Bioactive Compound and Antioxidant Activity of Methanol Extract *Mauli Bananas (Musa sp)* Stem

Maharani Laillyza Apriasari, Iskandar, and Eko Suhartono

***Abstract*—Banana is one of the most widely distributed and consumed fruit in tropical and subtropical countries. Considering to nutritional aspects, it is one of the worlds leading food crops with a high source of minerals, vitamins, carbohydrates, flavonoids, phenolic compounds etc. The current study was performed to evaluate the bioactive compound and antioxidant activity in *Mauli Bananas (Musa sp)* stem. *Mauli bananas* stem exctract contain a biocative compound with tannin has a highest level and followed by ascorbic acid, saponin, β-carotene, total flavonoid, lycopene, alkaloid and flavonoid. The chellating effect of ferrous iron in *Mauli bananas* stem had a highest antioxidant activity than hydroxyl scavenging activity and hydrogen peroxide scavenging activity. The data obtained *Mauli bananas* stem contained a lot of biocative compound and also have moderate to potent antioxidant and/or free radical scavenging activity.**

***Index Terms*—Antioxidant, antioxidant activity, *Mauli bananas, musa sp*.**

I. Introduction

There are various problem in human body, and one of them is oxidative stress. Different air pollutions, smoking, UV radiation, etc. leads to oxidative stress of cells and caused various diseases like dermatitis, melanomas or photo aging of the skin, cancer, heart disease, inflammation, arthritis, brain dysfunction, cataracts, etc [1]. Oxidative stress occurs because of imbalance between oxidants and antioxidants and induced the increasing of Reactive Oxygen Species (ROS) [2].

The human body has several mechanisms to reduced oxidative stress, such as produced antioxidants, which are either naturally produced inside the body, or externally supplied through foods and/or supplements [3]. The mechanism of antioxidant is suppression of ROS formation by inhibition of enzymes or by chelation the trace elements which is involved in ROS production, scavenging ROS, and protect the antioxidant defense [4].

In this recent years, there are much attention has been focused on the activity of natural antioxidants present in fruits because potentially these components may reduce the level of oxidative stress [5]. Wolfe and Liu in their research; the Frequent consumption of fruits and vegetables is associated with a lowered risk of cancer, heart disease, hypertension and stroke [6].

Musa sp. (Musaceae) also known as banana are one of the interesting tropical fruits and important sources of food in the world [7], [8]. There are hundreds of banana varieties in the world, and in Indonesia itself there are over 230 varieties of banana [8]. One from 230 species of bananas in indonesia is *Mauli bananas. Mauli bananas* is one of the typical species of bananas which is only found only in South Kalimantan, Indonesia. *Mauli bananas* are very popular among the people because the flavour of that bananas is delicious and sweet [9].

All parts of banana plant are beneficial to mankind in the medical aspects and ornamental uses. The result of Tan EE Shian research showed that banana are known as a weak primary antioxidant source but a powerful secondary antioxidant source [5]. Banana fruit contains various antioxidant components such as ascorbic acid, vitamin E, β-carotene, and flavonoid [4]. The result of Singhal research on banana *(Musa acuminata)* peel exctract indicated that banana peel is potential source of bioactive compounds like flavonoids and polyphenols with wide range of medicinal properties in particular the high free radical scavenging activity [10]. In the other hand there is no literature shows the used of banana stem especially for antioxidant sources. Because of that in this research we determined the levels of bioactive compound and antioxidant activity on banana stem.

II. Determination of Antioxidant, Phtochemical and Antioxidant Activity

A. Chemical and Materials

1% metaphosphoric acid, 2,6-dichlorophenolindophenol, acetone–hexane, qurectin solution, 1mM FeCl3, 2 mM FeCl2, 0,1 N HCl, dried methanolic exctract, 0,008 M potassium ferrocyanide, distilled water, 0,1 M $FeCl_3$, acetic acid, ethanol, ammonium hydroxide, diethyl eter, n-butanol, methanol, 1mM 1,10- phenanthroline, 0.2 M phosphate buffer (pH 7.8), 0.17 M H2O2, NaNO2, and AlCl3 were from Sigma.

B. Plant Material

Stem of *Mauli bananas* was collected from South Kalimantan Province, Indonesia. Botanical identification was performed by Department of Biological Science, Faculty of Science, Lambung Mnagkurat University. The active principles in the *Mauli bananas* stem were extracted by maceration.

Manuscript received October 15, 2013; revised December 20, 2013.

Maharani Laillyza Apriasari is with School of Medicine, Lambung Mangkurat university, South Kalimantan, Indonesian (e-mail: maharaniroxy@gmail.com).

Eko Suhartono is with Medical Chemistry and Biochemistry Department, School of Medicine, Lambung Mangkurat of University, South Kalimantan, Indonesian (e-mail: ekoantioxidant@gmail.com).

Iskandar is with Research Unit Mutiara Bunda Mother and Child Hospital, Martapura of South Kalimantan, Indonesian (e-mail: Iskandarthalib@gmail.com).

C. Total Flavonoid Content

Flavonoids is determine using Aluminum chloride colorimetric method [11]. The calibration curve is made by preparing quercetin solutions at different concentrations. Each experiment is carried out in triplicate and results averaged expressed as mean ± SD.

D. 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) is mixed with 9 ml of 2,6-dichlorophenolindophenol and the absorbance is measured within 30 min at 515 nm against a blank. Content of ascorbic acid is 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 [12].

E. 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 [12].

F. Tannin Content

Tannin measured according to the method of Van-Burden and Robinson (1981) 500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min [13].

G. Alkaloid Content

Alkaloid determination using Harborne (1973) method: 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a waterbath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed [13].

H. Saponin Content

The method used by Obadoni and Ochuko (2001). The samples were ground and 20 g of each were put into a conical flask and 100 cm3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4h with continuous stirring at about 55 ℃. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 ℃. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated [13].

60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a waterbath. After evoporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage [13].

I. Flavonoid Content

Flavonoid determination by the method of Bohm and Kocipai- Abyazan (1994). 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight [13].

J. Hydroxyl Radical Scavenging Activity

The scavenging activity for hydroxyl radicals was measured with Fenton reaction [14]. The absorbance of the mixture at 560nm 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.

K. Chellating Effect of Ferrous Iron

The chelating effect of ferrous ions was estimated by the method of Hung-Ju Chou *et al*. [15] 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.

L. Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging was determined according to the method of Ruch *et al* [16]. 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 triplicateand results averaged expressed as mean ± SD.

III. Result and Discussion

The bioactive compound in *Mauli bananas* stem was evaluated (Table I). The result showed that the *Mauli bananas* stem contained biaoctive compound. The highest bioactive compound in *Mauli bananas* stem is tannin, and then followed by ascorbic acid, saponin, β-carotene, toal

flavonoid, lycopene, alkaloid and flavonoid.

The antioxidant activity of *Mauli bananas* stem was evaluated. The result shows in Table II. The chellating of ferrous iron is a highest antioxidant activity in *Mauli bananas* stem and followed by hydrogen peroxide scavenging activity and hydroxyl scavenging activity.

TABLE I: ANTIOXIDANT AND PHYTOCHEMICAL CONTENT IN *MAULI BANANAS* STEM

Active Constituent	Concentration
Ascorbic acid (mg/mL)	0,44 ±0,001
β- Carotene (mg/100 mL)	0,066 ±0,012
Lycopene (mg/100 mL)	0,006 ±0,002
Total Flavonoid (mg EQ/ gr)	0,032 ±0,001
Saponin	14,494% ±1,032%
Alkaloid	0,347% ±0,003%
Flavonoid	0,253% ±0,002%
Tannin	67,594% ±2,171

TABLE II: ANTIOXIDANT ACTIVITY IN *MAULI BANANAS* STEM

Antioxidant Activity	IC 50 %*	R^2
Hydroxyl Scavenging Activity	0,543 %	0,964
Chellating Effect of Ferrous Iron	1,318 %	0,924
Hydrogen Peroxide Scavenging Activity	1,296 %	0,943

*) : *Musa acuminata* stem extract concentration that could inhibited 50% of hydroxyl radical, iron and hydrogen peroxide.

A. Ascorbic Acid

Ascorbic acid is a water soluble vitamin which is known as protect essential substances in the body such as proteins, lipids, carbohydrates and DNA and RNA from impact of free radicals [17]. Ascorbic acid contributes to a variety of other biochemical functions, such as amino acid, carnitine and cathecolamine biosynthesis for nervous sytem regulation, helps the body for iron absorp and to break down hystamine which is a component of allergic reaction [18].

Ascorbic acid facilitates intestinal absorption of iron and functions as a cellular antioxidant alone and coupled to the antioxidant activity of vitamin E [19]

As an antioxidant, ascorbic acid activity is expressed through a variety of mechanisms, which still await to be fully clarified. In principal it acts by scavenging ROS directly, and among these species probably the most important are superoxide and peroxynitrite. Secondly, it has been shown that ascorbate can recycle alpha-tocopherol, which in turn helps to prevent lipids oxidation. Without ascorbate, the alpha-tocopheroxyl radical can assume a prooxidant role and continue or even enchance the chain reaction of lipid peroxidation [20].

The important role of ascorbic acid within the network of recycling antioxidants has been extensively investigated and although known since long time, the studies on this vitamin are far to be complete. For example, we have recently highlighted its possible role in the transport of drugs, which do not cross itself the blood brain barrier. In these regards, it may act as a double targeting molecule, working both as a carrier and as antioxidant in neurodegenerative diseases [20].

B. β-Carotene and Lycopene

Carotenoids, such as lycopene and β-carotene, are natural constituents of many plants and may protect against disease [21]. As a carotenoid compound is a group of pigments and natural antioxidants, which can reduce free radicals, which cause the color yellow orange and red on the plant. Some benefits of carotenoids are vitamin A precursors, antioxidants, increase immunity, and metabolic alteration of cancer [22].

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 [23].

β-Carotene is commonly known as a radical 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 [24].

Several *in vitro*, animal and human experiments have demonstrated the antioxidant properties of carotenoids such as β-carotene. When human dermal fibroblasts were exposed to UVA exposure, β-carotene was able to suppress the up-regulation of heme oxygenase-1 gene expression in a dose dependent manner. 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 μM augmented UVA-induced haem oxygenase-1 induction indicating a pro-oxidant effect. Similarly, in another study β-carotene at a concentration of 10 μM increased the production of ROS and the levels of cellular oxidized glutathione in leukaemia and colon adenocarcinoma cell lines *in vitro*. 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. Recently reported human studies also support the pro-oxidant properties of β-carotene. Supplementation of β-carotene at pharmacological levels increased lung cancer incidences in smokers in the Alpha-Tocopherol Beta-Carotene (ATBC) trial and increased mortality from CVD in a group of smokers, former smokers and asbestos exposed individuals in the β-carotene and retinol efficiency trial (CARET). These observations suggest a possible biphasic response of β-carotene that promotes health when taken at dietary levels, but may have adverse effects when taken in higher amounts [23].

Lycopenes are bioflavonoids that are closely related to β-carotene. In human serum, lycopene is the dominant carotenoid and constitutes approximately 50 percent of all carotenoids found in the serum. Lycopene is a powerful antioxidant and it is about more powerful twice than β-carotene [25].

Lycopene, a member of the carotenoid family of phytochemicals is a lipid soluble antioxidant that is

synthesized by many plants and microorganisms but not by animals and human. It is a highly unsaturated open straight chain hydrocarbon consisting of 11 conjugated and 2 unconjugated double bonds. It is responsible for the red color of many fruits and vegetables such as the tomatoes [23].

The antioxidant property of lycopene has been the main focus of research to study its biological role. However, it has also been shown to exert its effect via other mechanisms that include gene function regulation, gap-junction communication, hormone and immune modulation, carcinogen metabolism and metabolic pathways involving phase II drug-metabolizing enzymes. An extensive review of both the antioxidant mechanisms and other molecular mechanisms has recently been reviews [23].

C. Other Bioactive Compound

In the past decades, secondary metabolites such as saponin, tannin, flavonoid, alkaloid and many others have contributed a lot in the production of new drugs to cure different types of diseases [26].

Tannins are believed to have some general antimicrobial and antioxidant activities where at low concentrations it can inhibit bacterial growth and act as an antifungal agent at higher concentrations. Tannins are known as polymeric phenolic substances because of its capability of precipitating gelatin from solution which is known as astringency [26]. Tannins do not act as pro-oxidants and in fact react very rapidly to quench the hydroxyl radical. *In vivo* studies have shown that tannin protein complexes in the gastrointestinal tract provide persistent antioxidant activity [27].

Saponins are natural detergents found in many plants. Saponins have detergent or surfactant properties because they contain both water-soluble and fat-soluble components. They consist of a fat-soluble nucleus, having either a steroid or triterpenoid structure, with one or more side chains of water soluble carbohydrates [28]. Recently, there have been a tremendous commercially driven promotion of saponins as dietary supplement and nutraceuticals and there is evidence of the presence of saponins in traditional medicine preparation [29]. Some studies suggest used of saponins in medical treatment such as epilepsy, excessive salivation, chlorosis and migraines [26].

Recently, it has been reported that flavonoids possess many pharmacological properties like antifungal, antioxidant, antiallergenic, anti-inflammatory, antithrombic, anticarcenogenic and hepatoprotective that narrows researcher interest to work on this secondary metabolites. Since ancient times, flavonoids have been used as antiinflammatory and for cosmetic purposes in the Chinese traditions. The inconsistent in the results obtained in the antimicrobial activities could be due to the absence of other active compounds like alkaloids [26].

Flavanoids possess two aromatic rings and are better scavengers. An important effect of flavonoid is the scavenging of oxygen-derived free radicals. Flavonoids such as quercetin diminish oxidative damage by enhancing the concentration of glutathione. In addition, quercetin blocks Ca^{2+} influx, thus blocking Ca^{2+} channels responsible for cell death. Multiple mechanisms of action have been also recently proposed in mediating cardiovascular effects of flavonoids. These include metal complexation, inhibition of xantine oxidase activity (which generates superoxide anions) as well as chemical quenching of ROS. Selected flavonoids scavenge the highly reactive oxygen derived radicals called peroxynitrite [27].

D. Hydroxyl Radical Scavenging Activity

OH radical is an extremely reactive free radical formed in the biological system and has been implicated as highly harm species in free radical pathology, ability of damaging almost included every molecule found in the living cells and intiator of lipid peroxidation [4].

Unlike superoxide, which can be detoxified by superoxide dismutase, the hydroxyl radical cannot be eliminated by an enzymatic reaction [27]. It has been reported that the diffrent exctract of banana has a hydroxyl radical scavenging activity [4].

The ability of the banana components to quench hydroxyl radical seems to be directly related to the prevention of lipid peroxidation and appears to be moderate scavenger of active oxygen species, thus reducing rate of chain reaction [4].

E. Chellating Effect of Ferrous Iron

Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydro peroxide decomposition reactions via Fenton reaction [3]. Fenton reaction is a reaction which is the hydroxyl radicals produced by the reaction between hydrogen peroxide with iron. The hydroxyl radical can react at diffusion-limited rates with various biomolecules, including lipids, proteins, and DNA. This implies that shielding of iron from molecular oxygen and the surrounding media is a critical event in preventing iron-mediated oxidative stress [30].

It has been demonstrated that in the presence of Fe(III) or Fe(III)–EDTA complex, endogenous reductants such as ascorbate, glutathione (GSH), and the reduced form of nicotineamide adenine dinucleotide (NADH), caused DNA damage at every type of nucleotide with a slight dominance by guanine. Specifically, NADH in the presence of Fe(III)–EDTA and H_2O_2 generated radical hydroxyl leading to formation of 8-oxo-dG. The DNA damage was inhibited by typical radical hydroxyl scavengers and by catalase, suggesting that these reductants cause DNA damage *via* the Fenton reaction [31]-[33]. It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion such as iron [27].

The research of Darsini (2012) suggests that in the banana extract contained some component that chellating the ferrous iron. The component can donors the electron that could react with free radical and convert them into more stable products [4]. Padam (2102) also reported that the diffrent extract of banana has a chellating effect of ferrous iron [33].

F. Hydrogen Peroxide Scavenging Activity

Environmental stresses are known to induce hydrogen peroxide and other toxic oxygen species production in cellular compartments and result in acceleration of lipid peroxidation and other oxidative damage. Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which

can donate electrons to H_2O_2, thus neutralizing it to water [3]. Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems [26].

In human body, hydrogen peroxide is produced in many different cell types, including fibroblast, vascular endothelial, smooth muscle, and inflammatory cells. It is known to act as a cellular signaling molecule within blood vessels, and it plays key roles in regulating vascular smooth muscle cell (VSMC) growth, differentiation, migration, and vascular inflammation. Hydrogen peroxide has been shown to cause constriction in a variety of vascular beds under quiescent conditions, and it can induce vasoconstriction in a number of arteries in vitro, including rat aorta, vena cava and pulmonary artery, canine basilar artery, and human placental arteries [34], [35].

IV. Conclusions

In this present study, the bioactive compound and antioxidant activity of *Mauli bananas (Musa sp)* stem has evaluated. The result of this study shows that *Mauli bananas* stem contained a bioactive compound such as ascorbic acid, *β*- carotene, lycopene, tannin, saponin, and flavonoid. *Mauli bananas* stem also have moderate to potent antioxidant activities and/or free radical scavenging activity.

References

[1] M. Agarwal, R. Gupta, and S. Upadhyaya, "Exctraction of polyphenol, flavonoid from Embilica officinalis, Citrus limon, Cucumis sativus and evaluation of their antioxidant activity," *Orient. J. Chem*., vol. 28, pp. 993-998, 2012.

[2] N. Apriani, E. Suhartono, and I. Z. Akbar, "Correlation between duration of hyperglycemia and Advanced Oxidation Protein Product (AOPP) level of bone on hyperglycemia white rats (*Rattus Norvegicus*) induced with streptozotocin (STZ)," *JKM*, vol. 11, pp. 48-55, 2011.

[3] M. A. Ebrahimzadeh, S. M. Nabavi, S. F. Nabavi, F. Bahramian, and A. R. Bekhradnia, "Antioxidant and free radical scavenging activity of *H. Officinalis L. Var. Angustifolius, V. Odorata, B. Hyrcana and C. Speciosum*," *Pak J. Pharm. Sci.*, vol. 23, pp. 29-34, January 2010.

[4] D. T. P. Darsini, V. Maheshu, M. Vishnupriya, and J. M. Sasikumar, "I*n Vitro* antioxidant activity of banana (*Musa* spp. ABB *cv*. Pisang Awak)," *Indian J. Biochem. Biophys.*, vol. 49, pp. 124-129, April 2012.

[5] T. E. Shian, A. Abdullah, K. H. Musa, M. Y. Maskat, and M. A. Ghani, "Antioxidant properties of three banana cultivars (*Musa Acuminata 'Berangan', 'Mas',* and *'Raja'*) extracts," *Sains Malaysiana*, vol. 41, no. 3, 2012.

[6] K. Wolfe *et al*., "Antioxidant activity of apple peels," *J. Agric. Food Chem.*, vol. 51, no. 3, pp. 609–614, 2003.

[7] P. Karuppiah and M. Mustaffa, "Antibacterial and antioxidant activities of Musa sp. Leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection," *Asian Pac J Trop Biomed*., vol. 3, no. 9, pp. 737-742, 2013.

[8] I. Orhan, "Biological activities of *Musa* species," *J. Fac. Pharm.*, vol. 30, no. 1, pp. 39-50, 2001.

[9] M. Yulianty *et al*., "Karyotype of *Mauli* bananas," *Bioscientiae.*, vol. 3, no. 2, pp. 103-109, July 2006.

[10] M. Singhal and P. Ratra, "Antioxidant activity, total flavonoid and total phenolic content of *Musa acuminata* peel exctracts," *Global J. Pharmacol*., vol. 7, no. 2, pp. 118-122, 2013.

[11] C. Chang, M. Yang, H. Wen, and J. Chern, "Estimation of total flavonoid content in propolis by two complementary colorimetric methods," *J. Food Drug Analaysis*., vol. 10, pp. 178-82, 2002.

[12] L. Barros, M. J. Ferreira, B. Queiro, I. Ferreira, and P. Baptista, "Total phenols, ascorbic acid, b-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities," *Food Chemistry*, vol. 103, pp. 413–419, 2007.

[13] H. O. Edeoga, D. E. Okwu, and B. O. Mbaebie, "Phytochemical constituents of some nigerian medicinal plants," *Afr. J. Biotechnol.*, vol. 4, no. 7, pp. 685-688, July 2005.

[14] G. P. Ganu, S. S. Jadhav, and A. D. Deshpande, "Antioxidant and antihyperglycemic potential of methanolic extract of bark of Mimus Elengi L in mice," *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, vol. 1, no. 3, pp. 65-77, 2010.

[15] H. J. Chou, J. T. Kuo, and E. S. Lin, "Comparative antioxidant properties of water extracts from different parts of beefsteak plant (Perilla frutescens)," *Journal of Food and Drug Analysis*, vol. 17, no. 6, pp. 489-96, 2009.

[16] R. J. Ruch, S. J. Cheng, and J. E. Klaunig, "Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea," *Carcinogenesis.*, vol. 10, pp. 1003–1008, 1989.

[17] O. O. Elekofehinti and I. J. Kade, "Aqueous extract of *Solanum anguivi* Lam. Fruits (African Egg Plant) inhibit Fe^{2+} and SNP induced lipid peroxidation in Rat's brain – in vitro," *Der Pharmacia Lettre.*, vol. 4, no. 5, pp. 1352-1359, 2012.

[18] M. Cioroi, "Study on L-ascorbic acid contetnts from exotic fruits," *Cercetări Agronomice în Moldova Anul XXXX.*, vol. 1, no. 129, pp. 23-27, 2007.

[19] K. M. Phillips, M. T. Tarrago, S. E. Gebhardt, J. Exler, K. Y. Patterson, D. B. Haytowitz, P. R. Pehrsson, and J. M. Holden, "Stability of vitamin C in frozen raw fruit and vegetable homogenates," *Journal of Food Composition and Analysis*, vol. 23, pp. 253–259, 2010.

[20] S. Vertuani, A. Angela, and M. Stefano, "The antioxidants and pro-antioxidants network: An overview," *Current Pharmaceutical Design*, vol. 10, pp. 1677-1694, 2004.

[21] K. Muzandu, K. E. Bohi, Z. Shaban, M. Ishizuka, A. Kazusaka, and S. Fujita, "Lycopene and beta carotene ameliorate catechol estrogen mediated DNA damage," *Jpn. J. Vet. Res.*, vol. 52, no. 4, pp. 173–184, 2005.

[22] D. T. Panjaitan, B. P. Prasetyo, and L. Limantara, "The role of carotenoid to prevent a free radical inside the body," Ph.D. dissertation, Dept. Biology., Satya Wacana christian Univ., Malang, Indonesia, 2012.

[23] A. V. Rao and L. G. Rao, "Carotenoids and human health," *Pharmacological Research*, vol. 55, pp. 207-216, 2007.

[24] V. Chiosa, C. Mandravel, J. C. S. Kleinjans, and E. Moonen, "Determination of β-carotene concentration in orange and apple juice and in vitamin supplemented drinks," *Chimie Anul XIV (serie nouă.)*, vol. I-II, pp. 253-258, 2005.

[25] A. Azis, "Development of HPLC analysis for detection of lycopene in tomato and crude palm oil," *University College of Engineering & Technology Malaysia*, 2006.

[26] V. Sumathy, S. J. Lachumy, Z. Zakaria, and S. Sasidharan, "*In vitro* bioactivity and phytochemical screening of *Musa acuminata* flower," *Pharmacologyonline*, vol. 2, pp. 118-127, 2011.

[27] M. Parimala and F. G. Shoba, "Phytochemical analysis and *in vitro* antioxidant acitivity of hydroalcoholic seed extract *of Nymphaea nouchali Burm. F*," *Asian Pac J Trop Biomed*, vol. 3, no. 11, pp. 887-895, 2013.

[28] P. R. Cheeke, "Actual and potential applications of Yucca schidigera and Quillaja saponaria saponins in human and animal nutrition," *J Anim Sci*., vol. 77, pp. 1-10, 2000.

[29] O. O. Elekofehinti, I. G. Adanlawo, A. Fakoya, J. A. Saliu, and S. A. Sodehinde, "Effects of saponin from solanum anguivi lam fruit on heart and kidney superoxide dismutase, catalase and malondialdehyde in rat," *Curr. Res. J. Biol. Sci*., vol. 4, no. 4, pp. 530-533, 2012.

[30] E. Suhartono, E. Viani, M. A. Rahmadhan, I. S. Gultom, M. F. Rakhman, and D. Indrawardhana, "Screening of medicinal plant fot total flavonoid and antioxidant activity in South Kalimantan of Indonesian," *International Journal of Chemical Engineering and Applications*, vol. 3, no. 4, pp. 297-299. 2012.

[31] J. M. Moreno, I. R. Gomez *et al*., "Mechanisms of hydrogen peroxide vasoconstriction in the isolated perfused rat kidney," *Physiol Pharmacol*, vol. 61, no. 3, pp. 325-332, 2010.

[32] M. Valko, H. Morris, and M. T. D. Cronin, "Metals, toxicity and oxidative damage," *Current Medicinal Chemistry*, vol. 12, no. 10, 2005.

[33] M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur, and J. Telser, "Free radicals and antioxidants in normal physiological function and human disease," *IJBCB*, vol. 39, pp. 44-84, 2007.

[34] B. S. Padam, H. S. Tin, F. Y. Chye, and M. I. Abdullah, "Antibacterial and antioxidative activities of the various solvent extracts of banana (*Musa paradisiaca cv. Mysore*) inflorescenses," *J. Biol. Sci*, vol. 12, no. 2, pp. 62-73, 2012.

[35] J. M. Moreno, I. R. Gomez et *al*., "Mechanisms of hydrogen peroxide vasoconstriction in the isolated perfused rat kidney," *Physiol Pharmacol*, vol. 61, no. 3, pp. 325-332, 2010.

Maharani Laillyza Apriasari was born in Surabaya, Indonesia, in April 1977. She received her dentist in 2000 and Oral Medicine Specialist in 2010 from Airlangga University, Surabaya, Indonesia. She has published more than 20 scientific journal or conference papers. In 2011, she has published the research in Federation Dental International joint meeting conference.

Eko Suhartono was born in Surabaya, Indonesia, in September 1968. He received his Drs. and M. Sc degrees in 1991 and 1998 from Gadjah Mada University, Yogyakarta, Indonesia. He currently study environmental science and technology graduate program in Brawijaya University, Malang, Indonesia. His research is mainly focused on free radical and natural product antioxidant, ecotoxicology. He has published more than 40 scientific journal or conference papers.

Iskandar was born in Jakarta, Indonesia, in April 1987. He received his medical doctor in 2011 from Lambung Mangkurat University, Banjarbaru, Indonesia. He is currently a general physician in Mutiara Bunda Mother and Child Hospital, Martapura, Indonesia. His first publication in 2012 and was presented at one scientific meeting in dubai UAE.