VOL. 11, NO. 17, SEPTEMBER 2016 ISSN 1819-6608
ARPN Journal of Engineering and Applied Sciences

www.arpnjournals.com

ANTIOXIDANT POWER OF ROSE ANTHOCYANIN PIGMENT

Elfi Anis Saati
Department of Science and Food Technology, Faculty of Agriculture and Animal Husbandry, Muhammadiyah University of Malang
Campus III UMM, Tlogomas Street Malang, Indonesia
E-Mail: elfiumm@yahoo.co.id

ABSTRACT

Rose is a major cut flower in Batu city - East Java Province, Indonesia. Crown of roses has been known to contain anthocyanin pigments from the group of flavonoids. Lately, polyphenolic flavonoid pigment was widely studied due to its high antioxidant power. In vivo studies of antioxidant power in liver were performed through SGOT (Serum Glutamic Oxaloacetic Transaminase) analyses. This study aimed to determine the antioxidant power of anthocyanin pigments contained in the crown of roses. Samples were obtained from rose farmers in the village of Sidomulyo, district of Batu, East Java. Pigment was firstly extracted using distilled water and citric acid solvent. The extract was then filtered using Whatman paper 41 and concentrated in a rotary vacuum evaporator at 50°C. Following fractionation of C18 (Shimadzu chromatograph: acetonitrile 100% and 4% formic acid), isolates obtained were tested in white rats for antioxidant power using activity of SGOT. Results showed that SGOT content of white rats treated with 100% anthocyanin isolates was decreased from 117.542 U/l to 18.267 U/l. This value have approached the normal level of rats without CCl_4 injection is 17.075 U/l.

Keywords: anthocyanin pigments rose flower, antioxidant power, SGOT.

INTRODUCTION

The environment in which we live every day is an environment rich in free radicals. Approximately 10,000 free radical attacks occur on every DNA (Deoxyribonucleic Acid) of human cells every day (Boyer and Liu, 2004). These free radicals are very dangerous because they play an important role on the occurrence of various diseases (Aw, 1999). Rachmawati (2003) mentioned that the increased production of free radicals may cause abnormalities in various target organs, *e.g.*liver.

Source of free radicals comes from very acute toxic compounds such as carbon tetrachloride (CCl_4). CCl_4 is a volatile organic chemical compounds and is one of the free radical forms from the reaction of CH_4 and Cl_2 with the aid of ultraviolet light. It can cause tissue and organ damages, especially liver and kidney (Ogeturk *et al.*, 2004). High doses administration of CCl_4 can cause damage on endoplasmic reticulum, accumulation of lipid, reduction in protein synthesis, disruption of oxidation process, and swelling of the liver which is leading to an increase in weight and SGOT (Serum Glutamic-Oxaloacetic Transaminase). In order to reduce the formation of free radicals, additional substances such as antioxidants are thus needed to elude free radical attacks.

Roses are flora that thrives in Indonesia and a major cut-flower in Batu, East Java Province. Utilization of roses in Indonesia is generally limited to be used as decorative and perfume base materials. Crown of roses have been known to contain anthocyanin pigments from the group of flavonoids. Lately, polyphenolic/flavonoid compounds contained in pigment of roses were widely studied due to its high antioxidant power exceeds other antioxidants. A study of Saati *et al.* (2007) has shown that fresh rose extracts or that has been on display for 4 to 6 days were potential to be used as coloring agent as well as antioxidant. Furthermore, according to Soni *et al.* (2009), a number of studies have indicated the potential impact of a group of flavonoids among others, such as reduce the risk of heart disease and cancer. Garźon et al. (2009) has postulated that chronic diseases such as diabetes mellitus and stroke can be prevented through the intake of foods rich in anthocyanin. The potential of the red pigment from rose petals, however, is not completely excavated.

The objective of this study was to determine the antioxidant power of anthocyanin pigments contained in the crown of roses. An alternative yet effective natural antioxidant which can replace harmful pigments such as Rhodamine B was expected to achieve.

THEORETICAL BASIS

Natural dyes that are safer can be obtained from carotenoid pigment, curcumin, anthocyanins, and other pigments. They are contained in the network of fruit, flowers, leaves, stems or roots of plants. According to Hudson (2007), natural antioxidant found in foods is derived from food additive groups specifically isolated from natural sources and added to the food. Flavonoids are type of pigments soluble in water. These include anthocyanins and anthoxanthin. Both pigments can be generated from the corolla and as its origin name "anthos" (Latin) it means flower.

Anthocyanins are pigments belong to flavonoid compounds. It contains two benzene rings connected by three carbon atoms and sealed by one oxygen atom between the two benzene rings. Anthocyanins are naturally occurred in pigments with the following colors: blue, purple, violet, magenta, and yellow. These water-soluble pigments can be found in flowers, fruit, and leaves of plants (Moss, 2002).

Determination of SGOT and SGPT (Serum Glutamic-Pyruvic Transaminase) enzyme activity is a specific and sensitive test for diagnosing liver diseases (hepato cellular damage) (Raju *et al.*, 2012). The normal value for SGOT measurement is 40 U karmen (17 mU per

cc). As for SGPT, the normal value is 35 U karmen (13 mU per cc). The normal ratio of SGOT to SGPT value is 1.15 (Dalimartha, 2004).

MATERIALS AND METHODS

Materials and equipment

This study used sample of crown of red roses obtained from flower farmers in the village of Sidomulyo, District of Batu, East Java Province. Other materials used were CCl_4 solution, corn oil, Whatman paper No. 41, cotton, and alcohol. White male rats *(Rattus norvegicus)*Wistar strains were used for SGOT examination along with enzyme and starter reagents (DiaSys Diagnostic Systems GmbH, Germany). Antioxidant power of common materials such as vitamins A, C, and E was used as comparison. Equipments needed were measuring cups, analytical balance, a dry blender, pan, vacuum dryers, test tubes, tweezers, scalpel, scissors, cuvettes, centrifuged, eppendorf tubes, and micropipette.

Materials preparation

Anthocyanin pigments were firstly extracted from the crown of roses using solvent aquadest and citric acid with ratio of 95 to 5. The extract was macerated and stored in a refrigerator for 30 minutes at temperature of 10 to 12ºC. The filtrate was then filtered with Whatman 41filter paper and concentrated in a rotary vacuum evaporator at temperature of 50ºC. Isolates were then obtained after fractionation using C18 column (Shimadzu: with 100% acetonitrile and 4% formic acid) and analyzed for its antioxidant power. The SGOT observations were conducted on healthy male rats *(Rattus norvegicus)* Wistar strain at the age of 6-8 weeks old.

Test preparation on rats

In this research, *in vivo* studies were performed in experimental animals namely Wistar rats *(Rattus norvegicus)* using a post-test only control group design. The selected white male rats *(Rattus norvegicus)*-Wistar strain were at the age of 6-8 weeks old and possessed healthy weight. After a week of adaption period, the rats were treated with BR1 (Broiler Ras, Comfeed) until its body weight achieved 200 g on average +10%. Rats were then randomly divided into 11 groups. The group's name represents the treatment given for the corresponding group, namely K-1 (control, rats without administration of CCl_4), K+1 (control, rats with administration of CCl_4), Vit A (rats treated with vitamin A at dose concentration of 50, 75, and 100%), Vit C (rats treated with vitamin C at dose concentration of 50, 75, and 100 %), Ev (rats treated with concentrated pigments at dose concentration of 50, 75, and 100%), and Ex (rats treated with isolated pigments at dose concentration of 50, 75, and 100%). Except for rats in K-1 group, CCl_4 solution was subsequently injected to rats in accordance with the treatment for 14 days. The CCl_4 solution was administered at a dose of 0.180 mL per 136 g body weight for three days. CCl_4 solution was prepared by dissolving CCl_4 (with a concentration of 50%) in corn oil with the ratio of 1 to 1 (5 mL CCl_4 and 5 mL corn oil).

Observation and data analysis

SGOT analyses were performed by dissecting and taking the blood of the rats' heart using a 3mL syringe. One mL of blood was taken and put in a eppendorf tube and weighed up until reached certain weight. The blood was the centrifuged at a speed of 6,000 rpm for 10 minutes. SGOT observation was conducted by taking 50 mL of serum and adding 1 mL of NaCl solution. A reagent (1500 mL) was added and left for 1 minute. Another reagent as much as 2125mL was then added and the solution was left for 5 minutes. The absorbance was observed spectrophotometrically at λ 365 nm with distilled water as blank. Data enzyme activity was obtained by multiplying the analysis value with factor of 397.1.

RESULTS AND DISCUSSIONS

SGOT and SGPT are metabolic products produced by organs that can serve as a sign of cell or tissue damage by free radicals. They are resulted from process-induced lipid peroxidation of free radicals such as carbon tetrachloride (CCl_4) given in this trial. SGOT values of groups of rats experienced different treatments were listed in Table 1 and Figure-1.

As shown Table-1, SGOT value of positive control group (K+1, 117.542 ± 10.91 U/L), administered with CCl_4 solution, was significantly higher ($p<0.05$) compared to that of the negative control group (K-1, 16.083 ± 0.993 U/L), without the administration of CCl_4solution.

The increase in levels of serum transaminase (SGOT and SGPT) by CCl_4 can be explained by Pato mechanism hepatotoxic. CCl_4 induced by subcutaneous injection will pass through the cardiovascular system and thus in liver cells cytochrome P-450 system will become reactive metabolites, *e.g.* CCl_3-, Cl, Cl_3COO-. The trichloromethyl radicals that covalently bind to the protein and unsaturated lipids, which is the basic ingredient of cell membranes, cause lipid peroxidation and thus cause cell death until eventually the liver cell experience necrosis. A very active liver metabolism therefore becomes impaired and levels of SGOT and SGPT increase (Lu, 1995).

Isolates of anthocyanin pigment from roses have shown considerable power in decreasing SGOT value of rats induced with CCl_4 to 18.267 ± 1.26 (*i.e.* Ex 100%). This achievement has exceeded the power of common antioxidants, such as vitamin A at 50, 75, and 100% dose concentration (decreased to 64.330 ± 6.37, 48.446 ± 4.27, and 32.959 ± 3.68 U/L, respectively) and vitamin C at 50, 75, and 100% dose concentration (decreased to 72.669 ± 7.34; 57.182 ± 5.86, and 27.400 ± 3.03 U/L, respectively). Anthocyanin pigments in the form of concentrated extract (Ev 100%) managed to decrease SGOT value until 58.8%, *i.e.* from 117.542 ± 10.91 to 48.446 ± 4.32 U/L. This is very encouraging because it shows that by consuming the concentrated pigment of red roses can replace both antioxidant-vitamin supplements (vitamins A and C) and reduce the value of SGOT on rats administered with CCl_4

closely to the value of that the control rats (K-1 group, with AST of 16.083 ± 0.993 U/L).

Table-1. Values of SGOT (U/L) of groups of rats undertaken various treatment.

Group samples	Absorbance at λ 365 nm	SGOT (U/L)
K-1	0.041	16.083 ± 0.993
K+1	0.296	117.542 ± 10.91
Vit A 50%	0.162	64.330 ± 6.37
Vit A 75%	0.122	48.446 ± 4.27
Vit A 100%	0.083	32.959 ± 3.68
Vit C 50%	0.183	72.669 ± 7.34
Vit C 75%	0.144	57.182 ± 5.86
Vit C 100%	0.069	27.400 ± 3.03
Ev 50%	0.206	81.803 ± 7.87
Ev 75%	0.166	65.919 ± 6.28
Ev 100%	0.122	48.446 ± 4.32
Ex 50%	0.126	50.035 ± 5.08
Ex 75%	0.082	32.562 ± 3.34
Ex 100%	0.046	18.267 ± 1.26
SGOT threshold		40

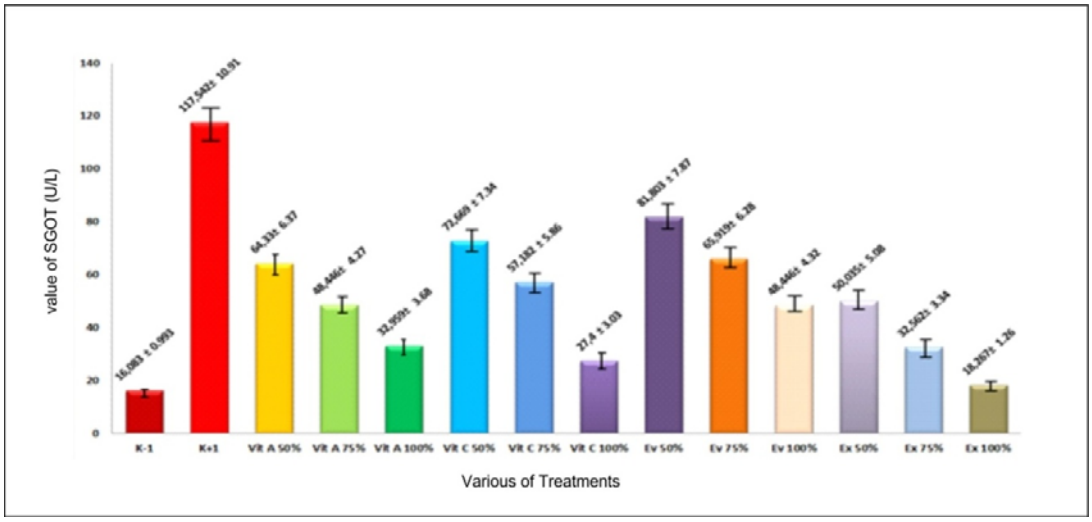

Figure-1. Comparison of SGOT values of rats with various treatments.

Anthocyanin compounds contained in isolated and concentrated pigments of red roses (*Rosa damascena Mill.*) can prevent and slow down the oxidation of lipids, although the substrate concentrations are lower than the oxidized substrate. The protein covalently bonding with unsaturated lipids is shielded by the trichloro methyl radical (Francis, 1999). The process of cell death and degradation of the liver metabolism function can thus be avoided. SGOT and SGPT value will also be dropping back towards their normal levels.

This study showed that free radicals increased the level of SGOT on rats induced with carbon tetrachloride. Administration of isolated and concentrated extracts of anthocyanin pigments from red roses has significantly reduced SGOT levels of Wistar rats at the optimum dose of 100% (equals to 5 mg/day). At these doses, SGOT values have closely reached the SGOT value of negative control group without injection of carbon tetrachloride (K-1). From this research, it can also be proven that red rose extract in the form of effervescent tablet gave significant effect ($p<0.05$) in lowering SGOT levels of rats exposed to carbon tetrachloride.

CONCLUSIONS

Results showed that SGOT value of white rats treated with 100% anthocyanin isolates could be lowered from 117.542 U/l to 18.267 U/l; whilst for creatinine value could be decreased from 1.243 mg/dl to 0.474 mg/dl. These values have approached the normal levels of rats without CCl_4 injection, i.e. 17.075 U/l for SGOT and 0.432 mg/dl for creatinine.

ACKNOWLEDGEMENT

Authors would like to acknowledge the financial support from the Directorate General of Higher Education (DIKTI) through Competitive Research Grant in the year of 2006/2007 and 2010-2015. The result of this research had been patented with certificate No. IDP 000034662 (Saati and Wachid, 2014) entitled "Natural Food Colorant from Rose and Its Processing" and Patent No. P00200900698 entitled "Effervescent Tablet from Rose Pigments".

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