

Evaluation of Antioxidant Activity of Some Selected Tropical Fruits in South Kalimantan, Indonesia

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ABSTRACT

In the present study antioxidant and antioxidant activity of some tropical fruits was evaluated. Antioxidants are compounds or molecules that can scavenging and prevent free radicals and reactive oxygen species that can caused a cell damage. Fruit was known as a source of antioxidant. South Kalimantan Indonesia, has a variety of fruit such as mentega, nangka, timun suri and kuranji. Study for evaluating the antioxidant levels and activity of those fruit were never been investigated. Thus, our study aim to measure the antioxidant levels and antioxidant activity of those selected fruits. Ascorbic acid, lycopene, β -carotene levels and antioxidant activity of four selected tropical fruits was evaluated using spectrophotometer. The result of this studied suggest that the four selected tropical fruits is potential antioxidant because it contained ascorbic acid, β -carotene, lycopene and had effect of scavenging radical hydroxyl, hydrogen peroxide and chellating ferrous iron.

Keywords: *antioxidant activity, ascorbic acid, β -carotene, lycopene*

INTRODUCTION

Fruits are source of antioxidants that are beneficial to health because it contains ascorbic acid, β -carotene, lycopene, and others that can scavenging the free radical [1, 2]. Free radicals are atoms or molecules containing unpaired electrons therefore unstable molecule. It can react with the cell membrane biomolecules such as lipids, proteins, and carbohydrates. Because of that, the presence of antioxidants is necessary to prevent or delay the damage [3, 4].

Antioxidants are compounds or molecules that can scavenging and prevent free radicals and reactive oxygen species generated from the metabolism [4]. Plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as peroxydase, catalase, superoxide dismutase and others. Insufficient levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells [5].

In South Kalimantan Indonesia is a tropical country that has a variety of fruits that are consumed for food and health [6]. For example methanol extract timun suri (*Cucumis sativus*) can be useful as an anti-inflammatory, analgesic, and antioxidants [7, 8]. Nangka (*Artocarpus heterolphyllus*) can be useful as anti-bacterial, anti-diabetic, anti-inflammatory, antioxidant and anti-helminthics [9]. Mentega (*Diospyros blancoi*) can be useful as anti-diarrhea and antioxidant [10].

The antioxidant properties mechanism of fruits through the inhibiting initiation and breaking chain propagation or suppressing formation of ROS by binding to the metal ions, reducing hydrogen peroxide, and quenching superoxide and singlet oxygen [5]. The antioxidant activity of those fruits have not been investigated, therefore, many study should be performed. In this present study, we investigate ascorbic acid, β -carotene, lycopene and antioxidant activity of kuranji, mentega, timun suri, and nangka.

MATERIALS AND METHODS

Chemical and Materials

1% metaphosphoric acid, 2,6-dichloro phenolindophenol, acetone-hexane, 1mM FeCl₃, 2 mM FeCl₂,

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1mM ortophenanthroline, 0.2 M phosphate buffer (pH 7.8), 0.17 M H₂O₂, NaNO₂, and AlCl₃ were from Sigma.

Fruit Materials

Four types of tropical fruits and used as reference were studied. They were kuranji (*Dialium indum* L), mentega (*Diospyros blancoi*), timun suri (*Cucumis sativus*), and nangka (*Artocarpus heterolphyllus*).

Ascorbic Acid Content

Ascorbic acid was determined according to the method of Klein and Perry (1982). The dried methanolic extract (100 mg) was extracted with 10 ml of 1% metaphosphoric acid for 45 minute at room temperature and filtered through whatman no. 4 filter paper. The filtrate (1 ml) was mixed with 9 ml of 2,6-dichlorophenolindophenol and the absorbance was measured within 30 min at 515 nm against a blank. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (0.020–0.12 mg/ml). The assays were carried out in triplicate; the results were mean values ± standard deviations and expressed as mg of ascorbic acid/g of extract [11].

β-Carotene and Lycopene Content

β-Carotene and lycopene were determined according to the method of Nagata and Yamashita (1992). The dried methanolic extract (100 mg) was vigorously shaken with 10 ml of acetone–hexane mixture (4:6) for 1 min and filtered through whatman no. 4 filter paper. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm. Contents of β-carotene and lycopene were calculated according to the following equations: lycopene (mg/100 ml) = -0.0458 A₆₆₃ + 0.372 A₅₀₅-0.0806 A₄₅₃ ; β-carotene (mg/100 ml) = 0.216 A₆₆₃- 0.304 A₅₀₅+ 0.452 A₄₅₃. The assays were carried out in triplicate; the results were mean values ± standard deviations and expressed as mg of carotenoid/g of extract [11].

Hydroxyl Radical Scavenging Activity

The scavenging activity for hydroxyl radicals was measured with Fenton reaction [12]. The absorbance of the mixture at 560 nm was measured with a spectrophotometer. Hydroxyl radical scavenging activity was calculated using the equation: (1 - absorbance of sample/ absorbance of control) × 100. Each experiment was carried out in triplicate and results averaged expressed as mean ± SD.

Chelating Effect of Ferrous Iron

The chelating effect of ferrous ions was estimated by the method of Hung-Ju Chou et al. [13]. The absorbance of the mixture was measured at 562 nm. Chelating effect was calculated using the equation: (1 - absorbance of sample/ absorbance of control) × 100. Each experiment was carried out in triplicate and results averaged expressed as mean ± SD.

Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging was determined according to the method of Ruch et al. [14]. The absorbance value of the reaction mixture was recorded at 230 nm. Hydrogen peroxide scavenging activity was calculated using the equation: (1 - absorbance of sample/ absorbance of control) × 100. Each experiment was carried out in triplicate and results averaged expressed as mean ± SD.

RESULTS AND DISCUSSION

Ascorbic Acid

Ascorbic acid is known as vitamin C is a substance commonly found in fruits [13-14]. Some studies suggest that the consumption of fruits and vegetables are associated with reduced risks of diseases [15]. Elderly people who take vitamin C and vitamin E supplements have a 50% lower risk of dying prematurely from disease than do people who do not supplement A Californian study has concluded people who consume more than 750 mg/d of vitamin C

Table 1. Ascorbic Acid, β-Carotene and Lycopene of Four Selected Fruits

Local Name	Scientific Name	Ascorbic Acid (mg/100mg)	β-Carotene (mg/100ml)	Lycopene (mg/100ml)
Mentega	<i>Diospyros blancoi</i>	1.320 ± 0.010	0.196 ± 0.010	0.333 ± 0.012
Kuranji	<i>Dialium indum</i> L	0.176 ± 0.012	0.128 ± 0.013	0.016 ± 0.001
Timun Suri	<i>Cucumis Sativus</i>	0.352 ± 0.011	0.125 ± 0.012	0.010 ± 0.002
Nangka	<i>Artocarpus heterolphyllus</i>	0.440 ± 0.012	0.192 ± 0.021	0.072 ± 0.001

reduce their risk of dying prematurely by 60% [15].

The result of ascorbic acid levels is shown in table 1. All four selected fruits contains ascorbic acid, and mentega had a highest levels of ascorbic acid followed by jackfruit, timun suri and kuranji. The levels of ascorbic acid showed that the four selected fruits has a potential antioxidant activity.

Ascorbic Acid is a water-soluble antioxidant. It was first isolated in 1928, by the Hungarian biochemist and nobel prize winner Szent-Gyorgyi. It is an unstable, easily oxidized acid and can be destroyed by oxygen, alkali and high temperature [16].

Ascorbic acid has been shown to play important role in several physiological processes in plants and fruits, including growth, differentiation, and metabolism [17]. Body requires ascorbic acid for normal physiological functions. It helps in the metabolism of tyrosine, folic acid and tryptophan. It helps to lower blood cholesterol and contributes to the synthesis of the amino acids carnitine and catecholamine that regulate nervous system. It is needed for tissue growth and wound healing. It helps in the formation of neurotransmitters and increases the absorption of iron in the gut. Being an antioxidant, it protects the body from the harmful effects of free radicals and pollutants [16].

Since ascorbic acid is water soluble, it can work both inside and outside the cells to combat free radical damages. Free radicals seek out an electron pair to regain their stability. Ascorbic acid is an excellent source of electrons therefore it can donate electrons to free that radicals such as hydroxyl and superoxide radicals and quench their reactivity [16].

Ascorbic acid prevents free radical damage in the lungs and may even help to protect the central nervous

High doses given after but not before the injury successfully suppressed preventing edema [16].

β-Carotene and Lycopene

Carotenoids, such as lycopene and β-carotene, are natural constituents of many plants and may protect against disease. Carotenoids are a family of pigmented compounds that are synthesized by plants and microorganisms but not animals. In plants, they contribute to the photosynthetic machinery and protect them against photo-damage [18].

Fruits and vegetables constitute the major sources of carotenoid in human diet. They are present as micro-components in fruits and vegetables and are responsible for their yellow, orange and red colors. Carotenoids are thought to be responsible for the beneficial properties of fruits and vegetables in preventing human diseases including cardiovascular diseases, cancer and other chronic diseases [18].

In recent years the antioxidant properties of carotenoids has been the major focus of research. More than 600 carotenoids have so far been identified in nature. However, only about 40 are present in a typical human diet. Of these 40 about 20 carotenoids have been identified in human blood and tissues. Close to 90% of the carotenoids in the diet and human body is represented by β-carotene, α-carotene, lycopene, lutein and cryptoxanthin [18].

The levels of β-carotene and lycopene in four selected fruits shown in table 1. The levels of β-carotene decreased in the order of mentega fruit > jackfruit > kuranji > timun suri, and the levels of lycopene decreased in the order of mentega fruit > jackfruit > kuranji > timun suri.

β-carotene is commonly known as a radical

Table 2. Antioxidant Activity of Four Selected Fruits

Fruits	% Scavenging Hidroksil	% Chelating Ferrous iron	% Scavenging H ₂ O ₂
Mentega	42.432 ± 1.120	28.191 ± 0.910	25.871 ± 1.673
Kuranji	45.918 ± 2.211	15.426 ± 1.110	24.378 ± 1.721
Timun Suri	40.893 ± 1.212	14.894 ± 1.817	26.866 ± 1.523
Nangka	36.623 ± 1.124	7.979 ± 1.213	12.438 ± 2.170

system problems from such damage. In a study of guinea pigs, pretreatment of ascorbic acid including effectively diminished the acute lung damage caused by the introduction of super oxide anion free oxygen radicals to the trachea. Ascorbic acid also has been tested as an antioxidant inflammatory reaction in mice.

scavenger and a physical scavenger of singlet oxygen and is believed to play an important role in the inhibition of initial stages of lipid peroxidation [21]. The antioxidant properties of β-carotene have been suggested as being the main mechanism by which they afford their beneficial effects. Recent studies are also

showing that carotenoids may mediate their effects via other mechanisms such as gap junction communication, cell growth regulation, modulating gene expression, immune response and as modulators of phase I and II drug metabolizing enzymes. However, carotenoids such as α - and β -carotene and β -cryptoxanthin have the added advantage of being able to be converted to Vitamin A and its related role in the development and disease prevention [18].

Several in vitro, animal and human experiments have demonstrated the antioxidant properties of carotenoids such as β -carotene. It is interesting to observe that β -carotene has also been reported to act as a pro-oxidant under certain situations. β -carotene at a concentration of 0.2 μ M augmented UVA-induced haem oxygenase-1 induction indicating a pro-oxidant effect. The pro-oxidant effect of β -carotene was also demonstrated in rats that showed increased activity of phase I enzymes in liver, kidney and intestine as well as increased oxidative stress [18].

Lycopene is present in many fruits and vegetables, with tomatoes and processed tomato products being among the richest sources. Several recent studies suggest that dietary lycopene is able to reduce the risk of chronic diseases such as cancer and cardiovascular diseases. Although several mechanisms have been implicated in health-beneficial effects of lycopene, such as modulation of intercellular gap junction communication, hormones, immune system and metabolic pathways, the antioxidant properties of lycopene are thought to be primarily involved in its preventive effects in chronic diseases. Because of its high number of conjugated dienes, lycopene is one of the most potent antioxidants, with a singlet-oxygen-quenching ability twice as high as that of β -carotene and 10 times higher than that of α -tocopherol [21].

Hydroxyl Radical Scavenging Activity

Unlike superoxide, which can be detoxified by superoxide dismutase, the hydroxyl radical cannot be eliminated by an enzymatic reaction. Mechanisms for scavenging peroxy radicals for the protection of cellular structures includes dietary antioxidants such as flavonoid and vitamin C [20, 21].

Radical hydroxyl is the most reactive among all ROS. It has a single unpaired electron, thus, it can react with oxygen in triplet ground state. Radical hydroxyl interacts with all biological molecules and causes subsequent cellular damages such as lipid peroxidation, protein damage, and membrane destruction. Because cells have no enzymatic

mechanism to eliminate radical hydroxyl, its excess production can eventually lead to cell death. The oxidation of organic substrates by radical hydroxyl may proceed by two possible reactions, either by addition of radical hydroxyl to organic molecules or due to abstraction of a hydrogen atom from it [17].

The hydroxyl radical scavenging activity of the various extracts was investigated in this study (table 2). All extracts almost have equal ability to scavenging the hydroxyl radical. Kuranji has the highest abilities to scavenging hydroxyl radical than mentega fruit, timun suri and jackfruit.

Chelating Effect of Ferrous Iron

The ferrous ion chelating activities of mentega fruit, kuranji, pasak timun suri, and jackfruit extracts are shown in table 2. The metal scavenging effect of these samples decreased in the order of mentega fruit > kuranji > timun suri > jackfruit.

Although iron is vital for life, it can be toxic when it is present in excess. Iron homeostasis is a complex process, as there are many different proteins that respond not only to the total body burden of iron, but also to stimuli such as hypoxia, anemia and inflammation [22].

About 65% of iron is bound to hemoglobin, 10% is a constituent of myoglobin, cytochromes, and iron-containing enzymes, and 25% is bound to the iron storage proteins, ferritin and hemosiderin. About 0.1% of body iron circulates in the plasma as an exchangeable pool, essentially all bound to transferrin. The process of chelation not only facilitates the transport of iron into cells, but also prevents iron-mediated free radical toxicity [22].

The iron-mediated free radical toxicity leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions via Fenton reaction [23]. Fe^{2+} caused the production of oxyradicals and lipid peroxidation, therefore the ability of substances to chelating iron can be use as a valuable antioxidant. It was reported that the presence of chelating metal such as iron is capable of generating free radicals from peroxides and may be implicated in human cardiovascular disease [1, 23, 24].

Many studies documented that mutations in superoxide dismutase enzymes and iron-uptake regulator may lead to excess levels of superoxide anion radicals and iron overload. Such a condition leads to the possibility of redox active iron to participate in organic and inorganic oxygen radical reactions, such as stimulating lipid peroxidation and catalyzing the

formation of damaging hydroxyl radicals with subsequent tissue damage [22].

Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because of it may increase the hydroxyl radical in the cells.

In mammalian cells, potential enzymatic sources of ROS include the mitochondrial electron transport chain, the arachidonic acid metabolizing enzymes lipoxygenase and cyclooxygenase, the cytochrome P450s, xanthine oxidase, NADPH oxidases, uncoupled nitric oxide synthase, peroxidases, and other hemoproteins. These systems primarily catalyze one electron reduction of molecular oxygen to form radical superoxide which rapidly inactivates NO[•] to form peroxynitrite. Under ambient conditions, some radical superoxide is dismutated to hydrogen peroxide spontaneously or catalyzed by superoxide dismutase. Of interest, loss of NO[•] could lead to enhanced formation of hydrogen peroxide. Some enzymes, such as xanthine oxidase and glucose oxidase, can directly produce hydrogen peroxide by donating two electrons to oxygen. In the presence of heavy metals, hydrogen peroxide undergoes Fenton reaction to form highly reactive hydroxyl radical [24].

Thus removing H₂O₂ is very important throughout food systems. The composition of hydrogen peroxide into water may occur according to the antioxidant compounds as the antioxidant component present in the extract are good electron donors, they may accelerate the conversion of H₂O₂ to H₂O [25-27].

The hydrogen peroxide scavenging activities of mentega fruit, kuranji, pasak timun suri, and jackfruit extracts are shown in table 2.

Table 2 showed that the four selected fruits have a potential hydrogen peroxide scavenging activity. Timun suri has a higher scavenging activity and followed by mentega fruit, kuranji and jackfruit.

CONCLUSIONS

In present study the ascorbic acid, β-carotene, lycopene and antioxidant activity of four selected tropical fruits was evaluated. The levels of ascorbic acid decreased in the order of mentega fruit > jackfruit > timun suri > kuranji. The levels of β-carotene and lycopene decreased in the order of mentega fruit > jackfruit > kuranji > timun suri. Mentega fruit have a highest chellating effect on ferrous iron followed by kuranji, timun suri and jackfruit. Kuranji have a

highest hydroxyl radical scavenging activity and followed by mentega fruit, timun suri and jackfruit. For hydrogen peroxide scavenging activity, timun suri have a highest activity than mentega fruit, kuranji and jackfruit.

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