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Phytochemical Screening and Antioxidant Activity of Selekop (*Lepisanthes amoena*) Fruit

Heriad Daud Salusu[1,2*)], Farida Ariani[2)], Ernita Obeth[3)], Mark Rayment[4)], Edy Budiarso[5)], Irawan Wijaya Kusuma[6)] and Enos Tangke Arung[6)]

[1)] Faculty of Forestry Mulawarman University Samarinda East Kalimantan Indonesia

[2)] Lab. of Wood Properties and Product Analysis Samarinda State Polytechnic of Agriculture Indonesia

[3)] School of Processing Technology of Agricultural Product Samarinda State Polytechnic of Agriculture Indonesia

[4)] School of Environment Natural Resources and Geography Bangor University United Kingdom

[5)] Lab. of Wood Preservative Faculty of Forestry Mulawarman University Indonesia

[6)] Lab. of Forest Product Chemistry Faculty of Forestry Mulawarman University Indonesia

[*)] Corresponding author E-mail:rissalusu@yahoo.com

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ABSTRACT

Selekop (*Lepisanthes amoena* (Hassk.) Leenh.) plant leaves are used by the Dayak tribe of East Kalimantan as traditional cosmetics. Selekop fruit is also edible, but not well known. This study was conducted to obtain the phytochemical content and antioxidant assay in flesh, seed and pericarp extracts from the fruit of Selekop. Phytochemical analysis was conducted on ethanol extract for identification of flavonoid, alkaloid, tannin, saponin, triterpenoid and steroid. The antioxidant activity was done by DPPH assay with ascorbic acid as positive control. The flesh contained flavonoid, saponin, and tannin; the seed contained flavonoid, alkaloid, saponin, triterpenoid, and tannin; and the pericarp contained flavonoid, alkaloid, saponin, triterpenoid, and tannin. Analysis of antioxidant activity revealed the following *Inhibitory Concentration* (IC_{50} values): 122.51 ppm of flesh, 63.30 ppm of seed, 53.21 ppm of pericarp and 3.06 ppm of ascorbic acid. Based on these results, the ethanol extract of the seed and the flesh had a phytochemical content and antioxidant activity which was better than the flesh extract from Selekop fuit.

Keywords: antioxidant; flesh; pericarp; phytochemical; seed; Selekop

INTRODUCTION

Fruits obtained from forests are considered unimportant or non-profitable in comparison to other forest products such as timber, rattan and resin. Durian, mango, rambutan and mangosteen are some of the most popular forest fruit species in Indonesia while more other species are generally known only by the local residents around the forest. Increasing the value and benefits of forest fruit or local fruit has now become a challenge following the entry and domination of imported fruits in Indonesian markets. One effort that can be done to improve the image of forest fruits is by conducting more research on the nutrients and bioactivity contained in the fruits. Information on the benefits of certain forest fruits has traditionally been known only by the locals. The scientific research is not yet to be done. The Selekop fruit (*Lepisanthes amoena* (Hassk.) Leenh.) is a species of forest fruit that is not well identified. This fruit belongs to the family of Sapindaceae. In some regions in East Kalimantan it is known as Kokang or Kukang. In Western Java it is called Langir, while in South Kalimantan the locals call it Rembia. In general, the Dayak tribe of East Kalimantan uses the leaves as traditional cosmetic ingredients.

To date, there has not been a single report on the benefits of this fruit except for its being an edible fruit. As a cosmetic ingredient, (by various ethnic groups in Borneo) it is generally used in mixing powder, skin cleanser, shampoo and soap. It is used by squeezing the leaves and mix them with a little water to produce lather like soap. But now, because of modern cosmetics, shampoo and soap are available to the public so that the leaves are rarely used.

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This plant is considered of possessing a minor economic value, wildly grown in the forest and uncultivated (Milow, Malek, Edo, & Ong, 2014). Although the leaves of this plant have traditionally been known to be beneficial, the fruit is still unidentified and it requires further study about its benefits and contents especially bioactive components that have the potential for further development.

All bioactive food compounds are obtained from the plants; they are called the phytochemicals. Most of these compounds are active molecules and named as antioxidants. The antioxidants can diminish reactive oxygen, nitrogen, and free radicals. The reactive species cause many chronic diseases (Carlsen et al., 2010). Food from plants like vegetables, fruits, and whole grains, had abundant of bioactive compounds and give contribution to human health than basic nutrition to combat diseases. Zhang et al. (2015) suggested that more studies need to be done to identify the bio actives and mechanism of antioxidant phytochemicals actions. Thus, it is expected that more antioxidant phytochemicals in food as well as in medicinal plants will be explored not only for its positive but also potential undesirable effects to human.

Therefore, this research was carried out to determine the antioxidant activity and phytochemical in Selekop fruit extracts contained in the flesh, seed and pericarp in order to collect data and information to enhance the benefits of this fruit in the future.

MATERIALS AND METHODS

The research was conducted for two months (November-December 2015) in the Laboratory of Wood Properties and Product Analysis, State Agricultural Polytechnic Samarinda.

Materials

The tools used were a rotary vacuum evaporator, spectrophotometer, and other glass tools, while the material was fresh *Selekop* fruit separated into three different parts (i.e., flesh, seed and pericarp). In addition, a number of chemical materials namely etanol, acetone, aquades, solvent of DPPH (*1,1-diphenyl-2-picrylhydrazyl*), dimethyl sulfoxide (DMSO), acetic acid anhydride, sulfuric acid), Dragendorff reagent, Liebermann-Burchard regent, Molisch reagent, Sodium hydroxide, hydrochloric acid, lead acetate, and ascorbic acid were used in the analysis process.

Preparation of Fruit Extract

The extraction method used was cold extraction with etanol as a solvent. Cold extraction was done by dissolving the powder in the ratio of powder : solvent (1:10). The extraction was done by immersing each sample with etanol. The sample was then shaken using a shaker for 48 hours at room temperature, then filtered to separate the extract from fruit material. Furthermore, by using a rotary evaporator, the extract was evaporated at a temperature of 30-40 ^{0}C, to obtain a crude extract.

Phytochemical Screening

The secondary metabolites from each of the extracts were analyzed using phytocemical screening according to standard procedures such as flavonoids, tannins, alkaloids, triterpenoids/ steroids, and saponins analysis. For the tests for flavonoids (Kokate, 2001), a few drops of dilute sodium hydroxide (NaOH 1 %) were added into 1 ml of sample extract. The emergence of the clear yellow color in the extract solution, which turned colorless after the addition of dilute acid (HCl 1 %), indicated that flavonoids were present. Meanwhile, for the tests for tannins (Kokate, 2001), 10 ml of the extract solution was added to a 1 % solution of lead acetate. The tannins were tested positive if the yellow precipitate was formed. Furthermore, the tests for alkaloids (Kokate, 2001), 2 ml of HCl was added to 5 ml of the extract solution, then combined with 1 ml of Dragendorff solution. The color of the solution became orange or red indicating that the extract contained alkaloids. While the tests for triterpenoids and steroids (Harborne, 1987), Anhydride acetic acid was put about 10 drops and 2 drops of concentrated sulfuric acid were put into sequentially to 1 ml of sample test that had been dissolved in acetone. The test sample was shaken and left for a few minutes. If it turned to red and purple, the test would be declared positive for triterpenoids and if it turned green and blue, this would indicate a positive test for the presence of steroids. Moreover, for the tests for saponins (Harborne, 1987), 10 ml the hot water was put into 1 ml of sample test which had been dissolved in acetone. The solution was cooled and shaken for 10 seconds. The formation of a steady froth with a height of 1-10 cm would take approximately 10 minutes and remained even after the addition of 1 drop of 2N HCl, which indicated that the tested extracts contained saponins.

Antioxidants Activities

The testing of antioxidant activity used a spectrophotometer at room temperature (25 ^{0}C) with a wavelength of 514 nm and a solution of DPPH (1,1-diphenyl-2-picrylhydrazyl) acted as a free radical, and ascorbic acid as a positive control. The amount of antioxidant activity was characterized by the IC_{50} (inhibitory concentration), that is a concentration of the sample solution indicated a potential antioxidant that was needed to reduce DPPH by 50 % (Arung, Kusuma, Christy, Shimizu, & Kondo, 2009).

The activity of the antioxidant from the sample extract was determined based on the relative inhibition percentage compared to the control using the following equation:

$$\text{Relative Inhibition Activity}(\%) = \frac{A_{DPPH} - A_{sample}}{A_{DPPH}} \times 100\%$$

Where A_{DPPH} represents the absorption from DPPH and A_{sample} the absorption from the sample. The result was then integrated into the regression equation with extract concentration as the abscissa (x-axis) and the percentage of antioxidant inhibition as the ordinate (y-axis). The value of IC_{50} was calculated when the value of inhibition percentage reached 50 % using the equation y = ax + b.

RESULTS AND DISCUSSION

In this study, ethanol was used for extraction because it is an alcohol derivative that would easily dissolve the groups of secondary metabolites, both polar and non-polar. Furthermore, ethanol would not hydrolyze compounds; be volatile at a low boiling point, and good for thermolabile compounds. As stated by Harborne (1987), ethanol was good to be used for preliminary extraction.

Phytochemical testing was conducted to determine the content of secondary metabolites contained in the each of the extract in order to find out the potential utilization by testing the active compounds such as flavonoids, tannins, alkaloids, triterpenoids, steroids, and saponins.

Phytochemical contents in the crude extract of flesh, seed and pericarp from Selekop fruit are shown in Table 1. The data shows that the extract from flesh only contains flavonoids, tannins, and saponins, while the extracts from the seed and the pericarp contain almost all components except for steroids which were not found in all three parts of the fruit extract.

Meanwhile, flavonoids were detected in all parts of the fruit because these compounds belonged to the natural polyphenol group which can be found in many fruits, vegetables, nuts, seeds, flowers, leaves, barks, etc.

Furthermore, flavonoids belong to one group of aromatic compounds including polyphenols and antioxidants and the abundant numbers in nature, so they are more widely used than other compounds as antioxidants (Waluyo & Pasaribu, 2013).

Tannins are usually found in almost all green plants, and more in barks. The test results show all three parts (flesh, seed and percarp) of the fruit contain tannins. Taste astringent on some fruits including fruit Selekop indicates the presence of tannins. The more tannin content, the greater the antioxidant activity presents due to the fact that tannins contain polyphenolic compounds that have free radical catcher activities (Malangngi, Sangi, & Paendong, 2012).

Table 1. Phytochemical in extracts of flesh, seed and pericarp of Selekop

Phytochemicals	Parts of Selekop fruit		
	Flesh	Seed	Pericarp
Flavonoids	+	+	+
Alkaloids	-	+	+
Saponins	+	+	+
Tannins	+	+	+
Triterpenoids	-	+	+
Steroids	-	-	-

Remarks: + = present; - = absent

Alkaloid content in fruits were found only in the pericarp and seed but not in the flesh. Alkaloids are one of the secondary metabolites found in plants, which can be found in leaves, branches, seed, and bark, (Aksara, Musa, & Alio, 2013). Novelli, Lorena, & Antonella (2014) reported that alkaloid contained in *Ficus benjamina* L. crude extract, had a strong correlation with its antioxidant.

Similar to alkaloids content, triterpenoid was not found in the flesh but only in the seed and pericarp. Harborne (1987), stated that triterpenoids compounds generally found in resin and sap, classified as essential oils, were volatile and fragrant. Steroids were not detected in all of the extracts. Generall, steroids are found in leaf photosynthesis and are rarely found in fruits. Steroids in plants work to increase the rate of plant cell renewal, retard leaves aging (senescence), hamper leaves drop, and stimulate cell renewal in plant shoots.

Saponins were detected in all parts of the fruit. This confirms the statement of Fahrunnida & Pratiwi (2015), which reported that the highest amount of saponin was found on the fruit rather than on the leaves and leaf stalks in the case of starfruit.

Result of the analysis of the antioxidant activity based on IC_{50} values and compared with ascorbic acid as a positive control is shown in Table 2.

Based on IC_{50} values antioxidant of sample is presented in Table 2, as well as ascorbic acid as positive control.

Table 2. IC_{50} value of extract flesh, seed and pericarp on Selekop fruit

Samples	IC_{50} (ppm)
Flesh	122.51
Seed	63.30
Pericarp	53.21
Ascorbic acid	3.06

The strength of the antioxidant activity was determined based on IC_{50} value, referring to Jun et al. (2003) where the value of < 50 ppm is considered as strong, 50-100 ppm as active, 100-250 ppm as moderate, 250-500 ppm as weak, and > 500 ppm as inactive.

Based on IC_{50} of antioxidant activity for the pericarp, seed, and flesh was 53.21 ppm, 63.31 ppm (active category), and 122.51 ppm (medium category) respectively. Ascorbic acid as positive control with IC_{50} value of 3.06 ppm has strong antioxidant activity compared to the pericarp and seed. Data showed that the antioxidant activities of pericarp and seed were better than that of the flesh. Several previous studies on several species of fruit showed antioxidant activity in pericarp and seeds were better than that of the flesh. Contreras-Calderón, Calderón-Jaimes, Guerra-Hernández, & García-Villanova (2011) reported that the cashew, araza, coastal sapote, and algarrobo seeds had the strongest antioxidant and the highest value of total phenol as well as the algarrobo and coastal sapote peels. Similarly, the extract of peach peel contained minerals and phenolics, therefore had a strong antioxidant and benefit for food and nutrition (Manzoor, Anwar, Mahmood, Rashid, & Ashraf, 2012).

The overall results of this study indicated that the content of phytochemicals and antioxidants in pericarp and seeds were better than that of the flesh. The existence of phytochemical components and antioxidant activity expressed by Ahmed et al. (2014) in his study of *Calamus tenuis* Roxb suggested a correlation between antioxidant activity and the total flavonoid content. Regarding with phytochemicals, the peel and seed were higher than flesh and useful as reducing agents and radical scavengers. These antioxidants activities had a close relationship with its total phenols and flavonoids (Bakar, Karim, & Perisamy, 2015).

CONCLUSION AND SUGGESTION

This study showed that extract of pericarp, flesh and seed from Selekop fruit can serve as potential source of natural antioxidants. Out of the three extracts, seed in particular, prospective to be developed as a source of natural antioxidants and has the higher medicinal benefit. Further studies are required for the isolation and characterizing of antioxidant components as well as the active phytochemicals responsible their biological activities.

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