans, esters, steroids, flavonoids, terpenes, alkaloids, and propenylphenols. Various pharmacological activities in human such as hypoglycemic, anti-oxidant, anti-tuberculosis, anti-malarial and anti-cancer has been exerted by *P. sarmentosum*

(Sharifah, Sijam & Omar, 2016; Seyyedan et al., 2013). Nowadays, natural remedies are widely used in daily life due to cost effectiveness and absence of unfavorable or harmful effects. According to Maizura, Zakaria, Nor Anita and Othman (2013), the whole plant of aqueous extracted *P. sarmentosum* is safe for human consumption with dose less than 10 g of powder per kg of plant per oral uptake.

2.2 FLAVONOIDS

Flavonoids are secondary metabolites that found ubiquitously in the kingdom Plantae (Sak, 2014). They can be categorised into six groups, namely flavones, flavonols, flavanols, anthocyanidins, flavanones, and isoflavonoids (Thilakarathna & Rupasinghe, 2013). All the natural occurring flavonoids contained 15-carbon skeletons with a two aromatic rings (A & B) and heterocyclic ring (C) as shown in Figure 2.2. In plant, flavonoids play vital roles in protecting against UV radiation, regulating plant hormones, protecting against microbes and aiding in pollination (Amalesh, Gouranga & Kumar, 2011). Apart from their significant roles in the plants, flavonoids are important components in promoting human health. It exhibited diverse pharmacological activities in human body such as anti-bacterial, anti-viral, anti-inflammatory, anti-cancer and cardiovascular diseases (Kumar & Pandey, 2013; Procházková, Boušová & Wilhelmová, 2011).

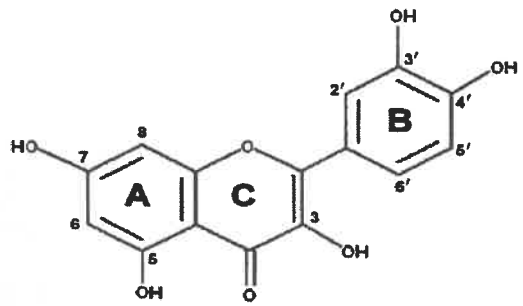

Figure 2.2 The structure of flavonoid (quercetin). A and B are aromatic rings while C is heterocyclic ring.

Flavonoids serve as the most potential components for application in cosmetology due to their anti-oxidant properties. The anti-oxidant strength of flavonoids depends on the arrangement of their functional groups such as hydroxyl