NUTRITIONAL ANALYSIS OF AQUEOUS EXTRACT OF SNAKE FRUIT SEEDS (SALACCA EDULIS REINW.) FOR DEVELOPMENT OF ANEMIA TREATMENT

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ABSTRACT
Anemia remains a global nutritional problem, which prevalence continues to increase every year. Children, female adolescents, and pregnant women are more susceptible to anemia than other age groups. Prolonged anemia has negative impacts on their life such as premature birth, low birth weight (LBW) babies and intrauterine fetal death. Snake fruit Pondoh (Salacca edulis Reinw) is an original Indonesian fruit, which is well cultivated at the Sleman regency, Yogyakarta. Snake fruit seeds contribute to 25-30% of the total fruit, which has not been established their health effect. Therefore, this study aims to analyze the nutritional content of aqueous extract of snake fruit seeds (AES). The powder of snake fruit seeds was made using a previous method, which was adopted from our research group. The powder was then extracted using hot aqueous with 65°C. A proximate analysis was used to determine the AES nutritional content. A hundred g AES contained 21.92% water, 41.10% carbohydrates, 12.66% protein, 4.51% fat, 0.62% tannins, 32.8 mg Iron, 10.25 mg zinc, 276.42 mg magnesium, and 495.48 mg vitamin C. In conclusion, the AES has complete macronutrients and contains high levels of Iron, Magnesium, and vitamin C, which becomes a potential nutraceutical for anemia treatment. Further research is required to confirm the beneficial effect of AES administration in female rats with anemia.

Keywords: aqueous extract; anemia; iron; snake fruit seed; vitamin c

INTRODUCTION
Anemia remains a global nutritional problem, which prevalence continues to increase every year (Natasia et al. 2021). Asia accounts for the highest prevalence of anemia among adult women globally with a prevalence in 2018 of 46.3% and increase in 2019 to 46.6% (WHO, 2022). Children, female adolescents, and pregnant women are at high risk of developing anemia (Ristanti et al. 2019). Indonesian’s prevalence of anemia of female adolescents in 2018 was 48.9%, pregnant women 46.9%, children aged under five year 48.1%, and children of school age 47.3% (RI Ministry of Health 2018). Normal blood hemoglobin levels in men are 13 g/dL, women 12 g/dL, pregnant women 11 g/dL, and school-age children 12 mg/dL (WHO, 2018). Anemia is caused by inadequate iron intake, decreased iron absorption, and increased iron requirements during pregnancy, growth, and breastfeeding (Krawiec and Pac-Kożuchowska 2020).

Anemia is characterized by symptoms of paleness, lethargy or fatigue (Krawiec and Pac-Kożuchowska 2020) and shortness of breath (Apriyanti, 2019). Prolonged anemia has negative impacts on their life, when pregnant will increase the risk of giving birth to premature babies, LBW babies, and intrauterine fetal deaths (Gusti et al. 2019). Our Indonesian government has made efforts to prevent anemia by administering Blood Supplement Tablets (BST) to female adolescents and pregnant women, but the achievements in iron supplement consumption have not met expectations (RI Ministry of Health 2018). People are reluctant to consume BST because of the effects they feel after taking the BST, such as nausea, diarrhea, and being forgetful. Currently, the
utilization of local food is an alternative to reduce anemia, one of the local fruit that can be used to prevent anemia is snake fruit seeds, known as salak Pondoh.

Snake fruit Pondoh is one of the local fruit that is originally from Indonesia and is easy to find and have a low price. Snake fruit pondoh seeds contribute 25-30% of the fruit with high nutritional content but are not been established their health effects. Previous research has tested snake fruit seed flour (SSF) with an iron content of 12.397 mg and zinc of 5.233 mg. The results from the vivo study of SSF at a dose of 3.72 g/100 gBW of female rats can increase hemoglobin levels for 4 weeks (Susanti et al. 2018). Intervention using SSF requires high doses with insignificant effect, so extraction is carried out to purify the bioactive substances in snake fruit seed. In this research, extraction was carried out using the deoxygenated method with aqueous as a solvent (Sukmawati V 2013). Iron has chemical properties that dissolve easily in aqueous, and has not been studied in previous studies. The extraction process is expected to increase the bioactive compounds contained in aqueous extract of snake fruit seeds (AES). Therefore, this study aims to analyze the nutritional and micronutrient content of iron, vitamin C, magnesium, and tannins in the aqueous extract of snake fruit seeds.

METHOD
This study uses a type of laboratory experimental research. The steps taken consisted of flouring, extraction, proximate analysis, and micronutrient analysis. The extraction process was carried out in the phytochemical laboratory at Setia Budi University, Surakarta. Proximate analysis, vitamin C, and tannins were carried out in food microbiology and biotechnology laboratory, Sebelas Maret University, Surakarta. Magnesium in AES was analyzed in Integrated Research and Testing Laboratory, Gadjah Madha University, Yogyakarta. Snake fruit seeds were obtained from a home food industry in Donokerto Village, Turi District, Sleman Regency, Yogyakarta. Snake fruit seeds that have the same size are washed with tap aqueous, then allowed to dry at room temperature. Snake fruit seeds are dried using direct sun exposure and then dried using a cabinet dryer for 8 hours and oven for 90 minutes. The dried snake fruit seeds are divided into two parts: small pieces and the SSF. Then, the simplicia of SSF was sifted using a 100 mesh sieve. Store using a tightly closed bottle before further analysis.

Extraction of snake fruit seeds based on modification of our group research and Sukmwawati 2013 using the deoxygenation method with aqueous as a solvent. In short, SSF was dissolved in aqueous (1:10) and then heated to 65°C for 30 minutes. The SSF solution was filtered using filter paper to form a filtrate. The filtrate was concentrated using a rotary vacuum evaporator at a temperature of 60°C, a speed of 80 rpm and a pressure of 175 mbar. If it is still in gel form, the thick extract is evaporated again to form a paste using an electric oven at 60°C for 24 hours. The proximate analysis consists of analysis of aqueous content, minerals, proteins, fats and carbohydrates. Analysis of aqueous content used the thermogravimetric method, fat used the soxhlet method, a protein used the kjehdal method, while carbohydrates used a different method, namely by calculating the difference in the protein, fat and carbohydrate content obtained.

Analysis of tannins on AES using a spectrophotometer. A total of 100 mg of sample was dissolved in 2 mL of distilled aqueous and filtered. Then, the supernatant was diluted with distilled aqueous to a concentration of 5 mL. Dilute AES by 300µL was added to 1.5 mL Folin Ciocalteau reagent and homogenized for 3 minutes. A total of 1.2 mL of 7.5% Na2CO3 solution was added to the
previous solution, then incubated for 1 hour at room temperature. Finally, the homogeneous solution is read using a spectrophotometer at a wavelength of 600 – 800 nm. Vitamin C analysis was examined using a spectrophotometer method. Briefly, 100 mg of AES was dissolved in 5 mL of aquabidest and measured directly using a spectrophotometer at a wavelength of 200-400 nm. Magnesium in analysis using the Atomic Absorption Spectrophotometer (AAS) method. In short, as much as 0.25 mg of sample was added to 10 mL of HNO3, then extruded with temperature 50°C until nearly dry, added 25 mL of distilled aqueous was, and filtered using a 50 mL volumetric flask, added distilled aqueous was until it reaches the mark, read using AAS at a wavelength of 248.4 nm.

RESULTS AND DISCUSSION

Extraction using deoxy method with an aqueous solvent. The decoction method is an extraction method using heating techniques. This method is easy and not expensive. The material used is in the form of SSF 1000 grams of simplicia used produced 174 g of AES, so the yield obtained was 17.4%.

Table 1.
Proximate analysis of the aqueous extract of snake fruit seeds.

<table>
<thead>
<tr>
<th>Content</th>
<th>Analytical method</th>
<th>AES Yield (%)</th>
<th>SSF*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>Thergravimetry</td>
<td>21.92</td>
<td>54.84</td>
</tr>
<tr>
<td>Fat</td>
<td>Soxhlet</td>
<td>4.51</td>
<td>0.48</td>
</tr>
<tr>
<td>Proteins</td>
<td>Kjedal</td>
<td>12.66</td>
<td>4.22</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>By Different</td>
<td>41.10</td>
<td>38.9</td>
</tr>
</tbody>
</table>


Table 1, the results of the proximate analysis showed that the fat, protein and carbohydrate. The content of AES was higher than that of SSF. During the extraction process, the bioactive compounds of snake fruit seeds are isolated which are dissolved in aqueous. Aqueous has strong hydrogen bonds and has a high solubility for many bioactive compounds (Plaza and Turner 2015).

Table 2.
Nutritional content of 100 grams AES

<table>
<thead>
<tr>
<th>Content</th>
<th>AES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron (mg)</td>
<td>32.8</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>495.48</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>10.25</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>276.421</td>
</tr>
<tr>
<td>Tannins (%)</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Table 2 shows the results of the analysis of nutritional content in AES. AES contains iron as much as 32.8 mg. These results are sufficient for daily iron needs based on Ministry of health in 2018 for female adolescents (15 mg/day). Iron in AES is higher than SSF (12.297 mg) (Susanti et al. 2018). Iron is a micromineral needed for the formation of red blood cells (Ganz 2013; Suharni et al. 2021). Iron has chemical properties easily soluble in aqueous and oxygen. Therefore, it is necessary to use aqueous solvent extraction to increase the concentration of iron. The absorption of iron in the body is influenced by the consumption of vitamin C (Krisnanda 2020). The higher the vitamin C, the faster the absorption of iron, because it provides an acidic environment that facilitates the reduction reaction of ferric iron to ferrous so that it is easily absorbed in the small intestine (Krisnanda 2020; Piskin et al. 2022). Vitamin C in our research was 495.48 mg. The results of the analysis of vitamin
C on AES exceed the daily requirement of vitamin C (90 mg/day) (Ministry of Health 2019), excess consumption of vitamin C can be excreted through the urine (Ministry of Health 2023). Vitamin C levels in AES are also known to be higher than the results of the analysis of vitamin C in SSF, only 152.21 mg (Susanti et al. 2018).

AES also contains zinc as much as 10.25 mg, sufficient for daily zinc needs (9 mg). Zinc plays a role in the synthesis of hemoglobin replacing iron when there is a decrease in iron in the body. Iron and zinc have a positive correlation, in anemia there is a decrease in iron in the body accompanied by a decrease in zinc (Fathy Abdelhaleim et al. 2019; Jeng and Chen 2022). The combination of iron and zinc in the administration of anemia therapy is more effective in the treatment of iron deficiency anemia (Karasu et al. 2018; Piskin et al. 2022). Apart from iron and zinc, AES also contains magnesium. Magnesium is the fourth most common mineral in the human body after calcium, potassium and sodium. Magnesium must be continuously replenished through food and aqueous intake because it cannot be synthesized by the body (Ismail and Ismail 2016). The daily requirement of magnesium in young women is 230 mg per day. The results of this study meet the daily requirement of magnesium (120%). The magnesium consumption limit according to the Food and nutrition board is 350 mg/day. A deficiency of magnesium can inhibit erythropoiesis and the synthesis of hemoglobin resulting in anemia (Huang et al. 2023). AES also contains tannins, but the results are still classified as safe for consumption because it is still below the tannin intake limit based on SNI 560 mg/bw. Tannin is one of the phenolic compounds as an anti-nutritional agent. When tannins are consumed together with iron and zinc, they will affect the bioavailability of iron and zinc. Tannins will bind non-heme iron which comes from vegetable ingredients so that iron cannot be absorbed into the body (Natasia et al. 2021).

CONCLUSION
Analysis results of AES contains iron, magnesium, and vitamin C which are able to meet nutritional daily needs based on the Ministry of Health 2018, so that it can be used as an alternative ingredient for preventing anemia. In addition, AES contains low tannins so it does not inhibit the absorption of iron in the body. The hope is that AES can be used for iron supplementation which can be combined with other ingredients. Further research is required to confirm the beneficial effect of AES administration in female rats with anemia.

REFERENCES


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