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1st INTERNATIONAL CONFERENCE ON BIODIVERSITY, FOOD SECURITY AND HEALTH

22-23 November 2016
Gadjah Mada University, Yogyakarta

Editor:
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PREFACE FROM ORGANIZING COMMITTEE

The 1st International Conference on Biodiversity, Food Security, and Health took place at the Grha Sabha Pramana and University Club Hotel, Gadjah Mada University from Tuesday to Wednesday, 22 to 23 November 2016, and was attended by approximately 125 participants coming from Indonesia, Malaysia, Vietnam, India, Nigeria, Philiphine, Thailand, as well as other countries. This conference was organized by Center for Food and Nutrition Studies in collaboration with Faculty of Agricultural Technology UGM. This conference was also in conjunction with the 10th Global Conference of Regional Centres of Expertise.

The conference was opened by Prof. Dr. Suratman, MS., Vice Rector for Research and Community Services UGM and Prof. Dr. Ir. Umar Santoso, M.Sc., the Head of Center for Food and Nutrition Studies UGM. The keynote speaker for this conference was Dr. Drs. Sugeng Priyanto, M.Si. from Ministry of Environment and Forestry Indonesia. The invited speakers of this conference were Prof. Gerard Christopher Bodeker from Global Initiative For Traditional Systems (GIFTS) of Health, Oxford, UK, Dr. Unnikrishnan Payyappallimana from UNU-IAS, Prof. Dr. Ir. Eni Harmayani, M.Sc. (UGM), Prof. Dr. Ir. Murdijati Gardjito, MS. (UGM), Prof. Mohammad Na'iem (UGM), Prof. Subagus Wahyuono (UGM), and Dr. Ir. Arman Wijanarko, M.Sc. from PT. Pagilaran (tea plantation and industry).

There were 85 oral presentation and 16 poster presentation which were divided into 4 sub-theme namely Agrobiodiversity and Agroforestry; Food Security and Safety; Food Technology; and Human Health and Nutrition. Several papers were included in this proceeding while others will be published in the Indonesian Food and Nutrition Progress Journal. The success of the 1st ICBFSH can be attributed to the efforts of the Steering Committee, chaired by Prof. Dr. Ir. Eni Harmayani, M.Sc.; the Organizing Committee, chaired by Dr. Lily Arsanti Lestari; and our sponsor Publisher and Publication Board UGM and PT Tiga Pilar Sejahtera. Hopefully this

proceeding can give more information of biodiversity to the academician, researcher, institution, and also to the community.

Chairperson of the Organizing Committee

Dr. Lily Arsanti Lestari

PREFACE FROM EDITOR

About three years past - in July 2014, the UN General Assembly's Open Working Group (OWG) on Sustainable Development Goals (SDGs) forwarded a proposal for the SDGs to the Assembly. The proposal contained 17 goals covering a broad range of sustainable development issues, one of the issue is **Life on Land**, *i.e.*, to protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt **biodiversity loss**.

Biodiversity is variety and variability of life on Earth. The number and variety of plants, animals and other organisms that exist is known as biodiversity. It is an essential component of nature and it ensures the survival of human by providing food, medicines, fuel, shelter, and other resources to mankind. If the biodiversity is lost then our life is threatened. Biodiversity's relevance to human health is becoming a global issue, as scientific evidence builds on the global health implications of biodiversity loss.

Biodiversity plays an important on the sustainable productivity of soils and provides the genetic resources for all crops, livestock, and marine species harvested for food, thus biodiversity directly influence on food security. By definition, **food security** is an existence *"when all people at all times have access to sufficient, safe, nutritious food to maintain a healthy and active life"* (WFS, 1996*)*. Food security is threatened by biodiversity loss because the production and availability of food depend on plant, animal and other organism life. Biodiversity and **health** are linked at many levels; the ecosystem, with food production as an ecosystem service; the species in the ecosystem and the genetic diversity within species. Nutritional composition between foods and among varieties/cultivars/breeds of the same food can differ significantly, affecting micronutrient availability in the diet. Healthy local diets, with adequate average levels of nutrients intake, requires maintenance of high biodiversity levels. Biodiversity provides critical support for drug discovery and the availability of medicinal resources.

Based on the importance of three keywords mentioned above, *Center for Food and Nutrition Studies (CFNS) UGM* in collaboration with *Faculty of Agricultural Technology UGM* held the ***1th International Conference on Biodiversity, Food Security and Health*** in 22-23 November 2016, this event was in conjunction with the *10th Global RCE Conference* held in Yogyakarta, Indonesia. The objectives are to improve awareness of the importance of biodiversity related to many aspects, and to contribute in making strategy to conserve biodiversity while improving food security and health, and to disseminate results of relevant research and development related with the topic of Conference.

This Proceeding contained papers and abstracts of papers that have been presented in oral or poster presentation in this Conference, but not all, some authors requested not to include their papers in the Proceeding for some rational reasons. There are 7 abstracts from the invited speakers and 35 original research papers in this Proceeding. The titles of the abstracts are **Traditional medicine and medicinal plant biodiversity** (by Prof. Gerard Bodeker), **Rehabilitation of degraded forest to support food and wood security program in Indonesia** (Prof. Mohammad Na'iem), **Searching bioctive compounds from Indonesia medicinal plants** (Prof Subagus Wahyuono), **Ethnobotany, Community Health & Nutrition - Policy-Practice Linkages** (Dr. Unnikrishnan Payyappallimana), **Traditional Food Conservation to Support Biodiversity and Sustainable Food and Nutrition Security** (Prof. Eni Harmayani), **Agrobiodiversity Concept, Its Relevance in Farmers Family Welfare** (Prof. Murdijati Gardjito), and **Biodiversity in Tropical Climate Tea Management System in Indonesia** (Dr. Arman Wijanarko).

The original research papers are divided into 4 sections, *i.e*, Section I : Agrobiodiversity and Agroforestry (AA), Section II : Food Security and Safety (FS), Section III: Food Technolgy (FT), and Section IV: Human Nutrition and Health (HN). In the Section I we can find papers related to agrobiodiversity or agroforestry aspects such as Conflicting or Combinative – Human and Natural Values at Kathotiya, Central India; Biodiversity Assesment of Mangrove in Pasuruan District, East Java; Traditional Red Rice Grain Characteristics Still Cultivated In Regencies of South Sulawesi, and so on. In the Section II we can read papers including Chemical, Biological Activity and Heavy Metal Content

of Sea Cucumbers from Karimunjawa and Lampung's Marine, Indonesia; Pathogenic Bacteria Contamination of Loin Bali Cattle That Slaughter at Modern and Traditional System; Promoting Sustainable Agriculture in Pekalongan, Indonesia: Coastal Farmers Choices; and so forth. Papers with aspect of food technology in the Section III included Exterior and Interior Egg Quality of Muscovy Duck (*Cairinamoschata*) Reared Traditionally in Yogyakarta; Copigmentation of Anthocyanin Extract of Java Prune (*Kopsiapruniformis*) Fruit with Quercetin to Increase the Colour Stability; Effect of Autoclaving-cooling Cycle on Resistant Starch Content and Functional Properties of Gayam(*Inocarfusfagifer* Forst.) Flour; Biofilm-forming Ability and Resistance to Disinfectants of Samples Collected from Seafood Processing Plants; and so on. Papers in the Section IV including Protective Effect of Tropical Fruit Juice on Histopathological Image of Rats Lung Exposed to Cigarette Smoke; Antioxidant Activity of the EthanolicExtracs of Peel and Flesh of *Coleus tuberosus;* and The Effectiveness of Various Salacca Vinegar as Therapeutic Agent for Management of Hyperglycemia and Dislipidemia on Diabetic Rats.

In conjuction with the Conference we held a round table discussion (RTD) that discuss a concept of strategy to conserve Biodiversity while improving food security and health especially in Indonesia; the conclusion of the RTD is in the last section of this Proceeding. We do hope that this Proceeding can be used as a scientific document and may have contribution to enrich the knowlegde and development of biodiversity especially that related with food securiy and health, and also may provide inspiration for further research in related topics.

Finally, the Editors would like to express deep gratitude to all invited speakers and contributors for their valuable contribution of papers and all participants of the Conference. Special thank is also expressed to all peer reviewers and editing staff for their hardwork that made this Proceeding could be realized.

May 2017

Umar Santoso - Chief Editor

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HN-03

ANTIOXIDANT ACTIVITY OF THE ETHANOLIC EXTRACS OF PEEL AND FLESH OF *COLEUS TUBEROSUS*

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Abstract

The aim of this study was to know total phenolic and flavonoid content and the ability of the scavenging of free radicals in chemical tubes and in biological systems. Determination of total phenolic and flavonoid contents were done by spectrophotometric method. The method used by the chemical of tube is 1.1-diphenyl-2picrylhydrazyl (DPPH) and biological systems by the method of cellular antioxidant activity based on the oxidation of 2 ', 7'-dichlorofluorescein-diacetate (DCFHDA) by reactive oxygen species (ROS) in Hela cancer cells. The results showed that total phenolic content on peel and flesh were 6.10 ± 0.02, and 1.77 ± 0.01 mg of GA/g of extract. respectively. Flavonoids content on peel and flesh were 3.08 ± 0.05, and 0.26 ±0.01 (mg of quercetin/g of extract, respectively. The ethanolic extract of peel of Coleus tuberosus (EEPC) has higher antioxidant activity than the ethanolic extract of flesh of Coleus tuberosus (EEFC) evaluated by DPPH and cellular antioxidant method. The IC_{50} of EEPC was 310.97±0.32 and FEC was 1290.00 ± 1.58. The decrease of percentage of ROS on 100, 200, 400 and 800 µg/ml of EEPC were 44.32±0.35; 52.52±0.24; 72.77±0.31, and 80.15±0.82 respectively. The decrease of percentage of ROS on 100, 200, 400 and 800 µg/ml of EEFC were 25.67±0.18; 42.98±0.22; 59.12±0.28, and 66.27±0.37, respectively. This results shows that the extract of Coleus tuberosus has potential as a source of natural antioxidants.

Keywords: antioxidant activity, Coleus tuberosus, flesh, peel

1. INTRODUCTION

Everyday, the human body always interact with reactive oxygen species (ROS), which is derived from UV rays, cigarette smoke, air pollution, radiation, drugs, metabolism of the human body, and inflammation that can react and cause damage and mutations in the cells, oxidizes carbohydrates, lipids, proteins and DNA (Borek *et al*., 2004). Reactive oxygen species ranked highest as the main cause of the disease. Included in the ROS are

superoxide anion radicals ($O_2 \bullet -$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$).

Although the human body has antioxidants defense system namely antioxidant enzymes (superoxide dismutase, Catalase, Glutathione Peroxidase), vitamin E, beta-carotene, vitamin C, which has a molecular structure that can donate electrons to molecules ROS without disturbing the stability of the molecule and can break the chain reaction from free radicals, but the body can still suffer from oxidative stress. Oxidative stress occurs in cells or tissue resulting from an imbalance between the production or concentration of ROS and antioxidant ability in cells that can lead to oxidative damage (Manda *et al*., 2009). Reactive oxygen species can be captured by the body's antioxidant defense system and phytochemical compounds, such as phenolic, triterpenic acids, flavonoids, carotenoids, vitamin found in fruits and vegetables.

Several studies have shown that phytochemical compounds from plants can act as an antioxidant that can prevent the accumulation of ROS and have a positive impact on the prevention of disease. Research showed that antioxidants may have a positive impact on the prevention of disease also showed an association shortage of consumption of fruits and vegetables against the increased risk of cancer. The consumption of fruits and vegetables provide a protective effect against the occurrence of degenerative diseases. The efforts to protect the body against free radicals is by increasing plasma antioxidant capacity. This can be done by consuming vegetables and fruits that contain phytochemical compounds that have the ability as an antioxidant (Garinstein *et al*., 2009).

The growing demand for natural antioxidants in the food and cosmetics industries, encourage efforts to find the source of natural antioxidants. Numerous scientific investigations point at consecutive rich sources of antioxidants, both fruits and vegetables, but only few of them involve waste parts of fruits or vegetables, i.e. peels. Fruits and vegetables wastes and by-products, which are formed in great amounts during industrial processing, represent a serious problem, as they exert an influence on environment and need to be managed and/or utilized. On the other hand, they are very rich in bioactive components, which are considered to have a beneficial effect on health. So that the necessary efforts to provide information regarding the antioxidative potential of the peel of the fruit or vegetable in the hope that

the skins of fruits and vegetables is not only a waste, but it can be a source of bioactive compounds that can be used as a source of natural antioxidants.

Coleus tuberosus is a minor tubers are included in family of *Lamiaceae*, sub-family of *Ocimeae* and Tribe of *Nepetoide*. *Coleus tuberosus* classified in the group that shaped tuber vegetables. Based on ethnobotanical and filogenik then *Coleus tuberosus* 1b included in the group,which means the use of *Coleus tuberosus* not only asfood but also used in disease treatment (Sunarjono, 2009). Coleus tuberosus is a crop of potential as an alternative source of food carbohydrates. Some research suggests that peel and flesh of *Coleus tuberosus* contain bioactive compounds such ursolic acid, oleanolic acid, phenol and flavonoids, maslinic acid, Phytosterol: stigmasterol, beta-sitosterol, kampesterol (Mooi *et al*., 2010). Several methods were developed to determine the quality and quantity of these compounds. The method used to measure the antioxidant activity both fruit and vegetables and dairy products are DPPH (Kumaran *et al*., 2006), cell culture models of cellular antioxidant activity (Wolfe *et al*., 2008a; Wolfe *et al*., 2008b)

This aim of study was to determine the ability of the scavenging of free radicals with chemical tubes and in biological systems. The method used by the chemical of tube is 1.1-diphenyl-2-picrylhydrazyl (DPPH) and biological systems by the method of cellular antioxidant activity based on the oxidation of 2',7'- dichlorofluorescein-diacetate (DCFH-DA) by reactive oxygen species (ROS) in Hela cancer cells. Using of the method cellular antioxidant expected to be able to describe the complexity of biological systems and is an important tool to check out the food, phytochemicals and dietary supplements for potential biological activity, because activity model cellular antioxidant is considering making compound by the cell, distribution and efficiency of protection against free radicals under physiological condition of the body.

2. MATERIALS AND METHOD

2.1. Chemicals

Ethanol, 1.1-Diphenyl-2-picryl hydrazyl (DPPH), The ethanolic extract of peel of C*oleus tuberosus* (EEPC) and The ethanolic extract of flesh of *Coleus tuberosus* (EEFC), RPMI, 2,7-diacetate dichlorofluorescein (DCFH-DA), PMA from Sigma-Aldrich, Fetal Bovine Serum (FBS) from Gibco.

HeLa was obtained from ATCC. All other reagents and solvents were of analytical reagent grade

2.2. Sample preparation

The peel and flesh were separated peeling the Coleus tuberosus to the thickness approximately of 1-1.5 mm. The peel and flesh then dried using a cabinet dryer at 40°C for 24 hours. The dried peel and flesh milled and filtered by sieve to the mesh size of 80. The raw material then stored in a freezer (-20°C).

2.3. Extraction process

The peel and flesh of *Coleus tuberosus* flour were macerated with ethanol for 7 days (1:5), and then filtered using Whattman No. 1 and then evaporated by N_2 gas. The extract then dissolved in 1 ml of methanol, mixed and filtered with millex 0.45 µm.

2.4. Determination of total phenolic contents

The concentration of phenolic *Coleus tuberosus* was determined using spectrophotometric method (Singleton *et al*., 1999). Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% NaHCO3. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$. The samples were incubated in a thermostat at 45°C for 45 min. The absorbance was determined using spectrophotometer at λmax = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was analyzed (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g ofextract)

2.5. Determination of flavonoids content

The content of flavonoids in the examined plant extracts was determined using spectrophotometric method (Quettier *et al*., 2000). The sample contained 1 ml of methanol solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% $AlCl_3$ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at λmax = 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of rutin and the calibration line was construed. Based on the measured absorbance, the concentration of flavonoids was analyzed (mg/ml) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of quercetin equivalent (mg of quercetin/g of extract).

2.6. Determination of free radical scavenging activity

Evaluation by DPPH refers to (Singh *et al*., 2009) 2 ml of DPPH (0.1 mM in methanol) added with 300µL the peel or flesh extract of *Coleus tuberosus* (100, 200 and 400 µg/ml) in methanol, after 30 minutes monitoring at λ 517nm. Ascorbic acid and BHT at concentration 10, 20 and 40µg/ml used as a control standard. Experiment was done triplicates. IC_{50} values for determining the concentration required to scavenging of 50% DPPH free radicals.

2.7. Cell culture

Human cervix cancer cells (HeLa) was obtained from ATCC. Cells were cultured in the RPMI, supplemented with 10% heat-inactivated Fetal Bovine Serum and penicillin (100 units/ml-streptomycin (100µg/ml), using 75 cm^2 flasks in a 37°C in humidified 5% CO_2 incubator.

2.7.1. Cellular antioxidant activity in HeLa cancer cells.

In this study, the effect of the ethanolic extract flesh of *Coleus tuberosus* (EEFC) and the ethanolic extract of peel of *Coleus tuberosus* (EEPC) on reduction of oxidative stress in HeLa cells were evaluated on cellular antioxidant. The basis of the method reported by Chang *et al*. (2001), 2-,7-Dichlorofluorescin diacetate (DCFH-DA), a peroxide-sensitive dye

was used for the evaluation of oxidative stress in cells based on oxidation of DCFH-DA by Reactive Oxygen Species. In this study, HeLa cells were cultured in RPMI supplemented with 10% fetal bovine serum (FBS), 100units/mL penicillin, and streptomycin in an incubator at 37 °C, 5% CO_2, 95% air humidity. The cell suspensions (200 µl at the concentration of 10^5 cells/well) were seeded in and incubated with EEFC and EEPC (100, 200, 400 and 800µg/ml) for 20 min. Then cells were co-incubated with 25µM DCFH-DA in the absence or presence of 100ng PMA in darkness at 37 °C for 30 min. After incubation, cells were collected and washed once with ice-cold phosphate buffered saline (PBS), resuspended in 200 µl of the same PBS, and placed on ice in darkness until flow cytometry was carried out. The amounts of intracellular hydrogen peroxide were detected by BD flow cytometer. At least 10000 cells were analyzed for each test, and the observed fluorescence reflects the intracellular hydrogen peroxide level.

In this test, oxidative stress is induced by addition of PMA in the extracellular medium of the HeLa cancer cells. The antioxidant activity express on the reduction percentage of ROS generated in HeLa by exogenous PMA was calculated by the monitoring of the emitted fluorescence intensity (Fi).

The following relation was used

$$(Fit_O - Fit1) \times 100/(Fit_O - Fit2)$$

with Fit_O: control with oxidative stress; $Fit1$: treat cells; $Fit2$: control without oxidative stress (Muanda *et al*., 2011).

2.8. Stastistical analysis

The experiments were conducted in triplicate. Data shown were mean ± SD of three replications. Testing was performed by Anova, if there was a real difference followed by Least Significant Different.

3. RESULTS AND DISCUSSION

3.1. Phenol and flavonoid

Phenol and flavonoid content of peel and flesh extract of *Coleus tuberosus* were investigated. The level of phenols and contains phenols and flavonoids from extract peel and flesh of *Coleus tuberosus* were higher than

the flesh. This was in line with some research that showed the peel has higher bioactive compounds compared parts of the flesh (Nurliyana *et al*., 2010).

Table 1. Phenol and flavonoid content on the extract of peel and flesh of *Coleustuberosus*

Part of tuber	Phenol (mg of GA/g of extract)	Flavonoid (mg of quercetin/g of extract)
Peel	6.10 ± 0.02[b]	3.08 ± 0.05[b]
Flesh	1.77 ± 0.01[a]	0.26± 0.01[a]

3.2. The antioxidant activity with DPPH method

Antioxidant activity could be tested by measuring the power of free radical scavenging. Radical DPPH method using synthetic 1.1-Diphenyl-2-picryl hydrazyl (DPPH). DPPH can be done easily, then the DPPH is currently used for the measurement of free- radical-scavenging (Singh *et al*., 2009). The peel and flesh extracts of *Coleus tuberosus* measured in the hydrogen donates its ability or scavenging the radical using free radical 1.1-diphenylpricrylhydrazyl (DPPH).

The ability of antioxidants with DPPH method on the ethanolic extract of peel of *Coleus tuberosus* (EEPC) and the ethanolic extract of flesh of *Coleus tuberosus* (EEFC) indicated by the IC_{50}(Table 2). Inhibitory concentration 50 (IC_{50}) demonstrated ability to scavenging free radicals (DPPH) by 50%, the smaller the IC_{50} showed that the higher antioxidant activity. Based on the IC_{50} ethanolic extract peel of *Coleus tuberosus* in this study was higher than in the flesh.

Tabel 2. IC_{50} the extract peel and flesh of Coleus tuberosus with DPPH method

Compound	IC50 (µg/ml)
The ethanolic extract of peel (EEPC)	1290.00 ± 1.58[d]
The ethanolic extract of flesh (EEFC)	310.97 ± 0.32[c]
BHT	50.12 ± 0.53[b]
Ascorbic acid	19.28 ± 15[a]

Note: different notation means significant different $p < 0.05$.

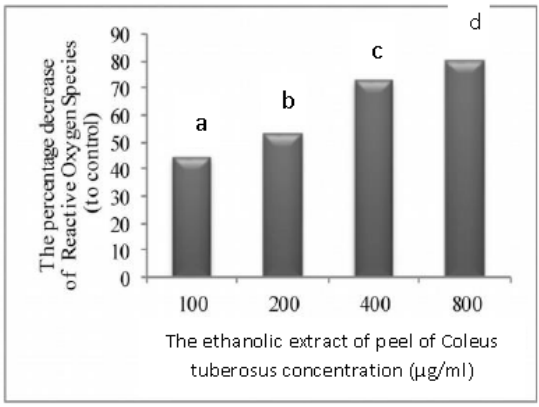
This study proved that the antioxidant activity (IC_{50}) of peel extract was higher than the flesh extract of *Coleus tuberosus*. The difference in antioxidant activity on the part of the flesh and the peel caused by bioactive

compounds such as phenolic acid, flavonoid. Phenol compounds have the ability to scavenging free radicals, it is evidenced by the strong correlation between the content of phenolic compounds and radical scavenging activity (O'Sullivan *et al*., 2011; Jung *et al*., 2011). The activity of scavenging free radicals was determined by the number of protons available for transfusion by the hydroxyl group and the structure of phenolic hydroxyl group on the benzene ring contribute to arrest free radicals (DPPH) (Fan *et al*., 2011).

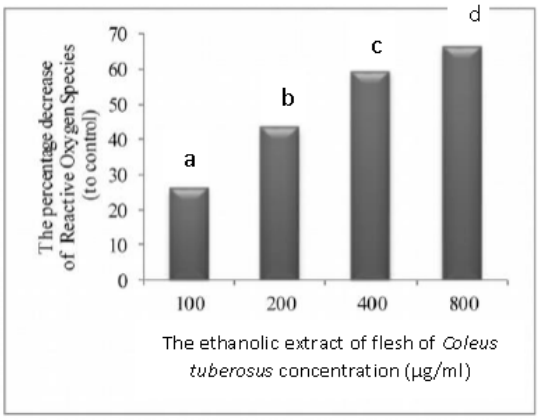
The difference in antioxidant activity between EEPC and EEFC based on DPPH method thought to be caused by differences in the content of bioactive compound (Table 1). The peel contains more bioactive compounds have a greater ability to transfer hydrogen atoms to free radicals (DPPH), so that the formation of diphenyl picrylhydrazyl compound was higher than in the flesh. The greater the DPPH compound formed showed greater antioxidant ability, especially scavenging free radicals. Research showed that the difference between the antioxidant activity of the peel and flesh of the fruit and vegetables caused by difference content of bioactive compounds (Nurliyana *et al*., 2010).

3.3. Cellular antioxidant activity

The principle of Celullar Antioxidant Activity/CAA is to know the antioxidant activity of a compound by measuring its ability to inhibit the oxidation of 2' 7'- dichlorfluorescein-diacetate (DCFH-DA) fluorescence 2' 7'-dichlorofluorescin (DCF) by ROS on cell culture. Cellular antioxidant activity in HeLa cells by treatment ethanolic extract peel and flesh of *Coleus tuberosus* can be seen in Figure 1.



A



B

Note: different notations indicate significant difference ($p < 0.05$).

Figure 1. Percentage reduction in reactive oxygen species (ROS) by EEPC (A) and EEFC (B) treatment in HeLa cells induced by Phorbol Miristate Acetate

Fig 1 (A, B) showed that the peel and flesh ethanolic extract of *Coleus tuberosus* able to reduce the formation of reactive oxygen species (ROS) in HeLa cells induced by PMA dose dependent manner. Part of the peel have the ability reduces ROS higher than the flesh of *Coleus tuberosus*. This was related to the difference of bioactive compounds in the peel and the flesh. Part of the peel contain phenol, flavonoids was higher flesh of the ethanolic extract of peel and flesh of *Coleus tuberosus* (Table 1).

Decrease of ROS in HeLa cells by the peel and flesh ethanolic extract of *Coleus tuberosus* allegedly through some mechanism, namely the ability of scavenging free radicals attack cell membrane and maintain the fluidity of cell membrane, repair and increase the antioxidant defense system (enzymatic reactions and non-enzymatic reactions).

Reactive oxygen species reduction mechanism in the peel and flesh ethanolic extract of *Coleus tuberosus* expected as mechanism of bioactive compounds contained it that scavenging the ROS attack the cell membrane. Increased ROS in the cells causing the cell membrane lipid undergoes oxidation so that the cell membrane permeability and fluidity changes. Phenolic compounds have the ability to maintain the fluidity of cell membranes to capture ROS, so the cellular communication signal level can run well including signal activation of antioxidant enzymes(NRF-2-ARE). Another mechanism underlying the antioxidative properties of phenolics is the ability of flavonoids to alter peroxidation kinetics by modification of the lipid packing order and to decrease fluidity of the membranes (Arora *et al*., 2000). These changes could sterically hinder diffusion of free radicals and restrict peroxidative reactions.

Increased expression of NRF-2-ARE increasing role in the cell's antioxidant defense system (SOD, CAT, GPx, glutathione, vitamin C, vitamin E and carotene). An increase in the cell's antioxidant defense system (SOD, CAT, GPx) give effect to the increased ability to neutralize superoxide anion radicals ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$) induced by PMA, so as to decrease the number of free radicals in the HeLa cells.

The peel and the flesh extract of *Coleus tuberosus* also have some kind of bioactive compounds such as phenolic and flavonoid compounds (Table 1). Phenol compounds have the ability to increase the antioxidant defense system (Giovanini *et al*., 2008; Verma *et al*., 2009). Increased cellular antioxidant activity may prevent 2.7 dichlorofluorescein diacetate-(DCFH) hydrocarbon and reduce the formation of 2.7-dichlorofluorescein diacetate fluorescent DCF (Salawu *et al*., 2011).

This study proves that the ethanolic extract peel of *Coleus tuberosus* able to reduce ROS is greater than the ethanol extract flesh of *Coleus tuberosus*. Differences in the ability to reduce ROS, one of them allegedly associated with differences in the content of phenol, flavonoids (Table1).

Some research has also shown that the peel has the ability of antioxidants that were higher than the portion of flesh. This difference is associated with the bioactive compounds in the peel section is higher than the portion of flesh. The antioxidant activity of apple peel part was higher than the flesh, this difference was associated with a significant positive correlation between the content of anthocyanin, flavonoids, phenol, with antioxidant activity (Vieira *et al*., 2009). The antioxidant activity of the peel of apple fruit is higher than the flesh. Comparative evaluation of antioxidant with the DPPH method and cellular antioxidant activity showed that the DPPH method (in vitro chemical) similar tendency, where the once the antioxidant activity of extract of peel the *Coleus tuberosus* greater than the flesh ethanolic extract of *Coleus tuberosus.*

Evaluation of antioxidant activity in vitro chemical (DPPH), the reaction tends to be on antioxidant compounds tested and free radicals are added. So the ability to scavenging of free radicals is highly dependent on the number of OH groups in its structure. While the evaluation of antioxidant activity using a biological system in this case is the HeLa cells, the antioxidant ability of a compound is not only a reaction between antioxidant compounds were tested by free radical compounds (PMA), but also involves other cellular mechanisms in the cell such as the cell membrane fluidity, d antioxidant defense system.

Thus, in vitro biological system evaluation in cells can describe the complexity of biological systems and is an important tool to check the food, phytochemicals and dietary supplements potential for biological activity, because the model considers the cellular antioxidant activity of the compound by the cell retrieval, distribution and efficiency protection against free radicals under physiological conditions. This study proves that the bioactive compounds that flavonoids and phenolic compounds contribute to the antioxidant activity of the peel and flesh ethanolic extract of *Coleus tuberosus*.

4. CONCLUSION

Evaluation of antioxidant activity for screening of free radical scavenging ability of a compound can be done by combining two methods, in vitro chemical (DPPH) and in vitro in biological systems (cell). This was done to provide a clear representation of the antioxidant potential of

a compound. Variations in having antioxidant method in strengthening the information obtained. This research is expected to provide information, that *Coleus tuberosus* as a vegetable is not only a source of carbohydrates but also have potential as natural anti oxidants.

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