Phytochemical, Haematinic and Antidiabetic Test of Pumpkin Extract *(Cucurbita moschata)*

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Abstract: Pumpkins contain natural phenolic compounds which are potential antioxidants and have a bioactivity as medicines. These compounds can be found in stems, leaves, flowers, and fruits. Pumpkin (Cucurbita moschata) is one of the antidiabetic and antihyperglycemic traditional food which can be utillized as substances for enteral formula. The polysaccharide and pectin contents are claimed to reduce blood glucose levels and control glycemic levels. The purpose of this study is to determine the effect of skin, mesocarp and pumpkin seeds (*Cucurbita moschata*) on hemoglobin and glucose levels. The study is an experimental study conducted at a laboratory with 24 male BALB/c mice which were given a methanol extract of mesocarp, seeds and pumpkin skin for 14 days in the integration laboratory of Sunan Ampel State Islamic University. Data analysis used the Anova One Way test. The results of the study indicate that there are differences in hemoglobin levels in each test group (p-value = 0,015). The LSD follow-up test indicates that the mesocarp and seed groups and the control and seed groups are two significantly different groups. However, there is no difference in glucose levels in each test group (p-value = 0,171).

1 INTRODUCTION

Pumpkins contain alkaloid compounds useful for stimulating the nervous system, increasing or decreasing blood pressure, and controlling microbial infections. Natural phenolic compounds of pumpkins are potential antioxidants and bioactivity as medicine. This compound can be found in stems, leaves, flowers, and fruits. In human body, flavonoid functions as an antioxidant, protection of cell structures, increasing the anti-inflammatory activity of vitamin C and antibiotics (Erfanur, 2014).

Pumpkins as accessible local food contain protein for consumption, high level digestibility (99%), beta-carotene (1,569 µg / 100g.), and Carotenoid particularly β-carotene, β-cryptoxanthin, lutein, and zeaxanthin (Rodriguez 2008). Carotene is known as a potential absorber of singlet oxygen species (ROS). The consumption of antioxidants for instance carotenoids, polyphenols, and tocopherols can prevent oxidative stress. (Tourniarire, 2009). The feeding of pumpkin flour to diabetic rats for 4 weeks significantly reduces insulin and blood glucose levels compared to the control group (Sedigheh, 2011).

Cucurbita moschata seed or *kuaci* (in Indonesian), is not only a tasty snack, but it also has a prevention effect of benign prostate enlargement. The seed also contains mineral elements: Zn (zinc) and Mg (magnesium), which are very important for the health of the reproductive organs, including the prostate. 100 g of *Cucurbita moschata* seeds contain 6.5 mg of Zn minerals (Widowati et al., 2008). The other contents in 100 g of pumpkin seeds are calories 515.00 cals, protein 30.60 g, fat 42.10 g, carbohydrates 13.80 g, sugar 5.30 g, calcium 54.00 mg, phosphorus 312.00 mg, iron 6.20 mg, and water 5,90 g

Pumpkins contain alkaloid compounds which are useful for stimulating the nervous system, increasing or decreasing blood pressure, and controlling microbial infections. It contains natural phenolic compounds which are potential antioxidants and have a bioactivity as medicines. The compound can be found in stems, leaves, flowers, and fruits. Flavonoids in the human body function as antioxidants, protecting cell structures and increasing the activity of anti-inflammatory vitamin C and antibiotics (Erfanur, 2014).

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The purpose of this study is to determine the phytochemical content of pumpkin and the effect of skin, mesocarp and pumpkin seeds *(Cucurbita moschata)* on hemoglobin and glucose levels.

2 METHOD

This research is experimental laboratory research employing quantitative method that fulfils the entire requirements to test the cause and effect relationship. This study controls the relevant conditions of the situation studied. The following step is observation of the effect of manipulated conditions. In this study, manipulated variables are extracts of some parts of the pumpkin fruits *(Cucurbita moschata),* for instance the skin of fruits, mesocarp, and seeds of the fruit. The next step is evaluation of the most significant part of pumpkin fruits on hemoglobin and glucose level as a dependent variable.

The sample of this study is the extracted pumpkin fruit *(Curcubita moschata)* which was extracted by the metanol maceration process. The experimental animals used were male mice *(Mus musculus)*. The extracting process of fruit pulp, skin, and seeds parts having constant weight was macerated by methanol-water (4:1) at temperature (25-28°C) for 10 hours. The maceration results were then filtered with 41 whatman paper and the filtrate was centrifuged for 10 minutes. In addition, the solvent evaporated using a rotary evaporator at a temperature of 65°C results condensed liquid of skin of fruits, mesocarp, and seeds of the fruit extracts

Extracted liquid per oral is provide by needle sonde at a 2 g / kg BW and a volume of 0,2 ml for 14 days. The adding of pumpkin extract to male mice *(Mus musculus)* is carried out as follows: the mice are grouped into four groups. Group I is a control group which is received aquades, Group II is the group which received the pumpkin bark extract, Group III is a group , which provided mesocarp extract pumpkin, Group IV is a group which provided an extract of pumpkin seeds. The research was conducted at the integrated laboratory of UIN SunanAmpel Surabaya.

3 RESULT AND DISCUSSION

In this study,extracts of skin, mesocarp, and seeds were tested by phytochemical tests. The tests are as follows:

3.1 Salkowski Test

The Salkowski test was conducted to determine the presence of sterols and triterpenoids. The initial step of this test was scaling a 0.5 gram sample. After scaling, the sample was added to a test tube and then added 1 ml of chloroform; it was then homogenized and filtered them. After the filtrate was obtained, the 3-5 drops of H2SO4 was added on its filtrate. The end of process was observation of sterol on filtrate. If filtrate's color is red, it indicates the existence of sterol. Contrarily, golden yellow on filtrate indicates the presence of triterpenoids.

Due to the colour conversion to golden yellow, the results of observation indicate that the mesocarp positively contained triterpenoid. Meanwhile, skin of fruits and seeds of the fruit did not contain triterpenoid and sterol.

3.1 Saponin Test

Saponin test was conducted by adding 5 ml aquades to 0.5 g the sample. If there is a foam, the sample contains positively saponin. The observation process indicate that the foam was formed. This foam indicates that the mesocarp and skin test contains saponin while the seeds do not contain saponin.

3.2 Wagner Test

In the Wagner test, 10 ml of HCl 2M was added to the sample before the heat process for 5 minutes. Subsequent cooling treatment on room temperature and filter process were then conducted successively. Filtrate as a result of filter process was then added with 5 ml of HCl 2M and 3-5 of wagner liquid. Sedimentation on the filter process indicates the contents of alkonoid compounds on the extract. The wagner test shows that pumpkin skins, seeds, mesocarp does not contain alkanoid.

3.3 Flavonoid Test

Flavanoid test began with the weighting of 0.5 g of pumpkin extract and filling it into a test tube. Sufficient methanol liquid and 3-5 FeCl3 then were added into the similar tube before conducting observation of the colour change. If the color of the mixture of pumpkin extracts with the liquids turns into green, red, purple, blue and black, these conditions indicate the flavanoid on the extract. The observation result of the skin, the mesocarp and pumpkin seeds find the color change to black indicates positive flavanoid on the extract.

3.4 Carbohydrate Test

The samples were diluted with methanol, 2 ml of the sample, then were mixed with 2 drops of molisch reagent until the color became brick red. Then, H2SO4 was added and the forming of purple ring was observed. Due to the absence of purple ring formation, carbohydrate compounds were absent of the skin, the mesocarp and pumpkin seeds.

3.5 Protein Test

The samples were diluted with methanol; 2 ml of the sample was then mixed with 1 drop of NaOH and 1 drop of CuSO 4 successively. Positive results indicate the formation of a purple ring or reddish discoloration. From the results of the observation, there is no color change in brick red on skin, mesocarp or seeds.

Figure 1: figure of pythochemicals test on the skin

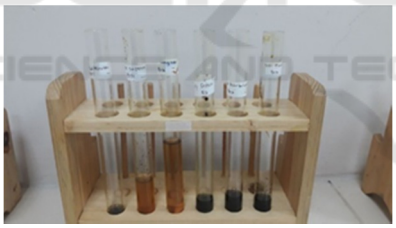

Figure 2: figure of pythochemicals test on seeds

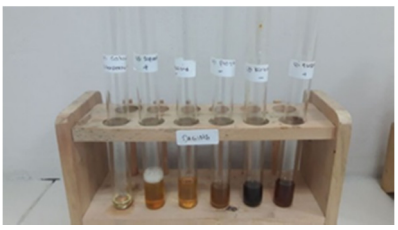

Figure 3: figure of pythochemicals test on the mesocarp

Based on qualitative phytochemical tests, the pumpkin skin methanol extract contained several chemical compounds such as saponins and flavonoids. In addition, the methanol extract of pumpkin mesocarp contains chemical compounds such as triterpenoid, saponin, and flavonoids. Moreover, pumpkin seed extract has flavanoid compound. This result is consistent with the research of El Adawi TA and Taha KM (2001), which indicates that the pulp of the pumpkin fruit or mesocarp has bioactive components such as polysaccharides, proteins, peptides, para-aminobenzoic acid. A component of phenol and sterols does not contain saponins, steroid and triterpenoid tannins. However the component contains secondary metabolites of flavonoids. The results of this study show that pulp of the pumpkin fruit or mesocarp contains triterpenoids and saponins. Based on Pabesak et al's study (2013), flavonoid compounds in natural phenolic compounds have bioactivity as a medicine (drug) and can potentially be used as an excellent antioxidant for cancer prevention. The total phenolic content in the pumpkin was 93 µg GAE / g.

Based on Raharjo et al's study (2011), pumpkin seeds contain nutrients such as amino acids, Zn (zinc), Mg (magnesium), essential fatty acids, vitamin E (tocopherol), carotenoids, steroids, cryptoxanthin, monocyclic sesquiterpenoid and trypsin inhibitors. Trypsin inhibitors could effectively inhibit peroxide, turning into free radical and oxidize unsaturated fatty acids in cell membranes. This evidence supports our results that methanol extract of pumpkin seeds contains flavonoid compounds which have a potential as an antioxidant. Several essential nutrients contained in various types of pumpkin extract which have been studied are antioxidant, antidiabetic, anti-obesity, antyhiperkolestrolemik, anti bacterial, and anti-prostate cancer.

The content of pumpkin polysaccharides macromolecules consists of galactose, glucose, arabinose, xylose and glucuronic acid which has chemical and pharmacological contents to support significantly pumpkin nutrition. Pumpkin fruit polysaccharides have shown high levels of insulin in the blood serum, reduced glucose levels in the blood, glucose tolerance improvise. Therefore, it can be developed as an oral antidiabetic agent (Chang et al 2014). Pumpkin polysaccharides can also inhibit the action of preoksida hydrogen, and as the result, it decreases cell viability. The appearance of lactate dehydrogenase and the formation of malondialdehyde indicate that the polysaccharide from the pumpkin has some benefits as a cytoprotective and antioxidant. This is consistent with the results of our study which showed no significant difference between skin, mesocarp and pumpkin seeds *(Cucurbita moschata)* group in increasing blood glucose levels in male mice.

The average results of measurement of the hemoglobin level in the mice fed by mesocarp extracts, skin and pumpkin seeds can be seen in Figure 4.

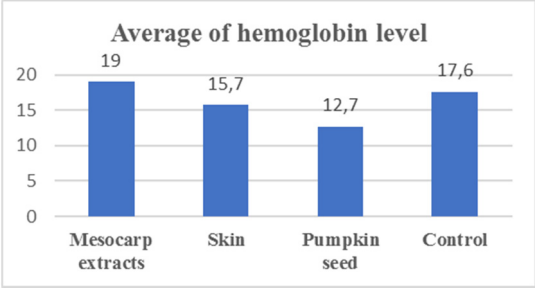

Figure 4 : Average of hemoglobin level

From Figure 4, the lowest mean hemoglobin (Hb) level in the pumpkin seed treatment group is 12.7 and the highest mean Hb level is the treatment group pumpkin mesocarp extract at 19

The average or mean of glucose levels in the control group such as mesocarp extracts, skin and pumpkin seeds are illustrated in Figure 5.

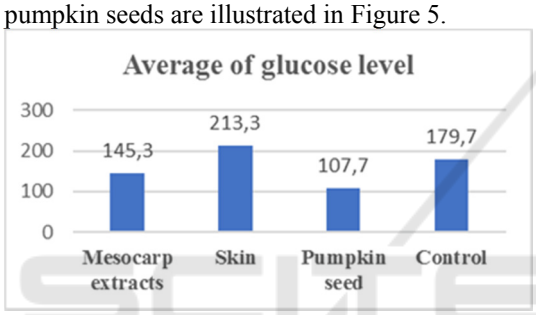

Figure 5: Average of glucose level

Based on the figure 5, the average of the lowest glucose levels is the treatment group which are given pumpkin seed extracts in the amount of 107,7 while the higher glucose level in the group receiving the pumpkin skin extract. Pumpkin has been utilized as anti-diabetic substance and components of bioactive compounds concentrated in the fruit (Behera, et al., 2012). Polysaccharide extracts of cucurbita moschata which were tested in the enzyme reaction lead to significant differences in non-competitive inhibition of alpha-glucosidase at a concentration of 0.7-0.9 mg/ml. It has also been shown that the powder of the species *Cucurbita moshata* duch has hypoglycemic compounds for patients with type 2 diabetes. Chen et al, (1994) reported that because of the polysaccharide content in humans. (Xiong and Cao, 2001, Norfezah, et al., 2011). According to Li et al's research, 2005, the mechanism of anti-diabetic pumpkin is derived from the activities of protein-bound polysaccharides which has been proven to reduce blood glucose concentrations, increase blood levels of serum insulin and improve tolerance to glucose in the alloxan-induced rat which destroys cell β and therefore, induce diabetes. It is believed that this effect may be due to the antioxidant properties of polysaccharides that protect cells ß of the pancreas.

Data on hemoglobin and glucose levels are normally distributed. The significance of hemoglobin is 0.950, which is greater than α (0.05). Similarly, the significance of glucose is 0.637, which is greater than α (0.05). Therefore, testing with Anova One can be continued because data needs must be normally distributed. Data on hemoglobin and glucose levels are homogeneous. The significance of hemoglobin is 0.300, which is greater than α (0.05). Similarly, the significance of glucose is 0.126, which is greater than α (0.05). Therefore, testing with Anova One can be continued because requirement of homogenous data are fulfilled

Based on the Anova One Way test, there were significant differences in glucose levels in the Mus Muculus group, with the mesocarp, seed and pumpkin skin extract in the control group. This is reinforced by the p-value (Sig.) The glucose level is 0.171, which is greater than α (0.05). However, there were significant differences in hemoglobin levels in the MusMuculus group in which mesocarp, seeds, and pumpkin extract were given to the control group. This is reinforced by the value of p (Sig). The hemoglobin level is 0.015, which is less than α (0.05).

The calculation of the multiple comparisons leads to the conclusion that there are at least 2 pairs of different groups. At the glucose levels, skin and seed groups show a significant difference (p-value = 0.042). Meanwhile, at the hemoglobin levels, the groups of mesocarp and seeds show a significant difference (value of p = 0,003). These results are similar to the control group and the seeds which also have a significant difference (value of p = 0.012).

The different parts of the plant have also been used as medicine in developed and developing countries. The leaves are used as antianemic, analgesic, and in some cases, have been used externally for the treatment of burns and other related diseases (Fu et al., 2006). It supports our findings that there is a slight improvement in the hemoglobin extract of the skin treatment group, pumpkin flesh (mesocarp), and pumpkin seeds than those of the contol group. The increase of hemoglobin levels shows that pumpkin extract has haemanitic activity on experimental animals. Research testing noodles enriched with inorganic , organic iron only, or combination with steamed yellow pumpkin as a fodder for mice has been conducted to evaluate the significant effects of yellow pumpkin to increase hemoglobin levels. The results show that enriched noodles with a combination of inorganic iron and steamed pumpkin have a significant role as a food vector and intervenes the iron deficiency that causes anemia in mice. In the research of Widowati et al., (2003), pumpkin fruit is found to contain 0.071 mg of

vitamin B6, riboflavin 0.128 mg, 18.792 mg of folic acid and 0.147 mg of copper (Cu), per 100 g. Pumpkin also contains carotenoids activity of 1569 g equivalent to 261.5 Vitamin A in the form of a dry powder containing glycine amino acid from 19.97 to 27.12 mg/100 g. Thus, in this study, it is possible that the micronutrient-micronutrient mechanisms help utilization of iron in the group provided by the pumpkin extract in hemoglobin synthesis. Thus, the level of hemoglobin in the group increases.

4 CONCLUSIONS

Pumpkins contain trypenoids, saponins, and flavonoids. There were differences in hemoglobin levels in each test group (p-value = 0,015). The LSD follow-up test indicates that the mesocarp and seed groups, as well as the control and seed groups, are two different groups. However, there was no difference in glucose levels in each test group (p value = 0.171).

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