

**NUTRIENT COMPOSITION AND ANTIOXIDANT PROPERTIES OF
'KEREKUP' FRUIT**

(Flacourtia jangomas)



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LIST OF ABBREVIATIONS

%	:	Percent
°C	:	Degree Celsius
AA	:	Ascorbic Acid
BHA	:	Butylated hydroxy anisole
BHT	:	Butylated hydroxytoluene
OG	:	Octyl gallate
PG	:	Propyl gallate
TBHQ	:	Tertiary butyl hydroquinone
TBHP	:	2,4,5-trihydroxybutyrophenone
ROS	:	Reactive Oxygen Species
DPPH	:	Diphenyl-2-picrylhydrazyl
TPC	:	Total Phenolic Content
TPTZ	:	2,4,6-tripyridyl-s-triazine
FRAP	:	Ferric reducing antioxidant power
cm	:	Centimeter
g	:	Gram
kg	:	Kilogram
M	:	Molar
m	:	Meter
mg	:	Milligram
mL	:	Millilitre
mm	:	Millimetre
NaOH	:	Sodium Hydroxide
H ₂ SO ₄	:	Sulphuric acid
O ₂	:	Oxygen
FW	:	Fresh weight
v/v	:	Volume per volume

ABSTRACT

NUTRIENT COMPOSITION AND ANTIOXIDANT PROPERTIES OF 'KEREKUP' FRUIT (*Flacourtia jangomas*)

This study aimed to determine nutritional composition, mineral constituents, ascorbic acid content and antioxidant activity of *Flacourtia jangomas* fruit. This fruit was rich in carbohydrate (12.67 g/100g). Calcium was the major minerals in 'kerekup' fruit (174.75 mg/100g). Ascorbic acid content in *Flacourtia jangomas* obtained from this study was 89.39 mg/100g. Water and ethanol extractions were used to extract the samples for the purpose of antioxidant assay. The antioxidant activities of extracts were measured using total phenolic content (TPC) by Folin-ciocalteu reagent method, 2,2-diphenyl-2-picrylhydrazyl radical scavenging assay (DPPH) and ferric reducing antioxidant power (FRAP) assay. The results showed the ethanol extracts gave the highest total phenolic content (2507.41 mg GAE/100g). The antioxidant activities of the extracts (using DPPH assay) were highly correlated with total phenolic content ($r = 1.000$) and also in reducing power of extracts using FRAP assay ($r = 0.968$). The high antioxidant activity with high ascorbic acid, carbohydrate content and calcium content suggested that the *Flacourtia jangomas* can be used as a good source for preparation of health drink.

ABSTRAK

KOMPOSISI NUTRIEN DAN AKTIVITI ANTIOKSIDAN BUAH KEREKUP (*Flacourtia jangomas*)

Tujuan kajian ini ialah untuk menentukan komposisi nutrien, kandungan mineral, kandungan asid askorbik dan aktiviti antioksidan buah kerekup (*Flacourtia jangomas*). Buah ini kaya dengan kandungan karbohidrat (12.67 g/100g). Kalsium adalah mineral utama dalam buah kerekup (174.75 mg/100g). Kandungan asid askorbik yang diperolehi dari kajian ini adalah 89.38 mg/100g. Pengestrakan air dan etanol telah digunakan untuk mengestrak sampel bagi tujuan kajian antioksidan. Aktiviti antioksidan dalam sampel telah ditentukan nilainya melalui kaedah kandungan jumlah fenol dengan menggunakan kaedah reagen Folin-ciocalteu, 2,2-diphenyl-2-picrylhydrazyl dengan kaedah meneutralkan radikal-radikal bebas (DPPH) dan kuasa penurunan antioksidan (FRAP). Keputusan kajian telah menunjukkan ekstrak etanol dalam buah kerekup mempunyai nilai kandungan jumlah fenol tertinggi (2507.41 mg GAE/100g). Aktiviti antioksidan dalam ekstrak (menggunakan DPPH) telah menunjukkan korelasi yang tinggi dengan kandungan jumlah fenol ($r = 1.000$) dan juga dalam kuasa penurunan sampel menggunakan kaedah FRAP ($r = 0.968$). *Flacourtia jangomas* dicadangkan untuk digunakan sebagai sumber yang baik bagi penyediaan minuman kesihatan kerana mempunyai kandungan aktiviti antioksidan, kalsium, karbohidrat dan asid askorbik yang tinggi.

CHAPTER 1

INTRODUCTION

1.1 Background

Consumption of various types of fruit provides excellent health benefits because they are a good source of phytochemicals that are good for preventing disease. Epidemiological studies have shown that there is a positive association between intake of vegetables and fruits and reduce cardiovascular diseases and certain cancer (Hu, 2003; Ikram et al., 2009).

Generally, Malaysians consume vegetables that are relatively abundant sources of antioxidant components with strong potential antioxidant activities (Amin and Lee, 2005; Amin *et al.*, 2006). Similar to vegetables, tropical and subtropical fruits such as ciku, star fruits and guava have been reported to be rich in antioxidants (Leong and Shui, 2002).

Besides the commonly consumed local fruits, some underutilized fruits are important in the Malaysian diet, especially in rural communities. Malaysia is one of the countries that have a rich diversity of underutilized fruits that grow wild in the region of Peninsular Malaysia, Sabah and Sarawak. Some of the underutilized fruits are rarely eaten, unknown and unfamiliar. Due to the broad spectrum of their flesh and skin colour, these underutilized fruits may have potential benefits to human health. In

addition, some of these fruits have the potential to be used and processed as food products for local consumption.

However, underutilized fruits have not received much attention as antioxidant sources compared to commercial fruits like guava, papaya and pineapple. This could be due to their lack of popularity among local communities, lack of information on nutritional compositions and physical qualities and the lack of promotional campaign for these fruits (Ikram *et al.*, 2009).

Currently, research and development activities on underutilized fruits species have become priority areas in developing and develop countries. They are characterized by the fact that they are locally abundant but globally rare, and the scientific information and knowledge about them is limited (Gruere *et al.*, 2009).

1.2 Problem Statement

There are a lots of research has been conducted on nutritional composition, antioxidant activities and total phenolic content of Malaysian underutilized fruits but no research is done on *Flacourtia jangomas*. Thus, this study aimed at exploiting the nutritional composition and antioxidant properties of “kerekup” fruits, *Flacourtia jangomas*. Information provided by this study may enrich the database of nutrient content for a wild fruit in Malaysia. Additionally, a better understanding of the nutraceutical and functional potential of this fruit will further contribute to conservation and enhancement of species.

1.3 Significance of Study

Flacourtia jangomas is chosen in this study because it has a high potential to serve as an antioxidant. *Flacourtia jangomas* is a large deciduous shrub or a small spreading tree up to 9m in height. The plant is used for a variety of astringent, acrid, sour, refrigerant, stomachic diarrhea, inflammation, skin disease, jaundice, tumours, nausea, dyspepsia and diabetes in South Indian traditional medicine (Singh *et al.*, 2010). Result of this study will be useful in order to give information on nutritional value and antioxidant properties of this fruit. Moreover, it can be used as a reference for further studies on *Flacourtia jangomas*. Thus this study will be carried out to determine the antioxidant properties, total phenolic content and nutritional composition of *Flacourtia jangomas*.

1.4 Objectives of study

The objectives for this study are :

- i. To analyse nutritional composition of *Flacourtia jangomas*
- ii. To determine antioxidant activity of *Flacourtia jangomas*
- iii. To determine the total phenolic content in *Flacourtia jangomas*

CHAPTER 2

LITERATURE REVIEW

2.1 Antioxidant

Antioxidants are chemical substances that donate an electron to the free radical and convert it to a harmless molecule. An antioxidant help organism deals with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability (Ali *et al.*, 2008).

Many observational epidemiological studies have shown that a high fruit and vegetable intake is associated with a lower cancer incidence specially cancers from the gastrointestinal tract (Johnson, 2004). This is due in part to the dietary antioxidant content of fruits and vegetables. Dietary antioxidants are believed to be effective in the prevention of oxidative stress related diseases (Kaur & Kapoor, 2001).

Plant foods provide a wide variety of dietary antioxidants, such as vitamins C and E, carotenoids, flavonoids and other phenolic compounds. The additive and synergistic effects of these antioxidants with other dietary compounds for example minerals may contribute to the health benefits of the diet (J.Serrano, 2007).

2.1.1 Roles of antioxidant

Antioxidants are nutrients or enzymes that help neutralize free radicals in the body. Free radicals are created as byproducts when the body uses oxygen. Free radicals can also enter the body via pollution, smoking, poor diet, pesticides or radiation. In some cases, excess exercise can cause over-production of free radicals. Free radicals are also known as reactive oxygen species (ROS), oxidants, or simply radicals. Most free radicals, with the exception of a few such as melanin, are chemically reactive and dangerous to health.

In vitro and in vivo experiments have revealed that antioxidants have substantial effects on the prevention of atherogenesis and cellular damage, with the capacity to act as an anti-tumor, anti-inflammatory and anti-allergy agents (Crozier *et al.*, 2009). A possible way to fight the diseases is to improve our body's antioxidant defenses. High consumption of fruits and vegetables has been associated with a lowered incidence of such degenerative diseases (Bajpai *et al.*, 2009).

2.1.2 Reactive oxygen species (ROS)

Reactive oxygen species (ROS) are chemically-reactive molecules containing oxygen. Examples include oxygen ions and peroxides. Reactive oxygen species are highly reactive due to the presence of unpaired valence shell electrons. ROS form as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling. However, during times of environmental stress (e.g., UV or heat exposure), ROS levels can increase dramatically. This may result in significant damage to cell structures. This cumulates into a situation known as oxidative stress. ROS are also generated by exogenous sources such as ionizing radiation.

Reactive oxygen species (ROS) formed in vivo, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damaging transient chemical species. These are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function. ROS are regulated by endogenous superoxide dismutase, glutathione peroxidase and catalase but due to over-production of reactive species, induced by exposure to external oxidant substances or a failure in the defense mechanism, damage to cell structures, DNA, lipids and proteins (Valko *et al.*, 2006).

At high concentration, ROS can be important mediators of damage to cell structures, including lipids and membrane, proteins and nucleic acid which is termed as oxidative stress (Poli *et al.*, 2004). The harmful effects of ROS are balanced by the antioxidant action of non-enzymatic antioxidants in addition to antioxidant enzymes (Halliwell, 2007).

2.1.3 Free radicals

Free radicals are very unstable and react quickly with other compounds, trying to capture the needed electron to gain stability. Generally, free radicals attack the nearest stable molecule, "stealing" its electron. When the "attacked" molecule loses its electron, it becomes a free radical itself, beginning a chain reaction. Once the process is started, it can cascade, finally resulting in the disruption of a living cell. Some free radicals arise normally during metabolism. However, environmental factors such as pollution, radiation, cigarette smoke and herbicides can also spawn free radicals.

Normally, the body can handle free radicals, but if antioxidants are unavailable, or if the free-radical production becomes excessive, damage can occur. According to Halliwell, oxidative stress is an imbalance in the redox status of a cell, between the production of reactive oxygen species (ROS) and antioxidant defence mechanisms, leading to damage, potential mutations and ultimately the formation of cancer. Defence against oxidative stress is therefore an important factor in preventing the development of many diseases. Dietary polyphenols are potent antioxidants, able to scavenge and intercept free radicals, preventing damage to cellular molecules (Mouskag *et al.*, 2005).

2.2 Types of antioxidant

There are two types of antioxidant which are natural antioxidant and synthetic antioxidant. Antioxidants derived from fruits, vegetables, spices and cereals are very effective and have reduced interference with the body's ability to use free radicals constructively (Wolfe *et al.*, 2003). Natural antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols) ascorbic acid and carotenoids. The quest for natural antioxidants for dietary, cosmetic and pharmaceutical uses has become a major industrial and scientific research challenge over the last two decades (Ali *et al.*, 2008). The other antioxidant is synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) commonly used in processed foods have side effects and are carcinogenic (Branen, 1995).

2.2.1 Natural antioxidants

Natural antioxidants are primarily plant phenolics that may occur in all parts of plants, such as fruits, vegetables, nuts, seeds, leaves, roots and barks (Pratt and Hudson, 1990). Plant phenolics are multifunctional and can act as reducing agents, free radical terminators, metal chelators and singlet oxygen quenchers. Many natural antioxidants have already been isolated from different kinds of plants, such as oilseeds, cereal crops, vegetables, leaves, roots, spices and herbs. All the phenolic classes have the structural requirements of free radical scavengers and have potential as food antioxidants (Ramarathnam, *et al.*, 1995).

One of the naturally occurring antioxidants most widely used in the food industries is ascorbic acid, with a varied chemistry. It has complex multi functional effects. Depending on conditions, ascorbic acid can act as an antioxidant, a pro-oxidant, a metal chelator, a reducing agent or an oxygen scavenger, ascorbic acid and its esters function as antioxidants by protecting double bonds and scavenging oxygen (Jayathilakan *et al.*, 2005).

2.2.2 Synthetic antioxidants

Synthetic antioxidants are widely used as food additives to prevent rancidification, owing to their high performance, low cost and wide availability. Therefore, many synthetic antioxidants such as butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ), 2,4,5-trihydroxybutyrophenone (THBP), di-tertbutyl-4-hydroxymethylphenol (IONOX-100), propyl gallate (PG), octyl gallate (OG), nordihydroguaiaretic acid (NDGA) and 4-hexylresorcinol (4HR) are used in edible vegetable oil and cosmetics (Guan *et al.*, 2005).

Among the synthetic types, the most frequently used to preserve food are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ). Tocopherols are also used as antioxidants for food, the order of antioxidant effectiveness being $\delta > \gamma > \beta > \alpha$. Reports revealing that BHA and BHT could be toxic, and the higher manufacturing costs and lower efficiency of natural antioxidants such as tocopherols, together with the increasing consciousness of consumers with regard to food additive safety, created a need for identifying alternative natural and probably safer sources of food antioxidants (Moure *et al*, 2001).

2.3 Phenolic compounds

Phenolic compounds are secondary metabolites, which have been associated with flavor and colour characteristics of fruits and vegetables of fruits and vegetables and are gaining considerable attention because of their potent antioxidant and health promoting properties (Kaur and Kapoor, 2011).

Phytochemicals, especially phenolics in fruits and vegetables are the major bioactive compounds known for health benefits. Plant phenolics are commonly found in both edible and non-edible parts of the plants and have been reported to have multiple biological effects, including antioxidant activity. The scavenging activity of phenolics is mainly due to their redox properties, which allows them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, many of the natural antioxidants exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory, anti-allergic, and antithrombotic and vasolidatory actions (Cook and Sammon, 1996).

2.4 Vitamin C

Fruits are excellent sources of antioxidant vitamins, as well as of other vitamins, minerals, flavonoids, and phytochemicals (Ismail & Fun, 2003). Vitamin C is the most important vitamin for human nutrition that is supplied by fruits and vegetables. L-Ascorbic acid (AA) is the main biologically active form of vitamin C. AA is reversibly oxidised to form *l*-dehydroascorbic acid (DHA), which also exhibits biological activity. Further oxidation generates diketogulonic acid, which has no biological function (Davey *et al.*, 2000). Since DHA can be easily converted into AA in the human body it is important to measure both AA and DHA in fruits and vegetables to know vitamin C activity (Lee and Kader, 2000).

Ascorbic acid is widely distributed in plant cells where plays many crucial roles in growth and metabolism. As a potent antioxidant, AA has the capacity to eliminate several different reactive oxygen species, keeps the membrane-bound antioxidant α -tocopherol in the reduced state, acts as a cofactor maintaining the activity of a number of enzymes (by keeping metal ions in the reduced state), appears to be the substrate for oxalate and tartrate biosynthesis and has a role in stress resistance (Davey *et al.*, 2000). Since humans cannot synthesise ascorbate, their main source of the vitamin is dietary fruit and vegetables. Fruits (especially citrus and some tropical) are the best sources of this vitamin. An accurate and specific determination of the nutrients content of fruits is extremely important to understand the relationship of dietary intake and human health (Hernandez *et al.*,2006).

2.5 Mineral analysis

Fruits and vegetables are valuable sources of minerals (Milton, 2003). Diets high in fruits and vegetables are also linked to decreased risk of diseases (diabetes, cancer, etc) and their consumption should be encouraged. The levels of essential minerals that occur in foods may change for many different reasons. Some of the sources of variation are biological, such as variety of plant, different fertilisations of a crop or different feeding principles for animals, as well as seasonal and annual factors. Other factors are related to differences in harvest, storage, and processing (Torelm and Danielsson, 1998). Minerals play a vital role in the proper development and good health of the human body and fruits are considered to be the chief source of minerals needed in the human diet (Hardisson *et al.*, 2000).

2.6 Underutilized fruits

Underutilized fruits may be defined as fruits that are rarely eaten, unknown and unfamiliar. Underutilized species, such as Barbados cherry (*Malpighia gliabra*), Himalayan chenopod grains (*Chenopodium species*) and bambara groundnut (*Vigna subterranean*), have been documented for their potential as superior food for human health due to their high nutritional values (IPGRI, 2002). In Malaysia, indigenous fruits are important sources for a better nutritional status and food security for rural communities. These fruits are easily grown in the local ecology and have less pest and disease problems as compared to introduced varieties (Hoe and Siong, 1999). In Malaysia, many kinds of underutilized fruits are available, such as nam-nam (*Cynometra sp.*), bidara (*Ziziphus sp.*), pulasan (*Nephelium sp.*) and others. These fruits are usually grown in orchards or fruit gardens around houses and some grow wild in the rain forest (Ikram *et al.*, 2009) These fruits are known by older folks to be associated with many nutritional and medicinal properties.

2.6.1 Nutritional composition of other underutilized fruits

'Dabai' fruits or *Canarium odontophyllum* is one of the popular underutilized fruits potentially to be developed as a specialty fruit of Sarawak. Nutritional composition (g/100 g FW) of 'dabai' fruits from different districts is given in Table 2.1. Lipid was the major macronutrient in 'dabai' fruits and did not differ among fruits from different districts (21.16 ± 4.71 to 25.76 ± 3.03 g/100 g FW).

Moisture accounted for 50.44 ± 0.24 to $51.91 \pm 0.94\%$ by FW of 'dabai' fruits from different districts. Meanwhile, the ash content of 'dabai' fruits ranged from 1.66 ± 0.26 to 1.89 ± 0.08 g/100 g FW. Both moisture and ash contents did not differ among 'dabai' fruits collected from different districts. Results also demonstrated that the red variety shown no difference with the purple variety in terms of their lipid, moisture and ash contents. Protein content varied in a lesser extent among 'dabai' fruits from different districts. While the highest protein content was found in purple 'dabai' fruits from Kanowit (5.20 ± 0.87 g/100 g FW), the lowest was in red 'dabai' fruits from Sarikei (3.45 ± 0.64 g/100 g FW). It was observed that total available carbohydrate content in the red variety and in purple 'dabai' fruits from Song (9.16 ± 0.15 and 8.97 ± 2.21 g/100 g FW) were almost two folds higher than that of Kapit and Kanowit (5.07 ± 0.82 and 4.45 ± 0.83 g/100 g FW). Red 'dabai' fruits were richer in available carbohydrate but with reduced amount of protein as compared to the purple variety.

Table 2.1 Nutritional composition of ‘dabai’ fruits collected from different districts

Nutrient	Kanowit	Kapit	Sarikei	Song
Moisture	51.30 ± 0.37 ^a	51.11 ± 0.06 ^a	51.91 ± 0.94 ^a	50.44 ± 0.24 ^a
Protein	5.20±0.87 ^a	4.56±0.87 ^{a,b}	3.45±0.64 ^a	4.35±1.15 ^{a,b}
Lipid	25.76±3.03 ^a	21.16±4.71 ^a	23.72±1.11 ^a	24.47±2.76 ^a
Ash	1.89±0.08 ^a	1.88±0.42 ^a	1.78±0.17 ^a	1.66±0.26 ^a

Source: Chew *et al.*, (2011)

Values with different letters are significantly different at $p < 0.05$ within the same row.

^a g/100 g fresh weight. Results are expressed in means ± standard deviation and (range).

2.6.2 *Flacourtia jangomas*

Flacourtia jangomas is a semi-wild trees which is distributed over the sub-tropical and tropical regions of Southeast Asia .The tree belong to the Flacortiaceae family (Kermasha *et al.*, 1987). *Flacourtia jangomas* is a large deciduous shrub or a small spreading tree up to 9 metre in height. The plant is used for a variety of astringent, acrid, sour, refrigerant, stomachic diarrhea, inflammation, skin disease, jaundice, tumors, nausea, dyspepsia and diabetes in South Indian traditional medicine.

The fruits are palatable, bright in colour and are eaten fresh during the summer season when they ripen. Commercial use of the fruits has not been made, and the tree remain uncultivated and neglected (Singh *et.al.*, 2010).



Figure 2.1 'Kerekup' fruits (*Flacourtia jangomas*)

CHAPTER 3

METHODOLOGY

3.1 Materials

The plants of fresh *Flacourtia jangomas* fruits were collected from local area in Bachok and Labok, Kelantan.

The list of chemicals and reagents of analytical grade used were ascorbic acid, acetic acid, boric acid, concentrated sulphuric acid, DPPH solid, ethanol 70%, Folin-Ciocalteu reagent, Ferum (III) chloride, gallic acid, hydrochloric acid (HCl), ICP multi-element standard solution IV-merck catalogue No.113550100, metaphosphoric acid, nitric acid, oxalic acid, petroleum ether, perchloric acid (HClO₄), pill (5 mg potassium sulphate + 5 mg selenium) sodium acetate, sodium bicarbonate, sodium carbonate, sodium hydroxide, TPTZ solid and 2,6-dichloropenolindophenol dye .

3.2 Solvent extraction

The extracts was prepared according to the method of Ikram et al., (2009) where the sample will be extracted with two different solvent which were water and 70% aqueous ethanol for two hours at 50° C using an orbital shaker at 200 rpm. The ratio between the samples to be extraction medium is 1:4. The mixtures were filtered through a filter by using a filter funnel. The extract was used for determination of antioxidant activity and total phenolic content.

3.3 Determination of total phenolic content