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Research Article

Antioxidant Activity of *Syzygium oleana*

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Abstract

Background and Objectives: *Syzygium oleana* (*S. oleana*) is a plant that can potentially serve as a natural food colorant. Anthocyanin present in fruit and red (young) leaves also functions as an antioxidant. The purpose of this study was to obtain information about the antioxidant activity of *S. oleana* and to measure the total polyphenol and anthocyanin content in its fruits and red leaves using 3 kinds of solvents: Water, methanol and ethanol. **Materials and Methods:** This study used the exploratory method. The total phenolic content of the *S. oleana* fruit and leaf extract was measured using gallic acid as a standard. The DPPH was used to measure the antioxidant activities of *S. oleana* fruit and leaf extracts. Anthocyanin pigment concentration was calculated. **Results:** The results showed that the antioxidant content is activated up to 3.87, 26.4 and 65.65% with water, methanol and ethanol solvents, respectively. The total polyphenol content in water, methanol and ethanol are 55.37, 78.87 and 122.10 mg mL^{-1}, respectively. Anthocyanin content measured in water, methanol and ethanol are 16.42, 19.20 and 15.16%, respectively. **Conclusion:** This study suggests that *S. oleana* can be applied as a food colorant and that the best solvent for extraction is methanol.

Key words: *Syzygium oleana,* antioxidant, polyphenol, anthocyanin, methanol, ethanol

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Competing Interest: The authors has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

With advances in science and technology, the industrial sector develops various food products. Food products are made in such a way to be attractive to consumers through the diversification of tastes, textures and colors. Color is a major factor that determines the demand for a food item. Food with added color will give a unique and interesting characteristic for consumers and it seems, the food may become more tasty. Therefore, many colors are used to color the food, such as red, blue, pink, orange, purple and yellow which can be naturally sourced. The natural color comes from many plant and animal resources. Some parts of the plant can be used as a natural coloring, for example the fruit (blueberry, papaya, orange, pineapple and others), leaves (tea, especially black tea, cinnamon, papaya and others) and tree bark, including that of the '*Secang*' tree, which has a red color. A variety of food colors and dyes are used in currently available foods and drinks. Some of these dyes are synthetic, while others are sourced from nature (natural). Natural colorants are obtained from natural materials. Natural colorant sources include riboflavin, chlorophylls, carotene and anthocyanins. Natural colorants are not very stable and can be characterized by their physiological activity[1]. Popularity of natural food colorants has increased compared to that of artificial dyes since the natural colorants enhance visual appeal while adding healthy phytochemicals[2]. For instance, the anthocyanin extract of black rice is capable of preventing and ameliorating hyperlipidemia and insulin resistance induced by a high fructose diet[3].

The main reasons to use color additives in food are to maintain color during exposure to light and temperature changes, to enhance the natural colors and therefore improve the attractiveness of the food, to provide color to colorless foodstuffs and to allow consumers to instantly identify the product visually[4]. Sales of natural colorants increased dramatically from $465 million in 2007 to over $600 million in 2011[5]. The use of color additives from natural sources is important to avoid some diseases that can be caused by synthetic colorants. The synthetic colorants can cause so many diseases such as cancer and tumors or other generative stress. The use of natural color not only gives an interesting color but also positively affects human health. Usually, the natural color can act as antioxidant, as many researchers have reported.

In light of the growing demand for natural colorants, the research community has developed great interest in the development of processes for the producing colorants from natural sources. However, there is very little information about the use of *S. oleana* as a colorant or antioxidant, even though the fruits have a dark purple color that makes them suitable as a food colorant. *S. oleana* is usually used as an ornamental plant that can grow as tall as 10 m, but even a short one still has many red colors and fruit. In Indonesia, *S. oleana* grows well in many places and the interesting red color of the young leaves gives beauty to its surroundings. People usually plant this plant in their home garden, in parks, at the city park and along the side of the road, since this plant also provide protection from sun light. *Syzygium oleana* has red-colored young leaves and it has fruit with a very dark purple color that taste sweet. The use of the red leaves and fruit is not reported yet and that is why the researchers are interested to study the antioxidant activity of its red leaves and fruit. The appearance of the fruit is similar to blueberries but smaller. This study will provide information about the anthocyanin and polyphenol content in the red leaves and fruit so they can be applied as food colorants. Because information about the use of the fruit is lacking, the fruit itself is not used for consumption or as a colorant. This study will be very useful for the uses of this plant, not only for consumption but to further describe its antioxidant properties and their beneficial health effects. Because the red leaves and fruits are not popular for consumption, in this article, the toxicity of this plant was also measured. In this study, the researcher will investigate which solvent is best used for extraction: Methanol or ethanol. Both methanol and ethanol are a very popular extraction solvents and they have a good abilities to dissolve some antioxidant compounds in some plants. In addition to methanol and ethanol, the researcher also used water for extraction because it is a safe solvent for use in food products. This study will show the differences between water, methanol and ethanol to dissolve color or other antioxidants in *S. oleana*. The effect of extraction on the anthocyanin composition in blueberries and cherries was analyzed with HPLC. The optimum method was identified to be 60% methanol for blueberries. Using an ultrasound-assisted method with 60% methanol was found to be optimal for cherries[6].

The present study focused on the specific chemical compounds in *S. oleana* fruits and leaves that are associated with antioxidant activity. This research used water, methanol and ethanol as solvents for extraction.

MATERIALS AND METHODS

Raw material: Young leaves and fruits of *S. oleana*.

Chemicals: Methanol, diphenylpicrylhydrazyl (DPPH), distilled water, gallic acid, Na_2CO_3, Folin-Ciocalteu reagent, HCl, $CH_3CO_2Na{\cdot}3H_2O$.

DPPH radical scavenging activity: The DPPH radical scavenging activity was monitored using the method originally developed by Blois[7]. A portion (0.1 mL) of the methanol extract solution (1.0 mg mL^{-1}) in a test tube was mixed well with 3.9 mL of methanol and 1.0 mL of a DPPH solution (1.0 mM in methanol). The mixture was kept at ambient temperature for 30 min prior to measurement of the absorbance at 517 nm (A517 nm). All measurements were done in triplicate.

$$\text{Percent DPPH-RSA} = \frac{(\text{Control absorbance} - \text{extract absorbance})}{\text{Control absorbance}} \times 100$$

Total polyphenol: The total phenolic content of the extract was measured by the Folin-Ciocalteu method[8], using gallic acid as a standard. A 0.1 mL sample of the extract solution was added to 2 mL of 2% Na_2CO_3 and mixed for 3 min. After adding 0.1 mL of 50% Folin-Ciocalteu reagent, the final mixture was left for 30 min before measuring the absorbance at 750 nm. All measurements were conducted in triplicate and the data were expressed as Gallic Acid Equivalent (GAE) per kg extract based on the calibration curve of gallic acid.

Anthocyanin content[9]: About 1.86 g of KCl was added to a beaker and approximately 980 mL of distilled water was added to it. Next, the pH was measured and adjusted it to (1.0$\pm$0.05) with HCl (approximately 6.3 mL). The mixture was transferred to 1 L volumetric flask and diluted to volume with distilled water. Furthermore, 54.43 g of $CH_3CO_2Na{\cdot}3H_2O$ was weighed in a beaker and added distilled water to approximately 960 mL. Next, the pH was measured and adjusted it to (4.5$\pm$0.05) with HCl (approximately 20 mL). Then, the mixture was transferred to a 1 L volumetric flask and diluted to volume with distilled water.

Next, anthocyanin pigment concentration was calculated, expressed as cyanidin-3-glucoside equivalents, as follows:

$$\frac{\text{Anthocyanin pigment}}{(\text{cyanidin-3-glucoside equivalents, mg L}^{-1})} = \frac{(A \times MW \times DF \times 1000)}{(\varepsilon \times d)}$$

where, A = (A520-A700 nm) pH 1.0-(A520-A700 nm) pH 4.5.

where, Molecular Weight (MW) = 449.2 g mol^{-1} for cyanidin-3-glucoside (cyd-3-glu), DF is dilution factor established in D, l is path length in cm, ϵ is 26,900 molar extinction coefficient, in L and mol^{-1} and cm^{-1}, for cyd-3-glu and 103 is factor for conversion from g to mg.

Statistical analysis: Standard Deviation (SD) was calculated using Excel 2003 for the antioxidant, total polyphenols and anthocyanin results.

RESULTS AND DISCUSSION

Antioxidant activity: The DPPH was used to measure the antioxidant activities of *S. oleana* fruit and leaf extracts, which have a deep purple color. The DPPH disappears upon reduction by an antioxidant compound, yielding a yellow colored solution. This could be taken as an indication of the hydrogen-donating ability of the samples[10]. The use of DPPH can estimate the antioxidant activity in a short time and the incubation time for DPPH solution and sample is 30 min. Thirty minutes of incubation is enough time for polyphenols and other antioxidants to react optimally with DPPH. Some compounds react very fast (2 min), but some antioxidants need more time (30-60 min). The change in the purple color shows the antioxidant property in plant, the purple color becomes less intense or even disappears because of the ability of antioxidants to scavenge the radical solvent. The antioxidant activities of the water, methanol and ethanol extracts in the *S. oleana* fruit are shown in Figs. 1 and 2. Antioxidant activities of the water, methanol and ethanol extracts in the *S. oleana* leaves are shown in Fig. 3.

The antioxidant activities of *S. oleana* fruit extracts ranged from 3-63% with water, methanol or ethanol. For concentrations of 1000 and 10,000 ppm, the water extract of *S. oleana* had almost no antioxidant property. The extract of *S. oleana* showed higher antioxidant activity for the ethanol extract than the methanol extract. Ethanol can better dissolve the antioxidants in *S. oleana* fruit than methanol and water. Ethanol is the best solvent to extract the antioxidants in the red leaves and fruit of *S. oleana* and ethanol is less toxic than methanol. The use of methanol is also dangerous for the environment and ethanol is more safe for the environment and for health. Water is a very safe solvent, but it cannot dissolve antioxidant or polyphenols in this plant well. Anthocyanin is water soluble but best dissolves in organic solvents such as ethanol and methanol. When using ethanol, before using the food colorant, the solvent should be evaporated in rotary evaporator. The deep purple color of the fruit of *S. oleana* could be due to the presence of anthocyanin. However, Fig. 5 shows that the anthocyanin content in *S. oleana* fruit was not influenced by the solvents used for extraction, meaning that the anthocyanin content in *S. oleana* fruit was not the only polyphenol responsible for the antioxidant activity. There might exist was another compound in *S. oleana* fruit that is a potent antioxidant besides anthocyanin.

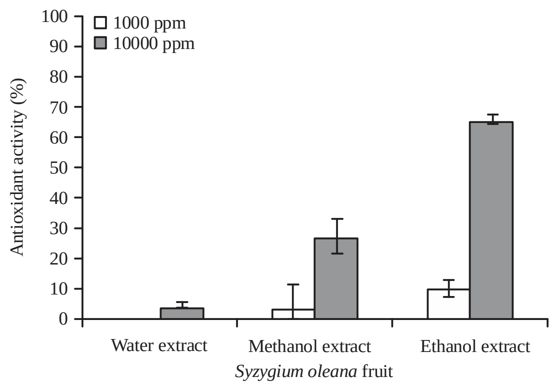

Fig. 1: Antioxidant Activities of water, methanol and ethanol extract of the *S. oleana* fruit

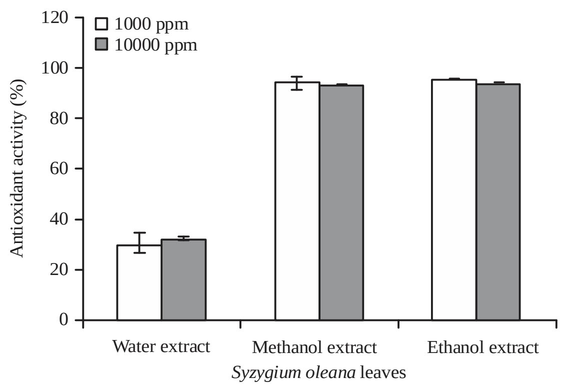

Fig. 2: Antioxidant Activities of *S. oleana* leaf water, methanol and ethanol extracts

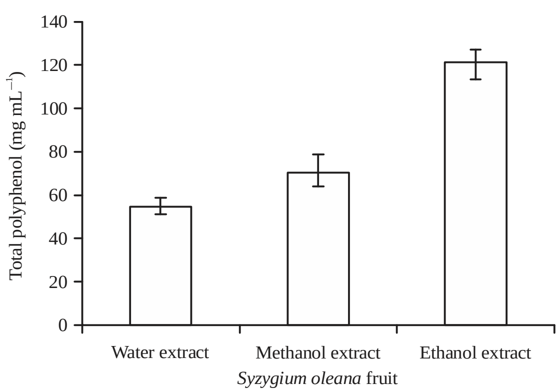

Fig. 3: Total Polyphenol contents of *S. oleana* fruit water, methanol and ethanol extracts

The antioxidant activities of *S. oleana* leaf water, methanol and ethanol extracts showed higher antioxidant activity than the fruit extract. The *S. oleana* leaf water extract showed the lowest antioxidant activity compared with the methanol and ethanol extracts, as was seen with antioxidant activity in the fruit extracts. In contrast to the *S. oleana* fruit, the leaves were potent as an antioxidant, as seen from the extract. The color of the leaves is red and the color of fruit extract is deep purple. The different colors mean there is a different anthocyanin content in the leaves and fruit. There is a need for investigation about the kinds of anthocyanin in this plant, because they have different capacities as antioxidants. From the antioxidant activity presented in Fig. 2, it is seen that *S. oleana* leaves have more antioxidant properties than the fruit extract. This study used the young, red colored leaves of *S. oleana*. The red color could be due to the presence of anthocyanin or another compound that gives a red color.

In candies made of fruit extract enriched with grape skin powder, the anthocyanin, flavonol and procyanidin contents increased and the candies remained stable during processing[11]. Grape skin powder is reported to be an important source of phenolic compounds, which act as natural antioxidant sources. Phenolic compounds are responsible for scavenging free radicals. Their correlation with antioxidant agents has been studied in fruits or vegetables[12].

Based on Fig. 3, it is evident that ethanol is the best solvent to dissolve the polyphenols in the fruit of *S. oleana* compared to methanol and water. The total polyphenol content in *S. oleana* leaves was not influenced by the solvents used for extraction, where the total polyphenols obtained with the use of water, methanol and ethanol did not vary much. This could be due to the presence of a larger portion of major polyphenol compounds in the leaves than in fruit. Fruits have more varied polyphenols that are dissolved well in ethanol. It is easier to separate them using ethanol and the rotary vacuum evaporator. This way the anthocyanin pigments in plants could be used for food coloring. Polymeric color is a widely used index for measuring anthocyanin degradation in aqueous extracts and juice[13].

Figure 4 shows that the highest total polyphenol content was in the ethanol extract. A previous study showed that the ethanol extract exhibited the highest antioxidant and phenolic content in the Kenitu fruit[14]. Other results regarding total polyphenols in leaves and fruit showed that, the polyphenol in fruit is easy to dissolve with all three kinds of solvent. However, the polyphenol in fruit has slight activity as an antioxidant. Antioxidant activity is very important to human health and is important for food product if we use them as colorants. For food products with high fat, this will help to make the shelf life longer because of the function of polyphenol as antioxidant. The use of *S. oleana* red leaves will increase the

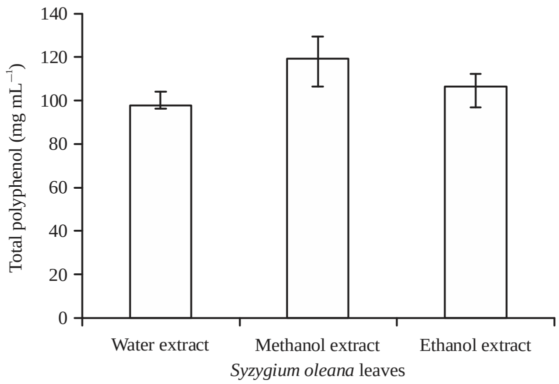

Fig. 4: Total polyphenol contents of *S. oleana* leaf water, methanol and ethanol extracts

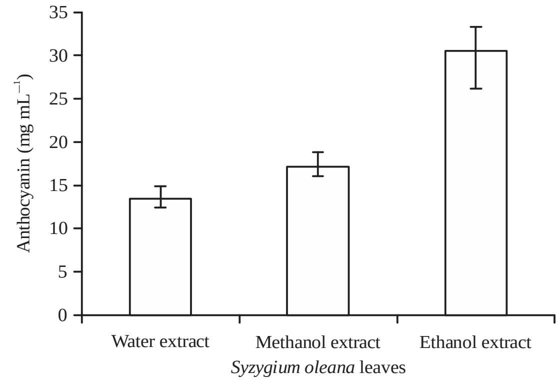

Fig. 6: Anthocyanin contents of *S. oleana* leaf water, methanol and ethanol extracts

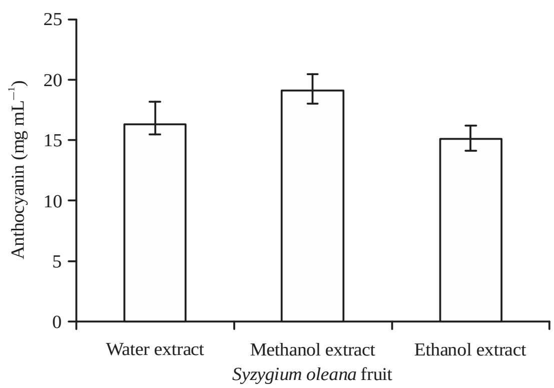

Fig. 5: Anthocyanin contents of *S. oleana* fruit water, methanol and ethanol extracts

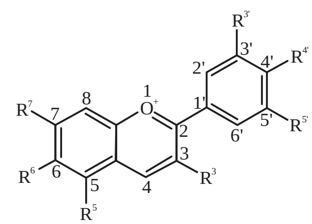

Fig. 7: Basic structure of anthocyanin

value for food products because of the unique color produced by it. The color will make the food eye catching and will function as an antioxidant that will reduce oxidative stress in humans.

Figure 5 shows that the anthocyanin content in *S. oleana* fruit was not influenced by the solvent used for extraction, but the total polyphenol content in Fig. 3 shows that ethanol is the best solvent to extract the *S. oleana* fruit. It can be concluded that there were other polyphenol compounds besides anthocyanin presence in the *S. oleana* fruit that dissolved well in ethanol. Based on above figure, anthocyanin dissolves well in water, methanol and ethanol, but compared with fruit, the red leaves have small amounts of anthocyanin.

Figure 6 shows that the anthocyanin content in *S. oleana* leaves dissolved in ethanol>methanol>water: 30.57, 17.27 and 13.49 mg mL^{-1} respectively. Based on Fig. 4, the polyphenols present in *S. oleana* leaves is the same in the various extractions but Fig. 6 shows that the anthocyanin content in *S. oleana* leaves varies depending on the solvent. It showed that anthocyanin is not the major compound in *S. oleana* leaves. It is suggested to investigate the polyphenols present in *S. oleana* leaves. Further investigation about the antioxidant properties both in leaves and fruit of *S. oleana* is necessary because Figs. 6 and 5 show that fruit with high anthocyanin content do not have high levels of antioxidants. There are seven kinds of anthocyanins: delphinidin ($C_{15}H_{11}O_7$), cyanidin ($C_{15}H_{11}O_6$), pelargonidin ($C_{15}H_{11}O_5$), malvidin ($C_{17}H_{15}O_7$), petudinin ($C_{16}H_{13}O_7$), petunidin ($C_{16}H_{13}O_6$) and fragarin ($C_{21}H_{21}O_{10}$).

Anthocyanin in colored rice (red and black rice) in West Sumatra and its application in a food product called 'Kembang Loyang' a traditional food that is made from rice, sugar and coconut milk and is deep fried, still has antioxidant activity and polyphenols[15,16]. Thus, the application of the *S. oleana* fruit will be a very good resource of food colorant and antioxidant in foods, such as bakery products, ice cream, bread, or fried food, because anthocyanin is quite stable at high temperatures, although the color of anthocyanin will change based on the pH. The basic structure of anthocyanin can be seen in Fig. 7.

By using the Brine Shrimp Test, it was observed that the extract of young leaves and fruit of *S. oleana* is not toxic (data not shown). Thus, the fruit of *S. oleana* which has not been

Fig. 8: Plant of *Syzygium oleana*

Fig. 9: Fruit of *Syzygium oleana*

consumed before, could be consumed freshly like common fruits including blueberries or blackberries. Additionally, they can be extracted optimally by using ethanol as a solvent, which is also environmentally safe. Natural, safe and functional foods provide a preventive, protective function against disease, in addition to adequate nutritional benefits[17]. The plant and fruit of S. oleana can be seen in Fig. 8 and Fig. 9.

SIGNIFICANCE STATEMENTS

This study discovered the antioxidant activity of *S. oleana* young leaves and fruit. The results showed that this plant could be a promising source of natural colorant that is safe and whose contents provide functional benefits for human health. The use of the pigment can be applied to some food products or the beverage industry. This study will help the researcher to find new information and a new source about the benefits of natural pigments from *S. oleana* young leaves and fruits.

CONCLUSION

Syzygium oleana fruit and leaves contain antioxidants, total polyphenols and anthocyanins and can be applied to food products (nontoxic). The best solvent extraction process used is ethanol. The results showed that the antioxidant content is activated up to 3.87, 26.4 and 65.65% with water, methanol and ethanol as a solvent, respectively. The total polyphenol content in water, methanol and ethanol are 55.37, 78.87 and 122.10 mg mL^{-1}, respectively. Anthocyanin content measured in water, methanol and ethanol are 16.42, 19.20 and 15.16%, respectively. Other polyphenol compounds in *S. oleana* should be investigated further.

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