PHYTOCHEMICAL CONTENT AND ANTIOXIDANT ACTIVITY IN FRUITS OF *Phyllanthus pectinatus* Hook.f. AND *Phyllanthus emblica* L.

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FACULTY OF SCIENCE
UNIVERSITI MALAYA
KUALA LUMPUR

2024

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DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF BIOTECHNOLOGY

INSTITUTE OF BIOLOGICAL SCIENCES
FACULTY OF SCIENCE
UNIVERSITI MALAYA
KUALA LUMPUR

2024

UNIVERSITI MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: **EMMA NATASYA BINTI AHMAD**

Matric No: **17029030 / SOC180014**

Name of Degree: **MASTER OF SCIENCE (BIOTECHNOLOGY)**

Title of Dissertation ("this Work"):

"PHYTOCHEMICAL CONTENT AND ANTIOXIDANT ACTIVITY IN FRUITS OF *Phyllanthus pectinatus* Hook.f. AND *Phyllanthus emblica* L."

Field of Study: **LIFE SCIENCE (BIOLOGY AND BIOCHEMISTRY)**

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PHYTOCHEMICAL CONTENT AND ANTIOXIDANT ACTIVITY IN FRUITS OF *Phyllanthus pectinatus* Hook.f. AND *Phyllanthus emblica* L.

ABSTRACT

Phyllanthus pectinatus is often mistakenly classified as *Phyllanthus emblica* since both species morphologically look very similar to each other. Unlike *P. emblica*, *P. pectinatus* have a very limited published work on its phytochemical constituents and biological activities. Therefore, the aim of this study is to explore the phytochemical content and antioxidant activities of the fruits of *P. pectinatus* in comparison with fruits of *P. emblica*. The phytochemicals, total phenolic content (TPC), total flavonoid content (TFC), ascorbic acid content (AAC) and the antioxidant potentials of both *P. pectinatus* fruit (PPF) *and P. emblica* fruit (PEF) extracts were determined. The phytochemical screening result showed the presence of phenols, flavonoids, tannins, phlobatannins, terpenoids and sterols in both PPF and PEF extracts while saponin was only detected in PPF extract. The PPF contained the highest TPC (1140 ± 1.41 mg GAE/g DW) and TFC (35.88 ± 3.17 mg QE/g DW). Meanwhile, the highest value of AAC was from PEF (291.60 ± 0.00 mg/100g). Furthermore, the result revealed that PPF had the highest antioxidant potential against 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and Ferric Reducing Antioxidant Power (FRAP). The TPC and TFC of both PPF and PEF showed strong positive correlation with total antioxidant activities. On the other hand, the correlation between AAC with all antioxidant assays were negative. The results suggested that both PPF and PEF are a good source of antioxidants. In addition, PPF can potentially be considered as a beneficial natural source of antioxidant.

Keywords: *Phyllanthus pectinatus*, *Phyllanthus emblica*, phenolic, flavonoid, ascorbic acid, antioxidant.

KANDUNGAN FITOKIMIA DAN AKTIVITI ANTIOKSIDAN DALAM BUAH *Phyllanthus pectinatus* Hook.f. DAN *Phyllanthus emblica* L.

ABSTRAK

Phyllanthus pectinatus sering dianggap sebagai *Phyllanthus emblica* kerana morfologi kedua-dua spesies kelihatan sangat serupa secara antara satu sama lain. Kajian mengenai kandungan fitokimia dan aktiviti biologi *P. pectinatus* adalah sangat terhad berbanding *P. emblica*. Oleh itu, matlamat kajian ini adalah untuk mengkaji kandungan fitokimia dan aktiviti antioksidan buah *P. pectinatus* dan *P. emblica*. Ujian fitokimia, penentuan jumlah kandungan fenolik (TPC), jumlah kandungan flavonoid (TFC), kandungan asid askorbik (AAC) dan potensi antioksidan kedua-dua ekstrak buah *P. pectinatus* (PPF) dan buah *P. emblica* (PEF) telah dijalankan. Hasil ujian fitokimia menunjukkan kehadiran fenol, flavonoid, tanin, phlobatannin, terpenoid dan sterol dalam kedua-dua ekstrak PPF dan PEF manakala saponin hanya dikesan dalam ekstrak PPF. PPF mengandungi TPC (1140 ± 1.41 mg GAE/g DW) dan TFC (35.88 ± 3.17 mg QE/g DW) tertinggi. Manakala, nilai AAC tertinggi adalah daripada PEF (291.60 ± 0.00 mg/100g). Selain itu, keputusan menunjukkan bahawa PPF mempunyai potensi antioksidan tertinggi terhadap 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) dan *'Ferric Reducing Antioxidant Power'* (FRAP). TPC dan TFC bagi kedua-dua PPF dan PEF menunjukkan korelasi positif yang kuat dengan jumlah aktiviti antioksidan. Sebaliknya, korelasi antara AAC dengan semua ujian antioksidan adalah negatif. Keputusan menunjukkan bahawa kedua-dua PPF dan PEF adalah sumber antioksidan yang baik. Selain itu, PPF dianggap berpotensi untuk dijadikan sumber antioksidan semulajadi yang bermanfaat.

Kata kunci: *Phyllanthus pectinatus*, *Phyllanthus emblica*, fenolik, flavonoid, asid askorbik, antioksidan.

ACKNOWLEDGEMENTS

First and foremost, I am grateful to Allah Almighty, the Most Gracious and Most Merciful for granting me the capability and providing me this opportunity to proceed this project successfully. This thesis appears in its current form due to the guidance and assistance of several people. Therefore, I would sincerely offer my thanks to all of them.

It is a genuine pleasure to express my deep gratitude and thanks to my supervisor, Dr. Sujatha Ramasamy. Her dedication and keen interest above all her overwhelming attitude to help her student had been solely responsible for completing my project. Her timely advice, kindness and scientific approach have helped me to accomplish my work. Besides my supervisor, I would like to give special thanks to my collegemates, Nazzim, Kak Faezah, Ainnsyah, Kak Wani, Kak Santha, Wani & Atid for their guidance, suggestions, teamwork and for letting my master journey be an enjoyable moment.

I owe a deep sense of gratitude to my mother, Salmi Shaarani, my father, Ahmad Md Nor, my family especially Kak Emmie, Abang Helmi, Abang Mail and Kak Sumayyah, and my friends, Hidayah, Ain and Khalidah for being such an amazing source of strength and support throughout my master journey.

A big thank you must go to my husband, Muhammad Ikhwan for the endless amount of support, love and encouragement to complete my master, and to my son, Raaif Ashraf, who is just an amazing boy – I am so blessed to be his mummy.

Finally, I am very thankful to all who have helped during my various stages of pursuing a master's degree. I could not have gotten through this journey without them. Thank you.

Emma Natasya Ahmad

Universiti Malaya, January 2024

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LIST OF SYMBOLS AND ABBREVIATIONS

α	:	Alpha
β	:	Beta
δ	:	Delta
γ	:	Gamma
<	:	Less than
>	:	More than
%	:	Percentage
ATP	:	Adenosine triphosphate
AAC	:	Ascorbic acid content
ABTS	:	2, 2-azino-bis-3-ethylbenzotiazoline-6-sulfonic acid
$^{\bullet}CCl_3$	:	Carbon trichloride
CAT	:	Catalase
^{o}C	:	Degree celcius
DCPIP	:	2,6-Dichlorophenolindophenol
MTT	:	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
DW	:	Dry weight
Fe^{3+}	:	Ferric
FRAP	:	Ferric reducing antioxidant power
Fe^{2+}	:	Ferrous
Fe	:	Ferrum/ Iron
$FeOO_2^{\bullet 2+}$	:	Ferryl ions
FW	:	Fresh weight

g	:	Gram
GAE	:	Gallic acid equivalents
GRx	:	Glutathione reductase
$GS^{\bullet}$	:	Glutathiyl
IC_{50}	:	Half-maximal inhibitory concentration
$X^{\bullet}$	:	Halogen
HPLC	:	High performance liquid chromatography
HCl	:	Hydrochloric acid
$H^{\bullet}$	:	Hydrogen
HAT	:	Hydrogen atom transfer
H_2O_2	:	Hydrogen peroxide
$HO^{\bullet}$	:	Hydroxyl radical
kg	:	Kilogram
LPO	:	Lipid peroxidation
MDA	:	Malondialdehyde
$^{\bullet}CH3$	:	Methyl
µg	:	Microgram
µL	:	Microlitre
µM	:	Micromole
mg	:	Milligram
mg/L	:	Milligram per litre
mg/mL	:	Milligram per millilitre
mm	:	Millimetre
mM	:	Millimolar
MW	:	Molar weight
nm	:	Nanometer

$NO^{\bullet}$	:	Nitric oxide
ANOVA	:	One-way analysis of variance
$ROO^{\bullet}$	:	Peroxyl radical
ONOO	:	Peroxynitrite anion
PEF	:	*Phyllanthus emblica* fruits
PPF	:	*Phyllanthus pectinatus* fruits
pH	:	Potential of hydrogen
QE	:	Quercetin equivalents
RNS	:	Reactive nitrogen species
ROS	:	Reactive oxygen species
SET	:	Single electron transfer
SE	:	Standard error
SPSS	:	Statistical package for the social sciences
$O_2^{\bullet-}$	:	Superoxide anion
SOD	:	Superoxide dismutase
TFC	:	Total flavonoid content
TPC	:	Total phenolic content
TCA	:	Trichloroacetic acid
TPTZ	:	2,4,6-tri(2-pyridyl)-s- triazine

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CHAPTER 1: INTRODUCTION

In addition to the COVID-19 pandemic, the production and consumption of functional foods have attracted a lot of interest recently due to their immune-boosting qualities, which can be used as a prophylactic tool against chronic illnesses. There is an enormous and ongoing need for a more sustainable, easily available, and nutrient-dense food supply due to the increase in the world's urban population. Emerging food technologies have great potential in producing bioactive and functional ingredients that support nutrition security and human health. Reducing nutritional inadequacies through the use of underutilized and neglected crops in product development and modifying the food matrix to improve nutrient bioavailability are the essential advances in research.

More than 50% of Malaysian consumers changed their eating patterns, consuming more plant-based foods, and majority of them expected restaurant and beverage operators to offer more healthy food options (Jie & Bakar, 2023). Fruits are currently the most appealing functional food category due to their usefulness, desirable nutrient and bioactive molecule content, convenience, and ability to satisfy consumer demands for shape, size, storage, and sensory qualities. Many consumers are looking for plant-based foods with health benefits (Granato *et al.,* 2023). There is an increase in the production of fruits, especially that rich in vitamins, minerals, and other natural ingredients.

Phyllanthus species (Family: Phyllanthaceae) had been widely used in Malaysia and other Asian countries as traditional medicine to treat many diseases. Studies shows that species belongs to the *Phyllanthus* genus is highly enriched with many phytoconstituents such as phenolics, lignans, flavonoids, alkaloids, and terpenes (Catapan *et al.,* 2000). Recently, due to their promising therapeutic potentials in treating many diseases, interest in the *Phyllanthus* species is increasing (Gul *et al*., 2022).

Phyllanthus emblica L. which popularly known as Indian gooseberry or amla is commonly used in Indian medicinal system (also known as Ayurvedic medical system) for centuries to treat many diseases. Essentially every part, from root to fruit of *P. emblica* are medicinally useful. The most studied part of *P. emblica* which is the fruit have been reported to possess many bioactive constituents that exhibit numerous biological activities (Tung *et al*., 2018). Extensive research reported various medicinal properties of *P. emblica* such as antimicrobial, antioxidant, hypolipidemic, antidiabetic, gastroprotective, antiulcerogenic, anticancer and hepatoprotective (Ahmad *et al*., 2021). Halim *et al*. (2022) reported that *P. emblica* fruit extract has high levels of total phenol and flavonoid and exhibit good antioxidant activity. Another study also demonstrated that gallic acid was found to be the major phytochemical in *P. emblica* fruit and the extract exhibited high antioxidant and antibacterial activity (Sheoran *et al*., 2019). The fruits of *P. emblica* is edible and are widely commercialized in the form of fresh fruits, health supplements, juices and as an ingredient in cosmetic products.

Phyllanthus pectinatus Hook.f. locally known as Pokok Melaka or Malacca tree is often mistakenly classified as *P. emblica* because both species morphologically look very similar to each other. Unlike *P. emblica*, *P. pectinatus* have a very limited published work on its phytochemical constituents and biological activities. The fruits of *P. pectinatus* is edible but is not widely consumed by the local peoples. To the date, the fruits of *P. pectinatus* is not commercialized and there is unknown any products made from this fruit.

Therefore, this study was carried out to explore the phytochemical content and antioxidant activities of the fruits of *P. pectinatus* in comparison with fruits of *P. emblica*.

The specific objectives of this study are as follows:

(i) to evaluate the phytochemical content of the fruits of *P. pectinatus* in comparison with the fruits of *P. emblica*; and

(ii) to assess the antioxidant of the fruits of *P. pectinatus* in comparison with the fruits of *P. emblica*.

CHAPTER 2: LITERATURE REVIEW

2.1 Therapeutic Potential of *Phyllanthus*

Phyllanthus is a large genus belongs to the Phyllanthaceae family. Phyllanthaceae plants are found in most subtropical and tropical regions of the world, including Asia, Africa, and America. About ten to eleven subgenera, such as *Kirganelia, Emblica, Phyllanthus, Conani, Xylophylla, Ericocus, Isocladus, Cicca, Gomphidium, Botryanthus,* and *Phyllanthodendron* make up the Phyllanthaceae family (Tan *et al*., 2020). Among all the subgenera, *Phyllanthus*, *Cicca* and *Kirganelia* contains important species that are extensively used as traditional medicine to treat numerous diseases including tumours, constipation, diabetes mellitus, indigestion, jaundice, arthritis, and indigestion.

Phyllanthus species are widely utilized in Malaysia and other Asian countries in the traditional medicine to treat many diseases for centuries. For example, *P. fraternus, P. niruri,* and *P. amarus* have been used traditionally to treat diseases such as diabetes, disturbances of the urinary bladder and kidney, hepatitis B virus and intestinal infections (Unander *et al*., 1995). Not only for medicinal purpose, *Phyllanthus* is also used in food industry, confectionaries, and as pesticides (Nisar *et al*., 2018). This genus consists of a diverse spurge family herbs, flowering trees, and shrubs. *Phyllanthus* plants are floriferous and deciduous with a leaf-like (phyllode) flattened green roots (Mao *et al*., 2016).

There are seventeen *Phyllanthus* species in Asia that are regarded as deobstruent, antiseptic agents, febrifuge, stomachic, diuretic, and considered to have astringent and bitter taste (Mao *et al*., 2016). In Peninsular Malaysia, there are 78 Phyllanthus species with morphological descriptions and records of their ethnobotanical use (Badron *et al.*, 2022). Some of the common species are *Phyllanthus amarus, P. acidus, P. niruri,*

P. pectinatus, P. albidiscus, P. elegans, P. emblica, P. filicifolius, P. reticulatus, P. roseus and *P. watsonii* (Tan *et al*., 2020). Figure 2.1 shows some of this *Phyllanthus* species.

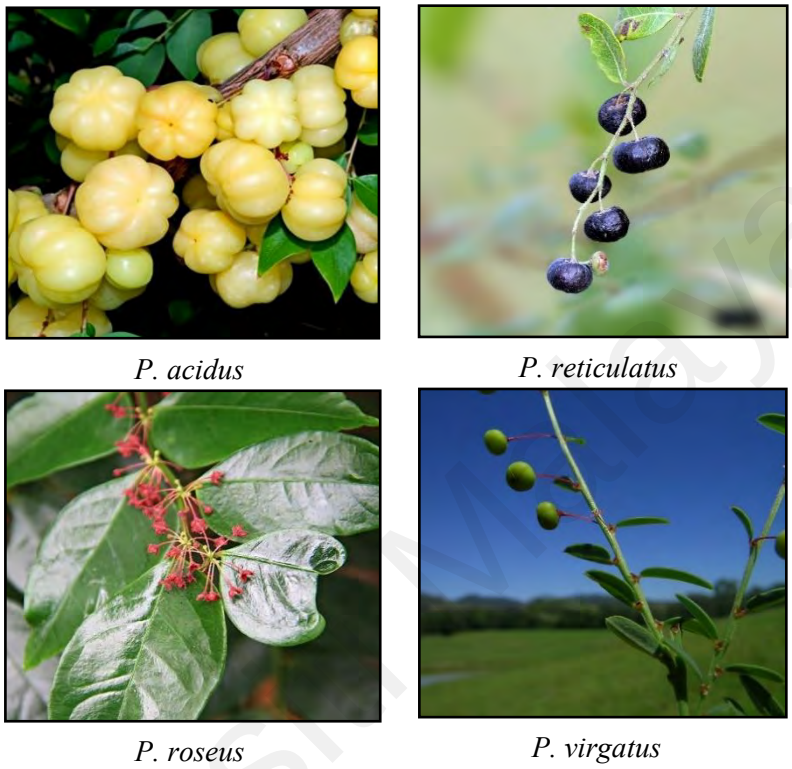

Figure 2.1: Images of some of the *Phyllanthus* species (Adapted from https://www.monaconatureencyclopedia.com, https://commons.wikimedia.org & https://www.flickr.com).

Interest in the genus *Phyllanthus* is growing among natural product researchers due to its promising therapeutic potentials for treating various diseases (Kumaran & Karunakaran, 2007). Studies shows that *Phyllanthus* species are enriched with more than 510 phytoconstituents such as phenolics, lignans, terpenes, flavonoids, alkaloids, and terpenes (Catapan *et al*., 2000). *Phyllanthus* species have been found to contain three main compounds: gallic acid, geraniin, and corilagin. Phyllanthin, geraniin and nirathin are among the mainly focused constituents in pharmacological research (Mao *et al*., 2016).

Different pharmacological activities have been observed in different parts of *Phyllanthus* species. Different parts of the plant may produce specific bioactive molecules thus exhibit different biological activities (Nisar *et al*., 2018). For example, according to Shimu *et al*. (2021), crude methanol extract of *P. acidus* fruit pulp has higher levels of antioxidant activity than seeds and contains a significant number of important phytochemicals, has a minimal toxic effect on normal cells and is a good source of lectin protein. The overall results suggested that the crude methanol extract of *P. acidus* fruit pulp can be regarded as a more promising medicinal source than its seed. Numerous studies on biological activities of *Phyllanthus* species have been reported including anticancer, antioxidant, immunomodulatory and antidiabetic activities (Dasiman & Bahari, 2021; Matou *et al*., 2021; Joujeh & Joujeh, 2023). Many studies reported on the antioxidative activity of *Phyllanthus* species (Joy & Kuttan, 1995; Asha *et al*., 2004).

According to Moniruzzaman *et al*. (2015) and Uddin *et al*. (2016a), *P. acidus* showed antioxidant activity and memory enhancing ability which suggest its potential in therapeutic strategy for treating neurodegenerative diseases. *P. reticulatus* also was reported to improve brain antioxidant enzymes, anti-acetylcholinesterase activity and cognitive functions which can be used in controlling neurodegenerative diseases (Uddin *et al.,* 2016b). *P. amarus* have been traditionally used to treat diabetes mellitus and cancer and studies shows that *P. amarus* extract can be potentially commercialized for its ability to isolate natural antioxidants and a few novel anticancer agents (Nguyen *et al*., 2017).

2.1.1 *Phyllanthus pectinatus* Hook.f.

Phyllanthus pectinatus Hook.f. (Figure 2.2) is locally known as Pokok Melaka or Malacca tree. *P. pectinatus* is native to Malay Archipelago Forest and it can be found commonly in the forests of Malacca state in Malaysia (Ng, 2000).

P. pectinatus is often mistakenly classified as *Phyllanthus emblica* because both species look very similar to each other. One of the differences between *P. pectinatus* and *P. emblica* is that the fruits of *P. pectinatus* are hanging at the end of the leafy shoots while fruits of *P. emblica* clustered at the base of robust leafy shoots. The flower structure, the shape of the seed and the bark is also different for both species (Ng, 2000).

Figure 2.2: *Phyllanthus pectinatus* Hook.f. (Adapted from Ng (2000) & https://nysp-runner-plants.blogspot.com).

Based on literature survey, there is lack of data reported on the phytochemical constituents and biological activities of *P. pectinatus*. A few of reported bioactivities of *P. pectinatus* are summarized in Table 2.1.

Table 2.1: Biological activities reported on *P. pectinatus.*

Plant part	Biological activities	Findings	References
Fruit	Cytotoxic	When *P. pectinatus* crude ethyl acetate extract was tested against human breast MCF7 cancer cells and human cervical Ca Ski cancer cells, it demonstrated strong cytotoxicity, with IC_{50} values of 18.1 ± 0.66 and 19.4 ± 0.53 μg/ml, respectively.	Ramasamy *et al*. (2011)
Leaves	Cytotoxic	*P. pectinatus* crude methanol and crude ethyl acetate extracts were cytotoxically active on human ovarian SKOV3 cancer cell with an IC_{50} value of 4.8 ± 1.04 and 5.8 ± 0.76 μg/ml, respectively.	Ramasamy *et al*. (2011)

2.1.2 *Phyllanthus emblica* L.

Phyllanthus emblica L. (Figure 2.3), commonly known as amalaka or Indian gooseberry, has been used in Indian medicinal system (also known as Ayurvedic system) for centuries to treat various diseases. Aside from Indian Ayurveda medicine, *P. emblica* is also used in Tibetan, Unani, and Turkish medicinal system. The native distribution of *P. emblica* is India but it can grow well in tropical and subtropical regions including Malaysia, Southeast Asia, China, Pakistan, Sri Lanka, Burma, Ceylon, and Uzbekistan (Thakur *et al*., 1989; Calixto *et al*., 1998; Dnyaneshwar *et al*., 2006; Singh *et al*., 2008). *P. emblica* is typically found in the highlands of northeast Kelantan, but it can also be found dispersed in lowland woods in Peninsular Malaysia (Pisar *et al*., 2018).


Figure 2.3: *Phyllanthus emblica* L. (Adapted from Ahmad *et al*. (2021) & www.indiamart.com).

The tree of *P. emblica* is small to medium in size with a height ranging between 1 – 8 meters. The leaves look like a pinnate leaf which is green in colour, closely set along the branches, elliptical, small, and simple. The flowers are greenish yellow in colour and the fruit have six vertical stripes, nearly spherical in shape and quite smooth on the surface. The branches are extending regularly (Scartezzini *et al*., 2006; Kuttan & Harikumar, 2011).

Essentially every part, from root to fruit of *P. emblica* plant are medicinally useful. The most studied part of *P. emblica* is its fruits. The fruits of *P. emblica* have been reported to possess many bioactive constituents that exhibit numerous biological activities (Tung *et al*., 2018). Extensive research has been done to study the medicinal properties such as antimicrobial, antioxidant, hypolipidemic, antidiabetic, gastroprotective, antiulcerogenic, anticancer and hepatoprotective properties of *P. emblica* (Ahmad *et al*., 2021). Table 2.2 summarized some of the scientific findings on the biological activities of *P. emblica*.

Previous phytochemical studies reported on the occurrence of various phenolics including tannins, emblicol, phyllembelic acid, tannins, phyllembelin curcuminoids and rutin in *P. emblica* (Onions, 1994). The fruits of *P. emblica* is also a rich source of important dietary nutrients such as vitamin C, minerals, and amino acids. Surprisingly,

the amount of vitamin C in the fruits of *P. emblica* is greater than that found in citrus fruits such as oranges, tangerines, and lemons (Ahmad *et al*., 2021).

Table 2.2: Biological activities reported on *P. emblica*.

Plant part	Biological activities	Findings	References
Branch	Anti-aging	In *in vitro* assays, the *P. emblica* extracts showed strong antioxidant and tyrosinase inhibitory activities. In cellular assays, at 0.1 mg/mL, *P. emblica* extracts suppressed melanin by inhibiting tyrosinase and tyrosinase-related protein-2 activities.	Chaikul *et al*. (2021)
Leaves	Antioxidant & Cytoprotection	With co-administration of *P. emblica* leaves extract, the histopathological damages of the bronchiolar exocrine Clara cells were restored to normal. *P. emblica* leaves extract contains caffeic acid, gallic acid, rutin, and kaempferol according to HPLC-DAD analysis.	Tahir *et al*. (2016)
Fruits	Anticancer	*P. emblica* fruit extract (0.2-1 mg/mL) effectively induces cell cycle arrest and death in cancer cells to halt their proliferation. *P. emblica* ethanol extract reduced the sustainability of cancer cells in a dose-dependent manner, according to the MTT assay. Jurkat cells, which are human T lymphocytes, showed the highest sensitivity to the extract (IC_{50} values varied 0.12–0.69 mg/mL).	Kumnerdkhonkaen *et al*. (2018)
Fruits	Anti-inflammatory	The increased levels of pro-inflammatory cytokines in lung tissue were significantly reduced by *P. emblica* extract treatment. *P. emblica* extract (5-10 g/kg) treatment significantly decreasing the COX-2 and HIF-α protein expressions. The extract also decreased	Wang *et al*. (2017)

		the levels of IL-1β and Lin28B and increased the expression of miR-101.	
Leaves	Antidiabetic	Oral treatment of the *P. emblica* extract resulted in a substantial ($P<0.05$) decrease in fasting blood glucose and a rise in insulin level when compared to diabetic rats. Significant reductions were also observed in all biochemical markers. Furthermore, in the kidney and liver of diabetic rats, a noteworthy rise was noted in reduced glutathione, superoxide dismutase, glutathione peroxidase, catalase, and decreased LPO levels.	Nain *et al*. (2012)
Fruits	Antimicrobial & Antioxidant	A wide range of secondary metabolites, such as flavonoids (38.50 ± 2.84 mg catechin equivalents/g), tannins (44.28 ± 3.09 mg tannic acid equivalents/g), and phenolics (59.18 ± 2.91 mg GAE/g), were discovered to be abundant in the aqueous fruit extract. Moreover, it works well against a range of pathogenic species that are found in food, such as gram-positive and gram-negative bacteria, and fungus.	Gunti *et al*. (2019)
Fruits	Antioxidant & Neuroprotective	The DPPH IC_{50} values for *P. emblica* extracts ranged from 6 to 158.9 μM, which was significantly higher than the DPPH IC_{50} value of the commercially available antioxidant butylated hydroxytoluene (371.4 μM). In a transgenic *Caenorhabditis elegans* model, ellagic acid 4-O-xyloside decreased β-amyloid-induced neurotoxicity by enhancing their survival rate by 28.3% as compared to the control group.	Rose *et al*. (2018)
Fruit	Anti-aging	Anti-cholinesterase activity was demonstrated by the fruit polyphenols of *P. emblica*, which scavenged free radicals and inhibited BuChE (IC_{50} 0.0542 ± 0.0054 mg/mL) and AChE (IC_{50} 0.2186 ±	Wu *et al*. (2022)

		0.0416 mg/mL) *in vitro*. The fruit of *P. emblica* exhibited a significant protective effect against the aging process in a *Caenorhabditis elegans* model, as evidenced by an increase in thermal resistance, an 18.53 percent increase in lifespan ($p < 0.05$), and a decrease in the activity of BuChE and AChE by 45.38% and 34.71% ($p < 0.01$), respectively. Alongside this, the levels of MDA decreased by 36.25% ($p < 0.05$), whereas the activities of the antioxidant enzymes SOD and CAT increased by 30.74% ($p > 0.05$) and 8.42% ($p > 0.05$), respectively.	
Fruit	Anti-inflammatory	Fisetin and gallic acid contained in *P. emblica* ethanol extract have the best anti-inflammatory potentials. They significantly reduced the levels of proinflammatory cytokines and nitric oxide in LPS-stimulated macrophages. Fisetin from *P. emblica* was the first to show significant anti-inflammatory effects.	Li *et al*. (2020)
Seed coat	Antibacterial & Antifungal	It has been found that *P. emblica* extract exhibited antifungal activity against *Fusarium oxysporum* and antibacterial activity against *Pseudomonas aeuroginosa*, *Burkholderia* spp. and *Klebsiella* spp.	Kaur *et al*. (2021)

2.2 Types of Antioxidants

Antioxidants are a man-made or natural molecules that scavenge free radicals and able to break off chain reaction that can cause damage to vital molecules (Sharma *et al*., 2018). In living things, antioxidants are responsible to prevent or delay excessive free radicals from damaging many biological molecules which can be harmful to the body (Surai *et al*., 2019). Numerous antioxidants (endogenous) are continually synthesized by living cells to maintain a low concentration of free radicals within the

body. Antioxidants can also be supplied externally (exogenous), through diet (Halliwell, 2011).

Enzymatic antioxidants and non-enzymatic antioxidants comprise the two categories of endogenous antioxidants in the body (Ifeanyi, 2018). Catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GRx) are some of the examples of antioxidant enzymes. These primary enzymes are directly involved in neutralising free radicals. Furthermore, non-enzymatic antioxidants can be divided into (i) nutrient antioxidants and (ii) metabolic antioxidants. Metabolic antioxidants (melatonin, uric acid, transferrin, bilirubin, etc.) are endogenous antioxidants which is produced by the metabolism in the body. Nutrient antioxidants including vitamin C, vitamin E, flavonoids, and carotenoids, can often be acquired exogenously via dietary sources or dietary supplements (Pham-Huy, 2008).

Synthetic antioxidants, produced through chemical processes, can induce both advantageous and detrimental biological responses in cells, organs, and molecules (Gulcin, 2020). Synthetic antioxidants are divided into two groups which is (i) primary and (ii) secondary synthetic antioxidants. Primary antioxidants involved in oxidation process by inhibiting the production of free radicals while secondary antioxidants involved in lipid oxidation process by breaking down hydroperoxides into constant and compound (Kahl, 1984; Bose *et al*., 2012; Bellés *et al*., 2019).

Antioxidants deliver its functions in many ways. As an example, catalase enhances the endogenous antioxidant defence by catalysing the breakdown of H_2O_2 into oxygen and water, a by-product of numerous normal metabolic processes (Schwentker *et al*., 2002). Next, glutathione inhibits the formation of radicals and is vital for maintaining the redox state of cells (Vance *et al*., 2013).

2.2.1 Classification of Free Radicals

Free radicals are chemical species that contain one or more unpaired electrons which makes it highly reactive, and it can exist independently. Free radicals can continuously be produced as a by-product of normal cellular metabolism (ATP production by mitochondria) whenever the body requires oxygen to produce energy (Pham-Huy, 2008). Free radicals can also be produced through environmental pollutants, pesticides, cigarette smoke, radiation, and others (Sharma *et al*., 2018).

Free radicals can be grouped as (i) reactive oxygen species (ROS), (ii) reactive nitrogen species (RNS) and (iii) miscellaneous species. ROS and RNS play a dual role as both beneficial and toxic compounds depending on their concentration levels (Valko *et al*., 2007). Low to moderate level of ROS and RNS produce beneficial roles on immune function and cellular response while at high concentration levels, they may cause deleterious effects (oxidative stress) that may damage all cell structures (Ifeanyi, 2018).

ROS may include superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$) and peroxyl radical ($ROO^{\bullet}$) (Droge, 2002; Van den Ende *et al*., 2011). Furthermore, RNS consist of peroxynitrite anion (ONOO) and nitric oxide ($NO^{\bullet}$) (Victor *et al*., 2004) while miscellaneous species consist of halogen ($X^{\bullet}$), hydrogen ($H^{\bullet}$), glutathiyl ($GS^{\bullet}$), methyl ($^{\bullet}CH3$), ferryl ions ($FeOO2^{\bullet 2+}$), and carbon trichloride ($^{\bullet}CCl_3$) (Sharma *et al*., 2018).

2.2.2 Plant as Antioxidant Agents

Plants have produced a vast range of active compounds, many of which have antioxidant potential (Kasote *et al.*, 2015). Some of the common plant used as a source of antioxidant are shown in Figure 2.4. The ability of plants to produce non-enzymatic

antioxidants is an innate trait. Meanwhile, plants experience an increase in reactive oxygen species (ROS) production in response to biotic and abiotic stressors, which induces oxidative stress. Plants enhance the synthesis and accumulation of various low molecular weight antioxidants (e.g., phenolic acids, vitamin C, and vitamin E) and high molecular weight antioxidant secondary metabolites (e.g., tannins) in response to heightened oxidative stress. According to the in vitro studies, most plants possess antioxidant properties through their ability to scavenge free radicals, act as reducing agents, and chelate metals (Kasote *et al*., 2015). A comprehensive overview of the generation of free radicals is demonstrated in Figure 2.5. Natural antioxidants produced by plants are necessary for plant to function normally, to adapt to their continuously changing environment and provide therapeutic properties for human health (Maury *et al*., 2020). There are three main classes of natural antioxidants from plants which include carotenoids, phenolic compounds and vitamins (Lourenço *et al*., 2019).

S. No.	Name of plants	Common English name	Family	Plant part used
1.	*Aegle marmelos*	Bengal quince	Rutaceae	Fruit pulp
2.	*Allium cepa*	Onion	Amaryllidaceae	Bulb
3.	*Aloe vera*	Indian aloe	Xanthorrhoeaceae	Leaf
4.	*Asparagus racemosus*	Satavar	Liliaceae	Shoot
5.	*Azadirachta indica*	Neem	Meliaceae	Leaf
6.	*Bacopa monniera*	Brahmi	Plantaginaceae	Leaf
7.	*Beta vulgaris*	Beet root	Amaranthaceae	Root
8.	*Camellia sinensis*	Green tea	Theaceae	Green tea
9.	*Cinnamomum tamala*	Tejpat	Lauraceae	Tejpat
10.	*Curcuma longa*	Turmeric	Zingiberaceae	Turmeric
11.	*Cuscuta reflexa*	Akashabela	Convolvulaceae	Stem
12.	*Daucus carota*	Carrot	Apiaceae	Root
13.	*Emblica officinalis*	Amla/Emblic	Euphorbiaceae	Fruit
14.	*Eucalyptus camaldulensis*	River red gum	Myrtaceae	Leaf
15.	*Foeniculum vulgare*	Saunf	Apiaceae	Fruit oil
16.	*Lavandula angustifolia*	Lavender	Lamiaceae	Aerial parts
17.	*Mangifera indica*	Aam	Anacardiaceae	Root
18.	*Murraya koenigii*	Curry tree	Rutaceae	Leaf
19.	*Ocimum sanctum*	Tulsi	Lamiaceae	Leaf
20.	*Phaseolus vulgaris*	Common bean	Fabaceae	Fruit
21.	*Piper nigrum*	Black pepper	Piperaceae	Fruit
22.	*Plantago asiatica*	Chinese plantain	Plantaginaceae	Seed
23.	*Prunus domestica*	Plums	Rosaceae	Fruit
24.	*Salvia officinalis*	Common sage	Lamiaceae	Aerial parts
25.	*Santalum album*	Sandalwood	Santalaceae	Heartwood, bark
26.	*Solanum nigrum*	Black nightshade	Solanaceae	Leaf
27.	*Solanum tuberosum*	Potato	Solanaceae	Tuber
28.	*Terminalia bellarica*	Behda	Combretaceae	Fruit
29.	*Withania somnifera*	Ashwagandha	Solanaceae	Root, leaf and seed
30.	*Zingiber officinale*	Ginger	Zingiberaceae	Rhizome

Figure 2.4: Common plants used as a potential source of antioxidants (Adapted from Sindhi *et al*., 2013).

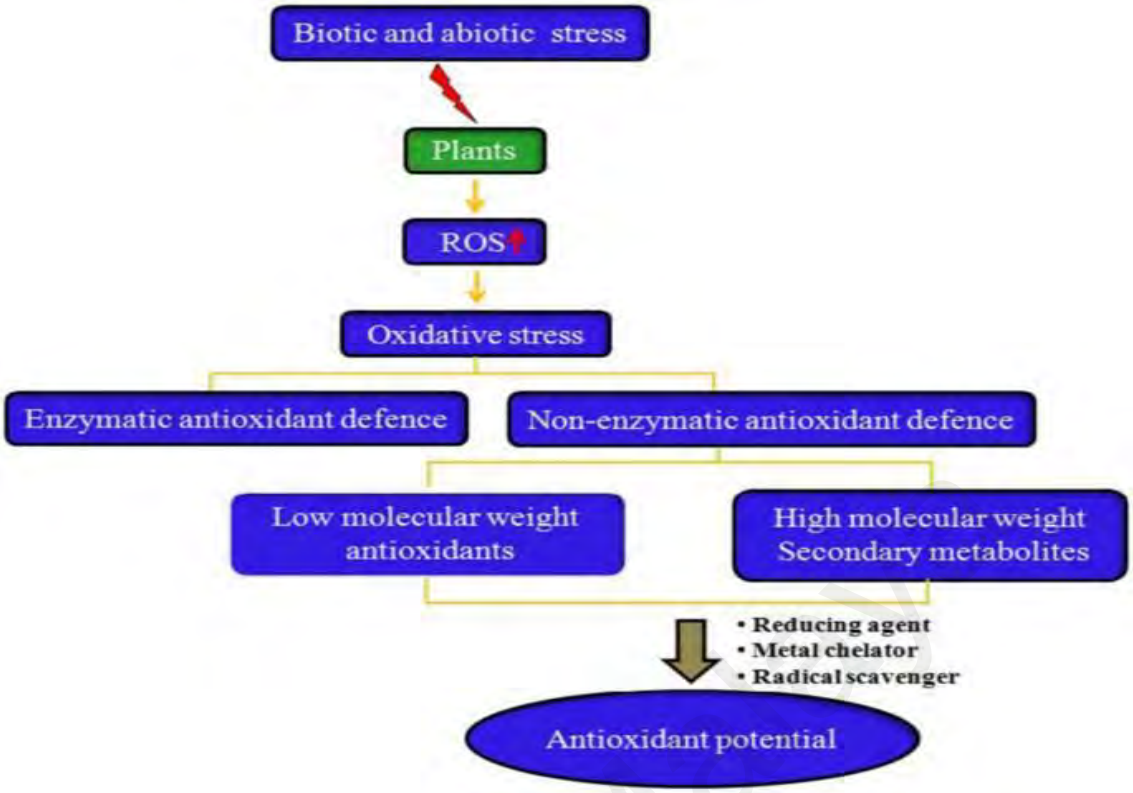

Figure 2.5: The overall process of free radicals' generation (Adapted from Kasote *et al.,* 2015).

Phenolic compounds demonstrate an extensive variety of structural variations, spanning from basic molecules such as vanillin, ferulic acid, and gallic acid to complex polyphenols including flavonoids and tannins. Phenolic compounds, in addition to being the primary plant compounds known for their antioxidant properties, also demonstrate antimicrobial and antifungal activity. Moreover, they exert significant influence over the flavours and textures of food products (Lourenço *et al*., 2019).

Vitamin C is one of the most essential antioxidant-potent vitamins found in plants. Vitamin C, which can be found naturally in most vegetables and fruits is water soluble. Another vitamin that has antioxidant properties in plant is Vitamin E. Vitamin E can be found in cereal grains and legumes. This lipid soluble vitamin comprises of eight chemical compounds (four tocotrienols (α, β, γ, and δ) and four tocopherols (α, β, γ, and δ)). However, the human body can only absorb α-tocopherol (Boschin & Arnoldi, 2011; Hussain *et al*., 2012; Lourenço *et al*., 2019). A diacetyl alcohol ring in vitamin E

contains hydroxyl hydrogen, which has a strong reducing effect on oxygen free radicals and efficiently prevents lipid peroxidation. As a result, vitamin E can lower oxidative stress, inhibit the formation of free radicals, and prevent the aging process from causing cognitive deterioration (Cui *et al*., 2020).

Carotenoids are mostly found in plant pigments. Main carotenoids with antioxidant capacities are lutein, lycopene, α-carotene and β-carotene (Omoni & Aluko, 2005; Lourenço *et al*., 2019). Their chemical structure made of a lot of conjugated double bonds. Therefore, the structure may be enriched with electrons while fighting free radicals and maintaining high chemical stability. Carotenoids also able to reduce lipid peroxidation, which can effectively delay the aging of the brain. Some of the examples of carotenoids in plants are lycopene, crocin and astaxanthin. The most effective antioxidant among flavonoids which is astaxanthin may be found in plants leaves and fruits (Cui *et al*., 2020).

The escalation of oxidative stress has been identified as a significant contributor in the development and advancement of critical ailments, including neurological and cardiovascular diseases (Kasote *et al.,* 2015). Given the diverse biological effects exhibited by natural antioxidants, including anti-aging, anticancer, anti-inflammatory, and anti-atherosclerosis properties (Xu *et al*., 2017), more studies should be focused on the antioxidant potential of plants.

2.2.3 Antioxidant Assays

Medicinal plant contains phytochemical compounds that possess antioxidant properties which can eliminate free radicals. To estimate the antioxidant potential of the compounds, few methods have been developed. These methods are typically used to measure the antioxidant capacity to chelate metal ions, to scavenge specific radicals or

hinder lipid peroxidation (Martínez *et al.,* 2012; Mahmud *et al*., 2019). Antioxidant activity assessment methods have been categorized into two groups which are single electron transfer (SET) reaction-based and hydrogen atom transfer (HAT) methods. Methods based on SET quantify the antioxidant potential of an antioxidant to reduce any compound which include free radicals, metals, and carbonyls while HAT-based methods quantify an antioxidant's ability to scavenge free radicals through the formation of stable compounds via hydrogen donation. (Kasote *et al*., 2015). ABTS and DPPH involve both SET and HAT mechanisms while FRAP assay only involve SET mechanism.

Due to its affordability and convenience, DPPH is one of the most extensively used methods for determining the antioxidant activities of plant samples. The DPPH assay quantifies the antioxidants present in the extract's ability to eliminate DPPH free radicals. The higher the antioxidant activity of the extract, the higher the percentage inhibition of DPPH free radicals.

ABTS radical scavenging assay is also one of the most widely used antioxidant assays for plant samples. The ABTS assay has been used to assess the antioxidant capacity of lipophilic and hydrophilic antioxidants. ABTS is a colourless compound which will change to blue-green colour when oxidized. The ABTS radical cation is produced when potassium persulfate oxidizes ABTS (Cano *et al*., 2023). Then, the antioxidants will donate hydrogen to the ABTS radical cation to neutralize it. The change of colour intensity will be measured using spectrophotometer (Krishnaiah *et al*., 2011).

FRAP assay is a simple and inexpensive antioxidant assay which analyse the ability of antioxidant compounds to reduce ferric ions (Fe^{3+}) in the FRAP reagent to ferrous ions (Fe^{2+}) in a chemical reaction (Shah & Modi, 2015). The reduction of ferric ions can be seen through the change of colour from yellow to blue and can be measured

using spectrophotometer (Wojtunik-Kulesza, 2020). An increased in absorbance indicates stronger reducing power.

2.3 Phytochemical and Its Benefits

Chemical compounds that are naturally produced by plants are called phytochemical. Phytochemicals provide plants their colour, flavour, and scent as well as a defence against predators and disease. Most of the phytochemicals are antioxidants which protect the cells from oxidative damage from the environment. Geographic distribution, species, age, part of the plant, cultivation method, harvesting season, and preservation method all have significant effects on the quantity and content of phytochemicals (Zhang *et al*., 2019).

Only a small fraction of the tens of thousands of phytochemicals found in plants have been extracted and identified (Singh & Chaudhuri, 2018; Xiao & Bai, 2019). Based on their chemical compositions and properties, phytochemicals are divided into six broad categories. These groups include phenolics, terpenoids, alkaloids, and other nitrogen-containing compounds as well as carbohydrates and lipids (Huang *et al*., 2016). Alkaloids, tannins, phlobatannins, steroids, saponins, cardiac glycosides, flavonoids, anthraquinones, terpenoids, and reducing sugars are some of the essential phytochemicals that can be found in therapeutic plants (Talema, 2020).

Phytochemical hold an enormous beneficial health effect on human beings. It appears to offer protection from a range of diseases by reducing oxidative stress. The average person consumes more than 1 g of phytochemicals every day since food and beverages such as fruit, nuts, vegetables, cereals, chocolate, tea, and juice contain phytochemicals (Zhang *et al*., 2019). Consuming plant-based foods that contain various phytochemicals helps the vascular endothelium to function better, reducing the risk of

diabetes and cardiovascular disorders such as high blood pressure. Phytochemicals also reduce the risk of obesity, cancer, and Alzheimer disease (Guan *et al*., 2021).

2.3.1 Phenolic Compound as Free Radical Scavenger

Phenolic compound is the largest category of plant secondary metabolite. Phenolic compounds are crucial to the development and defence of plants. It consists of a hydroxyl group that is directly bonded to an aromatic hydrocarbon group. Phenolic compounds fall into several groups, including stilbenes, flavonoids, phenolic acids, tannins, lignin, and lignans (Forni *et al*., 2019).

Flavonoids are the largest and most extensively researched phenols. Flavonoids is a widespread polyphenolic substance that appear in nature as glucosides, aglycones, and methylated derivatives. Its basic structure consists of a C6-C3-C6 carbon skeleton which comprise of two 6-carbon benzene rings that are connected by 3-carbon heterocyclic ring. There are more than 4,000 flavonoids have been identified and it can be classified into 12 subgroups which include flavones, isoflavones, flavanones, flavanols, dihydroflavonols, phlobaphenes, leucoanthocyanidins, proanthocyanidins, chalcones, aurones, anthocyanins, and stilbenes (Liu *et al*., 2021).

Flavonoids are mainly found in fruits, vegetables and beverages like coffee, tea, and fruit drinks. Recent research has focused on flavonoids because of their wide pharmacological and biological benefits. Almost every group of flavonoids is best known for their powerful antioxidant properties which provide the body with protection against reactive oxygen species and free radicals. Other biological effects of flavonoids have also been documented which include anti-inflammatory, anti-tumour, anti-microbial, and cytotoxic activities (Koche *et al*., 2018).

Phenolic acids refer to a phenolic compound that have one carboxylic acid group. They are mainly found in plants and human metabolites (Chen *et al*., 2020). Phenolic acids are classified as either hydroxycinnamic or hydroxybenzoic acids (Koche *et al*., 2018). Derivatives of cinnamic acid, hydroxycinnamic acids are frequently found in foods as simple esters with quinic acid or glucose. Caffeic, ferulic, sinapic and p-coumaric acids are some of the most common hydroxycinnamic acids. Hydroxybenzoic acids which derived from benzoic acid have a common structure of C6-C1. In onions, black radishes, and scarlet fruits, they are detected in soluble form and at low concentrations. Vanillic, p-hydroxybenzoic, syringic and protocatechuic acids are common examples of hydroxybenzoic acids (Kumar & Goel, 2019). Phenolic acids are thought to prevent excessive free radical from damaging the body thus preventing chronic diseases since they possess strong antioxidant potential (Chen *et al*., 2020).

2.3.2 Vitamin C as Free Radical Scavenger

Ascorbic acid, which is generally known as vitamin C is water soluble and has a molecular formula of $C_6H_8O_6$. It is an organic compound which commonly found in plants and possess great antioxidant potential. Bats, passeriform birds, guinea pigs and primates, including humans, are unable to synthesize vitamin C due to lack of L-gulono-1,4-lactone oxidase. Therefore, they are totally reliant on dietary vitamin C consumption. Some of the good sources of vitamin C include citrus fruit, Kakadu plum, broccoli, kiwi, strawberry, kale, star fruit, and guava (Ballaz & Rebec, 2019).

Three main functions of vitamin C in plants include a radical scavenger, an enzyme cofactor and an acceptor/donor in electron transport either in the chloroplast or plasma membrane (Davey *et al*., 2000). Vitamin C can be easily dehydrogenated and oxidized which makes it a very effective antioxidant and scavenger. This occurs due to

the chemical structure containing two enol hydroxyl groups (Cui *et al*., 2020). Vitamin C is essential in protecting cells from oxidative stress and preventing cell damage by neutralizing harmful free radical. Furthermore, apart from its antioxidant capacity, vitamin C exhibits various therapeutic attributes, including neuroprotective, antimicrobial, and anticancer properties (Ballaz & Rebec, 2019).

CHAPTER 3: METHODOLOGY

3.1 Preparation of *Phyllanthus* Fruit Extracts

3.1.1 *Phyllanthus* Fruits Collection and Identification

The fresh fruits of *Phyllanthus pectinatus* had been collected from Rimba Ilmu Botanical Garden during the fruiting season. The size of the *Phyllanthus pectinatus* fruits used in this study ranged from 2 cm to 2.5 cm while the size of *Phyllanthus emblica* fruits used ranged from 4 cm to 4.5 cm. Authentication of *P. pectinatus* (KLU47659) was carried out in the herbarium of the Rimba Ilmu Botanical Garden, Institute of Biological Sciences, Universiti Malaya by Dr. Sugumaran Manickam.

The fresh fruits of *Phyllanthus emblica* that was imported from India had been purchased from the grocery store in Puchong Jaya, Selangor. Preparation of the voucher specimen for *P. emblica* for further identification and authentication of the species is not conducted in this study. Voucher specimen preparation required that the plant material collected should be fertile and include all parts of the plant (fruits, flowers, and buds, as well as bark, leaves, juvenile or coppice foliage). *P. emblica* in this study is lacking with all the plant parts and only fruits were available. Incomplete specimens cannot be used and accepted for voucher specimen preparation.

3.1.2 Methanol Extraction of *Phyllanthus* Fruits

The fruits from *P. pectinatus* and *P. emblica* were pre-selected for the absence of visible infection or injury and the size and colour uniformity. After rinsing the fruits under flowing tap water, they were air-dried at room temperature. After removing the seeds, the fruits were subjected to a freeze-drying process. Next, the freeze-dried fruits

were ground to powder using dry blender and the grounded powder were kept at room temperature in a tightly sealed container until further extraction.

The powdered fruits of *P. pectinatus* and *P. emblica* was subjected to a maceration method described by Sapkota *et al*. (2022) with little modifications in which methanol was added in a ratio of 1:10 for a duration of 72 hours at room temperature. Following this, the extract-containing solvent was filtered via Whatman No. 1 filter paper (Whatman, England). The methanol filtrate was gathered, and any residual solvent was extracted by evaporating it under reduced pressure using a rotary evaporator (Buchi, Switzerland) at a temperature of 45 °C until completely dried. The yield for both *P. pectinatus* and *P. emblica* extracts were calculated. The dark-greenish methanol extracts of *P. pectinatus* and *P. emblica* were stored in closed glass container at 4 °C in refrigerator for further biological study. The percent yield of both extracts was calculated using following formula:

Yield (%): [(Weight of dried extract) / (Weight of dried sample)] × 100

3.2 Qualitative Analysis of Phytochemicals

The phytochemical screening for the main constituents of the methanol extract of *P. pectinatus* (PPF) and *P. emblica* (PEF) fruits was conducted with minor modifications to the methods described by Solihah *et al.* (2012).

3.2.1 Test for Phenols

A solution was prepared by adding 2 mL of each methanol extract of PPF and PEF to water and heating it between 45 and 50 °C. After that, 2 mL of 3% $FeCl_3$ (R &

M Chemicals, UK) was added into the solution containing the extract. Formation of green or blue colour indicated the presence of phenols.

3.2.2 Test for Flavonoids (I)

A solution was prepared by adding 1 mL of 10% lead (II) acetate (R & M Chemicals, UK) to 1 mL of each methanol extract of PPF and PEF. The mixture was then gently agitated. When a muddy brown precipitate formed, it was indicative of flavonoid presence.

3.2.3 Test for Flavonoids (II)

10% $FeCl_3$ was diluted with 1 mL of each methanol extract of PPF and PEF (R & M Chemicals, UK). The mixture was shaken. Precipitate with a woolly brown colour was indicative of flavonoid presence.

3.2.4 Test for Tannins

Methanol extracts of PPF and PEF, each with a volume of 1 mL, were combined with 1 mL of 3% $FeCl_3$ (R & M Chemicals, UK). A precipitate with a greenish-black colour indicated the existence of tannins.

3.2.5 Test for Phlobatannins

A volume of 2 mL of 1% hydrochloric acid was combined with 1 mL of each methanol extract of PPF and PEF (R & M Chemicals, UK) prior to boiling. A red precipitate formation indicated the existence of phlobatannins.

3.2.6 Test for Alkaloids

A 15-minute filtration was performed after a volume of 1 mL of each methanol extract of PPF and PEF was mixed with 5 mL of 1% hydrochloric acid (R & M Chemicals, UK) in a steam chamber set at 60 °C.

3.2.6.1 Test for Alkaloids (I)

1 mL of Dragendorff reagent was mixed into 1 mL of PPF and PEF filtrate. The detection of alkaloids appeared as a cloudy orange colour.

3.2.6.2 Test for Alkaloids (II)

1 mL of Mayer reagent was added into 1 mL of PPF and PEF filtrate. The presence of alkaloids was indicated by the appearance of a pale-yellow colour.

3.2.6.3 Test for Alkaloids (III)

1 mL of Wagner reagent was added into 1 mL of PPF and PEF filtrate. A turbid brown colour forming was served as an indication of the presence of alkaloids.

3.2.7 Test for Terpenoids

A mixture was prepared by combining 5 mL of each methanol extract of PPF and PEF with 2 mL of chloroform (Fisher Scientific, UK). Following this, 3 mL of sulphuric acid (R & M Chemicals, UK) in concentrated form was added with caution. The visible reddish-brown colour that formed between the upper and lower layers was indicative of the presence of terpenoids.

3.2.8 Test for Saponins

A mixture of 5 mL distilled water and approximately 0.2 mL of each methanol extract of PPF and PEF was prepared. It was vigorously agitated for five minutes. For saponins, the persistence of foams appearance served as an indicator.

3.2.9 Test for Sterols (Salkowski's Test)

2 mL of concentrated H_2SO_4 (R & M Chemicals, UK) were added into 2 mL of methanol extracts of PPF and PEF, respectively. Formation of a red precipitate suggested the existence of one or more steroidal ring compounds in the sample.

3.3 Quantification of Phytochemical Content

3.3.1 Total Phenolic Content

The methanol extracts of PPF and PEF were subjected to total phenolic content (TPC) analysis using Folin-Ciocalteu's method Sun *et al.* (2007) with little modifications. Ten minutes were elapsed after a volume of 10 µL of PPF and PEF extracts (10 mg/mL) was combined with 75 µL of 10% Folin-Ciocalteu reagent. 75 µL of a solution containing 2% sodium carbonate (Na_2CO_3) was subsequently added to the mixture. Using a microplate reader, the absorbance at 765 nm was determined following a 45-minute incubation at room temperature in the dark. In accordance with the gallic acid standard curve, the TPC was calculated in milligrams of gallic acid equivalents per gram of dried weight (mg GAE/g DW) of extract. The investigation was conducted with three replicates.

3.3.2 Total Flavonoid Content

The methanol extracts of PPF and PEF were subjected to an aluminium chloride colorimetric method described by Yusof *et al.* (2018) with minor modification to determine their total flavonoid content. 6 µL of 1M sodium acetate ($NaC_2H_3O_2.3H_2O$), 6 µL of 10% aluminium chloride hexahydrate ($AlCl_3.6H_2O$), 90 µL of 70% methanol, and 168 µL of distilled water were mixed together with 30 µL of 10 mg/mL PPF and PEF extracts. Following a 40-minute incubation period, the absorbance at 415 nm was quantified using a microplate reader. The total flavonoid content was expressed as quercetin equivalents in milligrams per gram of dried weight (mg QE/g DW) of extract, using a standard curve of quercetin. The investigation was conducted with three replicates.

3.3.3 Ascorbic Acid Content

The ascorbic acid content in both fresh fruits of *P. pectinatus* and *P. emblica* were determined using the 2,6-dichlorophenol-indophenol (DCPIP) method described by Shivembe & Ojinnaka (2017) with little modifications. A DCPIP solution (0.045%) was prepared using distilled water. Then, 0.2 mg/mL solution was prepared by dissolving 10 mg of L-ascorbic acid in 50 mL of 20% trichloroacetic acid (TCA). The 0.045% DCPIP solution was then standardised with a known concentration of ascorbic acid. Next, 2 mL of the fresh fruit's solution was titrated with a DCPIP solution to a pink end point. The ascorbic acid content was calculated using the following equation:

$$\text{Mass} = \text{MR (Ascorbic acid)} \times \text{C (DCPIP)} \times \text{V (DCPIP)}$$

Where MR is molar mass, C is concentration and V is volume. The experiment was performed in triplicate.

3.4 Antioxidant Properties of *Phyllanthus* Extracts

3.4.1 DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Activity Assay

The DPPH assay was performed on the methanol extracts of PPF and PEF, adhering slightly to the procedure outlined by Navanesan *et al.* (2015). A 96-well plate was prepared by adding 50 µL of PPF and PEF extracts (15.63–500.00 µg/mL) into each well. Each well contained 150 µL of a 0.3 mM DPPH solution. The mixture was then incubated for 30 minutes at room temperature in the dark. To prepare the untreated control, 50 µL of methanol was added to 150 µL of DPPH solution. Using a microplate reader, the absorbance of the reaction mixture in each well was determined at 515 nm. Standard ascorbic acid was used. Using the subsequent equation, the percentage of DPPH radical scavenging activity of PPF and PEF extracts was calculated:

$$\text{DPPH scavenging activity (\%)} = ((A_0 - A_1)/A_0 \times 100$$

Where A_0 is the absorbance of the control, A_1 is the absorbance of sample extract or standard. The assay was carried out with three replicates.

3.4.2 ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) Radical Scavenging Activity Assay

The ABTS scavenging assay was conducted on the methanol extracts of PPF and PEF, following the methodology described by Chatatikun & Chiabchalard (2017) with minor adjustments. To prepare $ABTS^{+\bullet}$ solution, 2.45 mM potassium peroxodisulfate ($K_2S_2O_8$) was mixed with 7 mM $ABTS^{\bullet}$ and the solution was kept at room temperature for a duration of 16 to 18 hours in the dark. The absorbance of $ABTS^{+\bullet}$ solution was adjusted to 0.70 ± 0.02 at 734 nm by diluting it with methanol. Next, a volume of 20 µL extract of PPF and PEF (15.63-500.00 µg/mL) was added to 180 µL of $ABTS^{+\bullet}$ solution and the mixture was incubated for 20 minutes in the dark.

The untreated control was prepared by adding 20 µL methanol into 180 µL $ABTS^{+\bullet}$ solution. Using the microplate reader, the absorbance was measured at 734 nm. The positive control used in this assay was ascorbic acid. The equation below was used to determine the scavenging activity of PPF and PEF extract:

$$ABTS^{+\bullet} \text{ scavenging activity } (\%) = ((A_0 - A_1)/A_0 \times 100$$

Where A_0 is the absorbance of the control, A_1 is the absorbance of sample extract or standard. The assay was carried out with three replicates.

3.4.3 FRAP (Ferric Reducing Antioxidant Power) Assay

The FRAP assay was carried out on the methanol extracts of PPF and PEF, adhering to the standard protocol with only minimal adjustments (Benzie & Strain, 1999). In 10:1:1 ratio, 10 mL of 300 mM of acetate buffer (pH 3.6), 1 mL of 20 mM iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) and 1 mL of 10 mM 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ) solution were mixed to freshly prepare the FRAP reagent during the assay. Next, the mixture was incubated in the water bath for 30 minutes at 37ºC. Then, a volume of 20 µL of PPF and PEF was mixed with 180 µL of FRAP reagent and the mixture was incubated for another 30 minutes in the dark at room temperature. A volume of 20 µL of methanol was added into FRAP reagent to replace *Phyllanthus* extract and used as untreated control. A microplate reader was used to measure the absorbance reading at 593 nm. Positive control for this assay was ascorbic acid while ferrous sulphate ($FeSO_4$) solution was used as standard. FRAP activity was expressed in milligrams of ferrous equivalent Fe (II) per gram of dried extract [mg Fe (II)/g]. The experiment was performed in triplicate.

3.5 Statistical Analysis

Three replicates for each measurement were taken in all experiments. The findings were summarized in the form of mean ± standard errors, and an analysis of variance (ANOVA) was used for the statistical analysis. The correlation between phenolic, flavonoid, and ascorbic acid contents with antioxidant activity of the extracts were determined using Pearson correlations test (IBM SPSS Statistic version 25).

CHAPTER 4: RESULT

4.1 Fruits Collection

In this study, two varieties of fruits derived from *Phyllanthus* species: *Phyllanthus pectinatus* and *Phyllanthus emblica* were used. The fruits of *P. pectinatus* were obtained from the Rimba Ilmu Botanic Garden in Universiti Malaya (Figure 4.1) while *P. emblica* were purchased from grocery store in Puchong Jaya. *P. emblica* fruits (Figure 4.2) were imported from India.

Figure 4.1: Fruits of *Phyllanthus pectinatus*.

Figure 4.2: Fruits of *Phyllanthus emblica*.

4.2 Yield of *Phyllanthus* Fruit Extracts

Methanol extraction was performed on dried powder of *P. pectinatus* and *P. emblica* fruits (20 g each) to obtain methanol extract of *P. pectinatus* fruits (PPF) and methanol extract of *P. emblica* fruits (PEF). The total yield of PPF and PEF extracts in methanol were calculated and presented in Table 4.1. PPF produced a slightly higher yield of methanol extract at 66.2 % as compared to PEF (60.7%).

Table 4.1: Total yield of *P. pectinatus* and *P. emblica* fruits methanol extracts.

***Phyllanthus* extract**	**Yield (%)**
PPF	66.2
PEF	60.7

4.3 Phytochemical Analysis

4.3.1 Phytochemical Screening

Qualitative screening of phytochemical compounds was performed on methanolic extracts of both PPF and PEF. The results presented in Table 4.2 show that both PPF and PEF extracts contained phenols, flavonoids, tannins, phlobatannins, terpenoids, and sterols. However, saponins was only detected in PPF extract and no alkaloids detected in both PPF and PEF methanolic extracts.

Table 4.2: Qualitative screening of phytochemical compounds in *P. pectinatus* and *P. emblica* fruit extracts.

Phytochemicals	Extracts	
	PPF	PEF
Phenols	+	+
Flavonoids (I)	+	+
Flavonoids (II)	+	+
Tannins	+	+
Phlobatannins	-	-
Alkaloids (Dragendorff reagent)	-	-
Alkaloids (Mayer reagent)	-	-
Alkaloids (Wagner reagent)	-	-
Terpenoids	+	+
Saponins	+	-
Sterols (Salkowski test)	+	+

+ Present – Not Present

4.3.2 Total Phenolic, Flavonoid and Ascorbic Acid Content in Methanol Extracts of *P. pectinatus* and *P. emblica* Fruits

The total phenolic content (TPC) and total flavonoid content (TFC) of the extracts were determined. The Folin-Ciocalteu method was used to determine the TPC in methanol extracts of PPF and PEF, with gallic acid serving as the standard. Meanwhile, the TFC of both extracts was determined through an aluminium chloride colorimetric assay, with quercetin serving as the standard.

As shown in Table 4.3, the TPC and TFC of PPF and PEF methanol extracts were calculated using the regression equation of the calibration curve of gallic acid (Figure 4.3) and quercetin (Figure 4.4), respectively. The units of measurement for TPC and TFC were mg gallic acid (GAE) and mg quercetin (QE), respectively, per g dry weight of the sample. Comparing both species, the value of TPC in methanol extract of

PPF is 1140 ± 1.41 mg GAE/g, which is slightly higher than the methanol extract of PEF with value of 1111 ± 4.45 mg GAE/g. Both PPF and PEF methanol extracts exhibited high TPC values. Similar to TPC, methanol extracts of PPF exhibited the highest flavonoid content, measuring 35.88 ± 3.17 mg QE/g.

The ascorbic acid content of both PPF and PEF was determined by DCPIP titration method. As shown in Table 4.3, PEF exhibited significantly higher amount of ascorbic acid compared to PPF with a value of 291.60 ± 0.00 mg/100g.

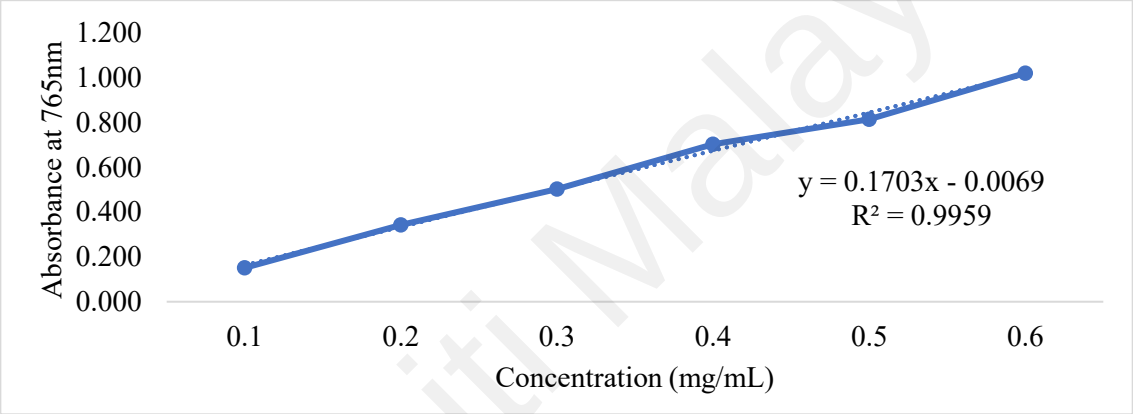

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Figure 4.3: Gallic acid standard curve.

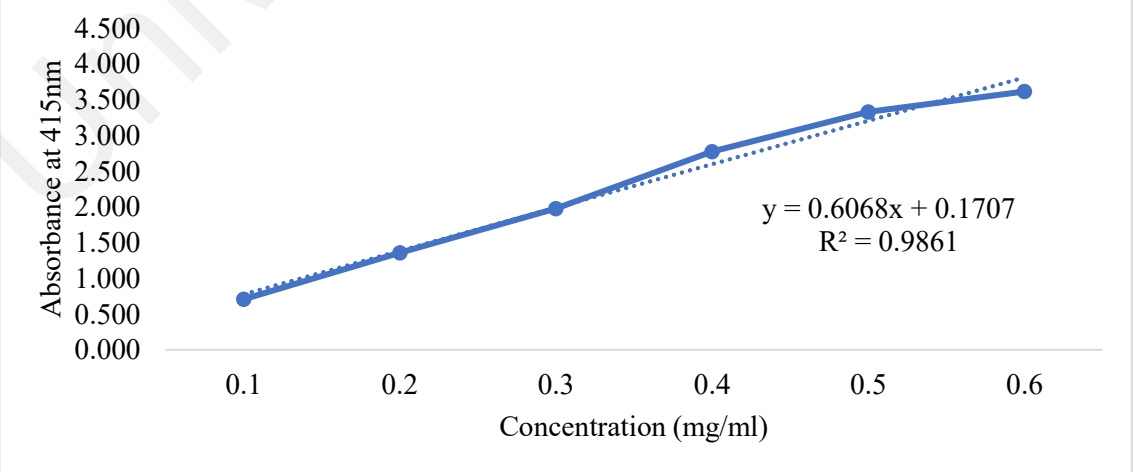

Figure 4.4: Quercetin standard curve.

Table 4.3: Total phenolic, flavonoid and ascorbic acid content in methanol extracts of PPF and PEF.

Extracts	Total Phenolic Content (mg GAE/g DW)	Total Flavonoid Content (mg QE/g DW)	Ascorbic Acid Content (mg/100 g)
PPF	1140 ± 1.41	35.88 ± 3.17	19.44 ± 0.00
PEF	1111 ± 4.45	5.95 ± 0.10	291.60 ± 0.00

Notes: The mean values ± standard error (SE) of three replicates are represented as the data.

4.4 Antioxidant Assay

4.4.1 1,1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity of *P. pectinatus* and *P. emblica* Fruit Extracts

In this study, both methanol extracts of PPF and PEF DPPH radical scavenging activity were measured. Figure 4.5 shows the percentage inhibition of DPPH free radical treated with different concentration of ascorbic acid which served as the positive control in this study. Figure 4.6 and 4.7 shows the percentage inhibition of DPPH free radical treated with different concentration of methanol extract of PPF and PEF. It is clearly shown that the concentration of the extracts positively correlates with the percentage inhibition of DPPH.

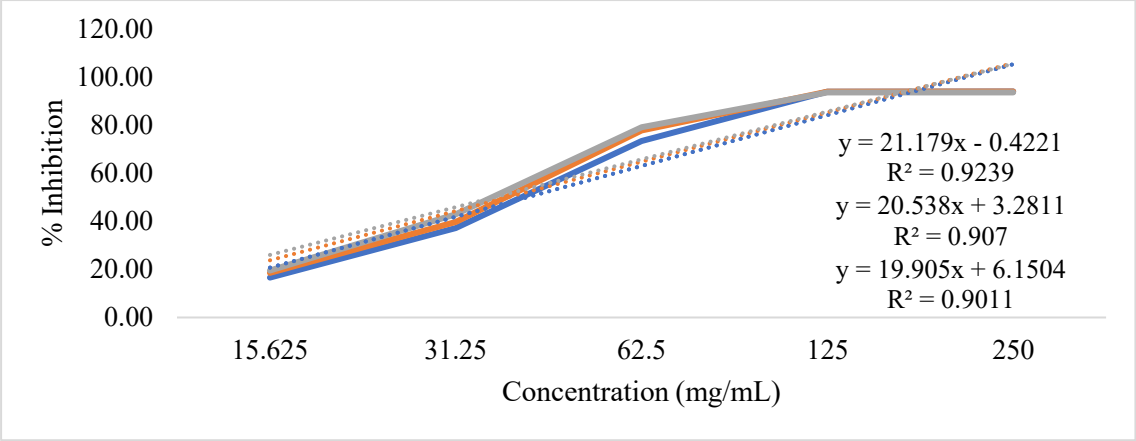

Figure 4.5: Percentage inhibition of DPPH free radical with different concentration of ascorbic acid.

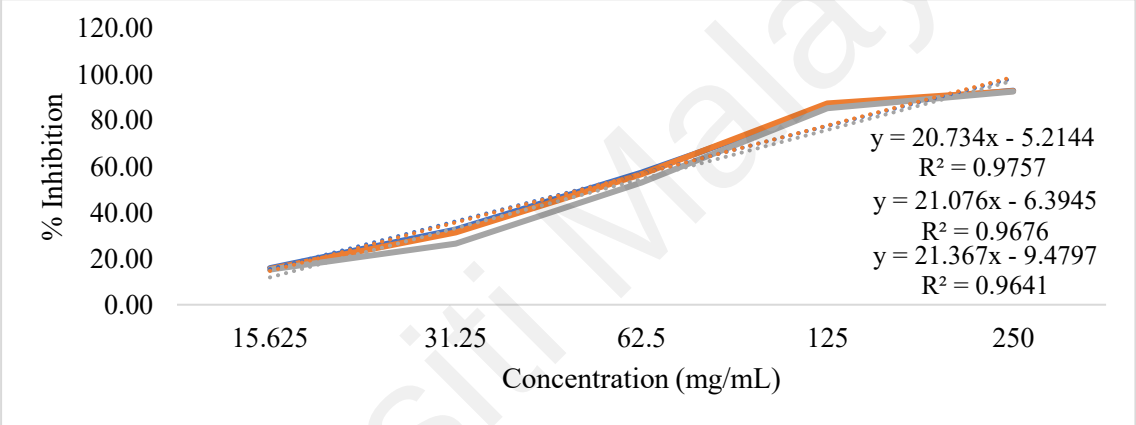

Figure 4.6: Percentage inhibition of DPPH free radical with different concentration of methanol extract of PPF.

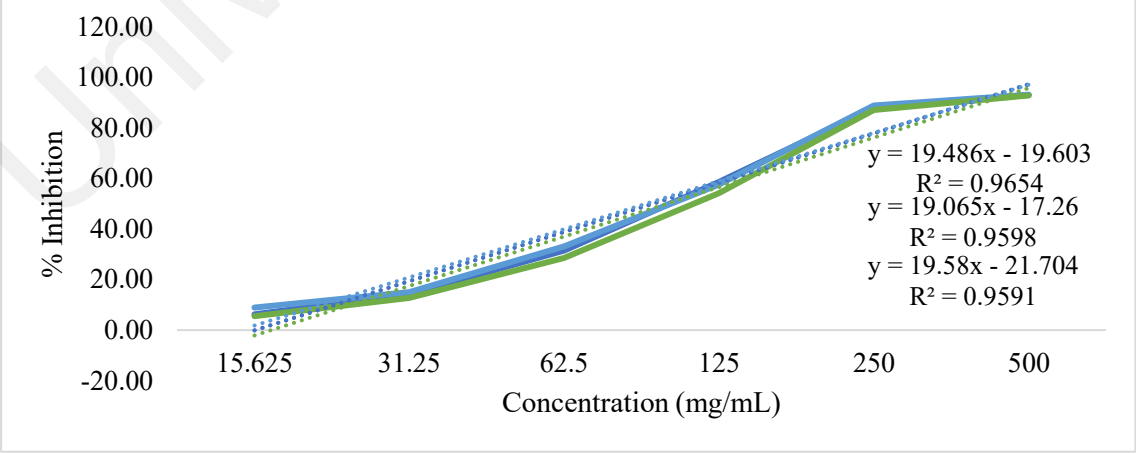

Figure 4.7: Percentage inhibition of DPPH free radical with different concentration of methanol extract of PEF.

The DPPH radical scavenging activity for methanol extracts of PPF and PEF is stated in IC_{50} value (concentration needed to inhibit 50% of DPPH radicals). The linear equation derived from the graph plot was used to calculate the IC_{50} value, which is summarized in Table 4.4.

Both methanol extracts of PPF and PEF exhibited excellent antioxidant activities depicted by their low IC_{50} value obtained from the assay. Methanol extract of PPF exhibited lower IC_{50} value of 2.71 ± 0.04 µg/mL than the methanol extract of PEF with IC_{50} value of 3.59 ± 0.04 µg/mL. The IC_{50} value obtained in this assay for ascorbic acid that serves as positive control is 2.29 ± 0.05 µg/mL.

Table 4.4: The IC_{50} of DPPH radical scavenging activity in ascorbic acid standard and methanol extracts of PPF and PEF.

Extracts	IC_{50} (µg/mL)
PPF	2.71 ± 0.04
PEF	3.59 ± 0.04
Ascorbic acid	2.29 ± 0.05

Notes: The mean values ± standard error (SE) of three replicates are represented as the data.

4.4.2 ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) Radical Scavenging Activity Assay

The present study carried out the ABTS assay to evaluate and compare the antioxidant scavenging capacity of PEF and PPF methanol extracts. Figure 4.8 shows the ABTS free radical percentage inhibition treated with various concentration of ascorbic acid, which served as the standard in this study. Figure 4.9 and 4.10 shows the percentage inhibition of ABTS free radical treated with different concentration of methanol extract of PPF and PEF. The ABTS radical scavenging activity for methanol

extracts of PPF and PEF is shown in IC_{50} value (concentration needed to inhibit 50% of ABTS radicals). The linear equation derived from the graph plot was used to calculate the IC_{50} value, which is summarized in Table 4.5.

The result reveals that the ABTS radical scavenging activity of PPF methanol extract with IC_{50} value of 2.62 ± 0.03 µg/mL was slightly more effective to that of PEF (2.83 ± 0.03 µg/mL). The IC_{50} value obtained in this assay for both PPF and PEF are low which indicates good antioxidant activity. The IC_{50} value obtained in this assay for ascorbic acid that serves as positive control is 2.13 ± 0.05 µg/mL.

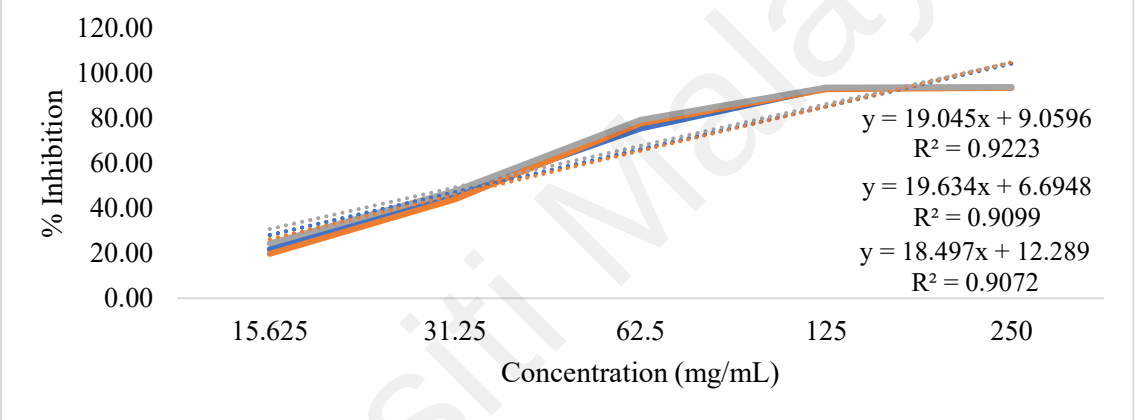

Figure 4.8: Percentage inhibition of ABTS free radical with different concentration of ascorbic acid.

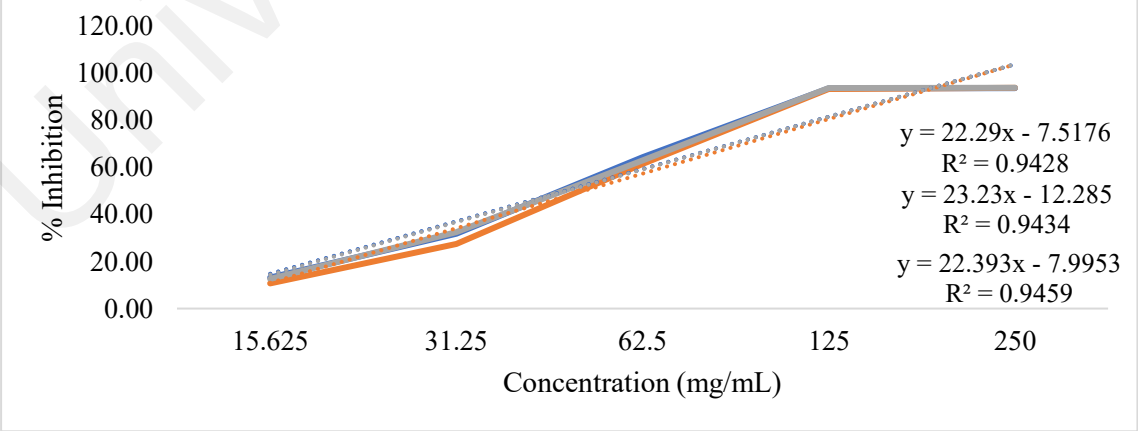

Figure 4.9: Percentage inhibition of ABTS free radical with different concentration of methanol extract of PPF.

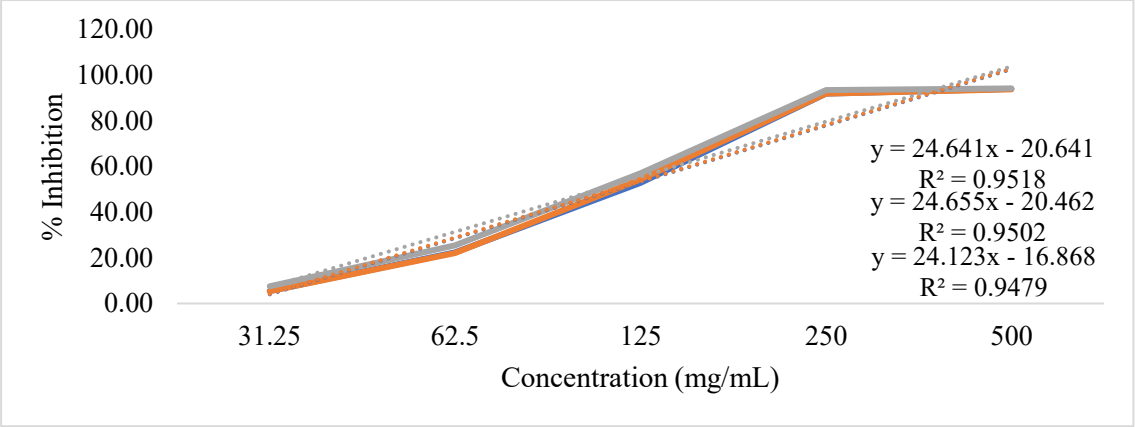

Figure 4.10: Percentage inhibition of ABTS free radical with different concentration of methanol extract of PEF.

Table 4.5: The IC_{50} of ABTS radical scavenging activity in methanol extracts of PPF, PEF and ascorbic acid standard.

Extracts	IC_{50} (µg/mL)
PPF	2.62 ± 0.03
PEF	2.83 ± 0.03
Ascorbic acid	2.13 ± 0.05

Notes: The mean values ± standard error (SE) of three replicates are represented as the data.

4.4.3 FRAP (Ferric Reducing Antioxidant Power) Assay

The reducing potential of PEF and PPF methanol extracts were measured using ferric reducing antioxidant power (FRAP) assay. $FeSO_4$ was used as the standard in this assay, whereas ascorbic acid served as the positive control. The absorbance readings measured at various concentrations of ferrous sulphate were used in the calibration curve's construction (Figure 4.11).

Table 4.6 demonstrates that the PPF methanol extract reduces FRAP more effectively than PEF with a value of 4.79 ± 0.05 mM Fe (II)/g. The FRAP reducing activity for ascorbic acid that serves as positive control is 1.67 ± 0.01 mM Fe (II)/g.

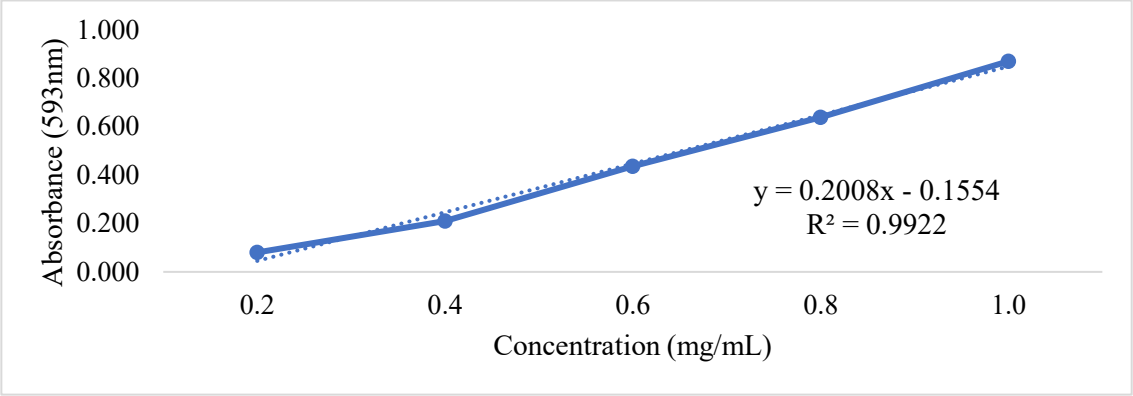

Figure 4.11: Standard calibration curve of FRAP assay.

Table 4.6: The FRAP activity in methanol extracts of PPF, PEF and ascorbic acid standard.

Extracts	(mM Fe (II)/g)
PPF	4.79 ± 0.05
PEF	3.22 ± 0.04
Ascorbic acid	1.67 ± 0.01

Notes: The mean values ± standard error (SE) of three replicates are represented as the data.

4.5 Correlation between TPC, TFC, AAC and Antioxidant Potential of *P. pectinatus* and *P. emblica* Fruit Extracts.

To understand the correlation between the antioxidant properties and phytochemical content, correlation analysis was conducted for both methanol extracts of PPF and PEF (Table 4.7). Strong negative correlations were observed between TPC and TFC of both extracts with DPPH and ABTS assays. This suggests that as TPC and TFC increase, the IC_{50} values of DPPH and ABTS will decrease. This relationship indicates that as TPC and TFC of both extracts increases, the DPPH and ABTS radical scavenging activity will increase proportionally. Even so, the correlation observed between TPC with ABTS was insignificant. Moreover, the FRAP reducing potential of the extracts were positive and highly correlated with TPC and TFC. This means that as

TPC and TFC of the extracts increase, the FRAP reducing activity will also increase. However, the correlation between AAC with all antioxidant assays in this study was in contrast with TPC and TFC.

Table 4.7: Pearson's correlation coefficients.

Parameters	TPC	TFC	AAC	DPPH	ABTS	FRAP
TPC	1					
TFC	0.918**	1				
AAC	-0.952**	-0.978**	1			
DPPH	-0.968**	-0.959**	0.992**	1		
ABTS	-0.809	-0.960**	0.925**	0.885*	1	
FRAP	0.942**	0.971**	-0.997**	-0.995**	-0.914*	1

** signifies that differences are statistically significant at $p < 0.01$; * signifies that differences are statistically significant at $p < 0.05$. Note: *TPC*: total phenolic content; *TFC*: total flavonoid content; *AAC*: ascorbic acid content; *DPPH*: 2,2-diphenyl-1-picrylhydrazyl assay; *ABTS*: 2,2-azino-bis(3-ethylbenzotiazoline-6-sulfonic acid); *FRAP*: ferric reducing antioxidant power.

CHAPTER 5: DISCUSSION

5.1 Phytochemical Extraction of *P. pectinatus* and *P. emblica* Fruits

In present study, the fruits of *P. pectinatus* and *P. emblica* were freeze dried before being subjected to methanol extraction. This is because freeze-drying method use low temperature and pressure to reduce the sample's moisture content. This method can help to prevent degradation and preserve phytochemical contents of the sample (Kumar *et al.,* 2022).

The types of solvent used in extraction and its polarity will influence the extraction efficiency (Taghizadeh *et al*., 2018). High polarity solvents including ethanol, methanol and water are frequently used in extraction to extract polar compounds in plant samples like phenolic and flavonoid. According to Idris *et al.* (2023), the most efficient solvent used for polar compound recovery in plant extract is methanol. Methanol is highly volatile which makes it easier to be evaporated after the extraction process. Methanol is also inexpensive, widely available, and versatile where it can be used to extract both hydrophobic and hydrophilic molecules (Osmic *et al*., 2018). Therefore, methanol solvent was used in this study to extract phytochemical compounds from both *P. pectinatus* and *P. emblica* fruits.

The percentage of extraction yield can be affected by the method of extraction, time of extraction, quantity of samples, solvent, pH, polarity, temperature used during extraction (Sharma & Singh, 2016). Liu *et al*. (2008) reported that the range of extraction yield of *P. emblica* from six various regions is from 21.0% to 39.4% which is lower than this study. High percentage of extraction yield from both *P. pectinatus* and *P. emblica* fruits in this study might be due to the freeze-drying method and methanol solvent used during extraction.

5.2 Phytochemical Screening

Qualitative phytochemical screening of both methanolic extracts of *P. pectinatus* and *P. emblica* fruits was carried out. Both *P. pectinatus* and *P. emblica* fruits extracts were found to contain phenols, flavonoids, tannins, phlobatannins, terpenoids, and sterols. However, saponins was only detected in *P. pectinatus* fruits extract and no alkaloids detected in both *P. pectinatus* and *P. emblica* fruits methanolic extracts.

P. emblica fruit extract was reported in Sumalatha (2013) to contain flavonoid, tannin, saponin, and alkaloids. Another study shows the presence of flavonoid, tannins, phenolics, saponins, alkaloid, glycosides and carbohydrates in methanolic *P. emblica* fruit extract (Sheoren *et al.,* 2019). This suggests that similar phytochemicals are present in *P. emblica* fruits regardless of the sample locations. However, the absence of saponin in *P. emblica* fruit and alkaloids in both *P. pectinatus* and *P. emblica* fruits extracts might be due to unfavourable environment that eventually affect the bioavailability of the phytochemicals in the fruits (Li *et al*., 2012).

5.3 Total Phenolic, Flavonoid and Ascorbic Acid Contents

The quantification of phenolic, flavonoid, and ascorbic acid content is crucial since chemical composition of the fruits significantly influence the plant biological activities.

The phenolic content (TPC) of the extracts is closely correlated to their antioxidant properties. This is because, phenolic compounds contain hydroxyl group which are responsible to scavenge free radicals (Wojdylo *et al*, 2007). Both *P. pectinatus* and *P. emblica* fruits methanol extracts exhibited high TPC values. However, the *P. pectinatus* fruits methanol extract has slightly higher TPC values than *P. emblica* fruits which were 1140 ± 1.41 and 1111 ± 4.45 mg GAE/g DW respectively. This shows

that methanol extract of *P. pectinatus* fruits can be considered as a good source of total phenolics compared to the *P. emblica* fruits. The result obtained in this study is in accordance with the study of Silva & Sirasa (2016) where the TPC value of methanolic extract of *P. emblica* fruit was found to be 915.7 ± 27.5 mg GAE/g. A good amount of phenolics in the extracts might significantly affect their antioxidant properties.

Flavonoids are considered as a major group of phenolic compounds found in plant which provide antioxidant properties. In this study, the *P. pectinatus* fruits methanol extract had high content of flavonoid compared to the *P. emblica* fruit methanol extract. In previous study, TFC of ethanol *P. emblica* extract was 5.816 ± 2.81 mg QE/g which is almost similar to the TFC of *P. emblica* fruit methanol extract in the present study (Halim *et al*., 2022). According to Liu *et al*. (2008), the TFC of *P. emblica* fruit methanol extracts from six regions in China were ranged between 20.3 to 38.7 mg QE/g. The TFC of *P. pectinatus* fruits methanol extract obtained in this study is in agreement with the TFC of *P. emblica* obtained by Liu *et al*. (2008). Interestingly, the TFC of *P. pectinatus* and *P. emblica* fruits methanol extracts differed significantly. This might be due to the geographical distribution that could affect the flavonoid content of *P. emblica* fruits since it was imported from India. The variation in flavonoid content may also be significantly affected by year-to-year variations, environmental, genetic diversity, and seasonal (Kumar & Roy, 2018).

Ascorbic acid is associated with lots of health benefits including antioxidant potential. Therefore, measuring the content of ascorbic acid in *P. emblica* and *P. pectinatus* fruits is essential to study its nutritional information. In this study, the ascorbic acid content of *P. emblica* fruits methanol extract (291.60 ± 0.00 mg/100 g) was way higher than methanol extract of *P. pectinatus* fruits (19.44 ± 0.00 mg/100 g). The result of this study is consistent with prior research in which the ascorbic acid content of twelve *P. emblica* fruits from Pakistan were reported to range between 217.7

± 3.1 to 400 ± 2.4 mg/100 g (Sabir *et al*., 2015). According to Gaire & Subedi (2014), the most abundant constituents of *P. emblica* fruit is ascorbic acid.

5.4 Antioxidant Activities of *P. pectinatus* and *P. emblica* Fruit Extracts

To better understand the antioxidant capacity of *P. pectinatus* and *P. emblica* fruits extract, DPPH, ABTS and FRAP assays were performed.

In this study, both methanol extracts of *P. pectinatus* and *P. emblica* fruits exhibited excellent antioxidant activities depicted by their low IC_{50} value obtained from the assay. Low IC_{50} values indicate higher DPPH radical scavenging activity in both *Phyllanthus* fruit extracts. High DPPH scavenging ability presented in this study may be associated with the presence of antioxidant secondary metabolites in the *Phyllanthus* extracts. There are other reported studies on the antioxidant activities from *P. emblica* fruit extracts. Low IC_{50} values were reported in the methanolic extracts of *P. emblica* fruits from Bilaspur, India (Sheoran *et al*., 2019) and *P. emblica* fruit ethanolic extracts in Halim *et al*. (2022) with a value of 4.09 ± 1.34 µg/ml and 7.626 ± 0.41 µg/ml respectively. According to previous research, the IC_{50} values obtained in Sheoran *et al*. (2019) is only slightly different from the value reported in this study. This indicates that this work is corresponding to other reported studies.

Both methanol extracts of *P. pectinatus* and *P. emblica* fruits in this study exhibited low IC_{50} values in ABTS assay which indicates high antioxidant activities. The present result aligns with the findings of Chaikul *et al.* (2021), who determined that the IC_{50} of *P. emblica* branch extract in ABTS assay is 2.83 ± 0.12 µg/mL. Another study reported that the IC_{50} values for ABTS assay in different *P. emblica* extracts were ranged between 3.91 ± 2.10 to 13.51 ± 0.67 µg/mL which is in line with this study (Laulloo *et al.*, 2018).

Data analysis revealed that the *P. pectinatus* fruits methanol extract exhibited the highest reducing power (4.79 ± 0.05 mM Fe (II)/g) as compared to *P. emblica* fruits methanol extract. However, the FRAP value of *P. emblica* fruit reported in Dutta *et al*. (2018) was way higher (FRAP value: 45.1 ± 0.9 mM GAE/g FW) than both methanol extracts of *P. pectinatus* and *P. emblica* fruits in this study. Another study also reported higher FRAP value of *P. emblica* fruit extract which is 28.92 ± 4.95 mM of Fe^{2+}/g (Mallawaarachchi *et al.,* 2019). According to Kunchana *et al.* (2021), the antioxidant and nutrient content of the fruits could be varied depending on its form (fresh/dried) thus FRAP values will be varied.

Although *P. pectinatus* and *P. emblica* are from the same genus, the environmental conditions where the plants were planted and the phytochemical content in each species might contribute to the slight differences in their scavenging abilities (Cosmulescu *et al.*, 2017).

5.5 Correlation between TPC, TFC, AAC and Antioxidant Potential of *P. pectinatus* and *P. emblica* Fruit Extracts

The antioxidant activities of *P. pectinatus* and *P. emblica* fruits were found to increase as the concentrations of TFC and TPC increased. Negative correlation was obtained with AAC. The result suggests that the phenolic and flavonoid content might be the primary sources of the antioxidant activity in both *P. pectinatus* and *P. emblica* fruits. The TPC and TFC positively correlate with all antioxidant assays, which indicate that TFC might contribute a significant amount of TPC since TPC contains non flavonoid and flavonoid polyphenols (Kim & Lee, 2020). The findings are consistent with prior research (Sabir *et al*., 2015; Kim & Lee, 2020; Ghosh *et al*., 2021; Muflihah *et al*., 2021).

Ascorbic acid possesses strong antioxidant properties, however, the antioxidant activities of both *P. pectinatus* and *P. emblica* fruits are not influenced by the presence of ascorbic acid. Other studies also reported a negative correlation between antioxidant activity and ascorbic acid (Almeida *et al.*, 2011; Soural *et al*., 2019; Wang *et al.*, 2022). Soural *et al.* (2019) stated that a better correlation with antioxidant activity can be achieved if the ascorbic acid is combined with other polyphenols. Based on Bahorun *et al.* (2004), the contribution of ascorbic acid alone to the total antioxidant activity of a plant is little to nothing thus the negative correlation is common. The combined and individual contributions of each polyphenol in *P. pectinatus* and *P. emblica* fruits towards the antioxidant activities needed to be studied in the future. Strong correlations between the results from different assays validates the antioxidant activities results (Kim & Lee, 2020).

CHAPTER 6: CONCLUSION

Since ancient times, people from many different cultures have used fruits as a form of foods and medicinal herbs. Fruits is frequently consumed in juice or supplement form. In this study, the phytochemical content, and the antioxidant capacities of both *P. pectinatus* and *P. emblica* fruits were evaluated. Based on the results, *P. pectinatus* fruits can be an excellent source of antioxidant. This is because the total phenolic and flavonoid content extracted from *P. pectinatus* fruits were high and their antioxidant potentials are almost the same as *P. emblica* fruits. A significantly strong correlation was found between the antioxidant activities with TPC and TFC which indicates that phenolic compounds contributed significantly to the antioxidant activities of both *P. pectinatus* and *P. emblica* fruits. The high nutrient content and potential health advantages widely reported in *P. emblica* fruits, has increase its popularity as a functional food and nutraceutical in many years. Vitamins, minerals, antioxidants, and other bioactive chemicals found in *P. emblica* fruits are abundant and have been linked to several health advantages. Additionally, some studies have suggested that consuming *P. emblica* fruits may benefit people with chronic illnesses like cancer, diabetes, and cardiovascular disease. This result in the present study implies that the *P. pectinatus* fruits may have the same potential as *P. emblica* fruits to be developed into functional products. However, more studies need to be carried out to explore other phytochemical constituents and biological activities of *P. pectinatus* fruits.

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