ANTIOXIDANT POWER OF ROSE ANTHOCYANIN PIGMENT

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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 and isolates was decreased from 117.542 U/l to 18.267 U/l. This value have approached the normal level of rats without CCl4 injection is 17.075 U/l. Results showed that SGOT content of white rats treated with 100% anthocyanin (Shimadzu chromatograph: acetonitrile 100% and 4% formic acid), isolates obtained were tested in white rats for antioxidant power using activity of SGOT. 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.

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 (CCl4). CCl4 is a volatile organic chemical compounds and is one of the free radical forms from the reaction of CH4 and Cl2 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 CCl4 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. Garzón 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
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 CCl4 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, 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 CCl4), K+1 (control, rats with administration of CCl4), 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, CCl4 solution was subsequently injected to rats in accordance with the treatment for 14 days. The CCl4 solution was administered at a dose of 0.180 mL per 136 g body weight for three days. CCl4 solution was prepared by dissolving CCl4 (with a concentration of 50%) in corn oil with the ratio of 1 to 1 (5 mL CCl4 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 an 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 (CCl4) 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 CCl4 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 CCl4 solution.

The increase in levels of serum transaminase (SGOT and SGPT) by CCl4 can be explained by Pato mechanism hepatotoxic. CCl4 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. CCl3-, Cl-, Cl3COO-. 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 CCl4 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 CCl4.
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.

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

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 CCl₄ injection, i.e. 17.075 U/l for SGOT and 0.432 mg/dl for creatinine.

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REFERENCES


