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Antioxidant and Anti-Melanogenic Activities of Pakoba Leaves (*Syzygium* sp) from North Celebes

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Abstract: In this research the potential of Pakoba leaf extract to be used as antioxidant and skin whitening (anti-melanogenic agent) is investigated. The antioxidant activity of Pakoba leaves were studied using DPPH method and the result showed that the 80 percent methanol crude extract has strong antioxidant activity with an IC50 value of 22.66 ± 1.02 µg/ml. The aqueous fraction of the sample has an IC50 value of 53.30 ± 1.42 µg/ml, followed by n-butanol fraction (53.63 ± 1.45 µg/ml) and chloroform fraction (511.54 ± 1.59 µg/ml). The anti-melanogenic activity of the crude methanol extract showed IC50 value of 316.56 ± 11.04 µg/ml. Thus, it is concluded that crude extract of Pakoba leaves shows good potential as the antioxidant source although it does not show good anti- melanogenic activity.

Keywords: syzygium, pakoba, antioxidant, antimelanogenic

1. Introduction

The skin care product m arket size is increasing from year to year. By 2024, the global skin care market is estimated (Bae-Harboc & Park, 2012) to be 180 billion U.S. dollars (Statista, 2018). The demand itself has been shifted from older consumers to a growing younger consumer base, where the skin lighteners will reach 23 billion U.S dollar and antioxidant product will reach 3.1 billion U.S dollar by 2020 (Researh Report Insights, 2018).

Skin is the largest part of the body which has the important role to protect the internal part from environment. The color of the skin is caused by the melanin, which is produced from epidermis melanocytes surrounded by keratinocyte and regulated by a closed paracrine system (Pillaiyar et al., 2017). There are two types of melanin: eumelanin (brown-black) and pheomelanin (red-yellow). Although melanin is a protective barrier against UV radiation from the sunlight, the melanogenesis can create unwanted side effects such as make the color of the skin not evenly distributed or even induce hyperpigmentation (Bae-Harboc & Park, 2012). Skin hyperpigmentation is not dangerous for health, but it can decrease the aesthetics value of face and body in some populations.

The melanogenesis process is initiated with the oxidation of L-tyrosine to dopaquinone (DQ) by tyrosinase enzyme (Gillbro & Olsson, 2011). After the dopaquinone is produced the synthesis pathway will be divided into two parts: eumelanin synthesis and pheomelanin synthesis. To synthesize eumelanin, which will make the skin darker, dopaquinone must first be converted to dopachrome. Dopachrome is converted spontaneously by tyrosine-related protein-2 (TYRP-2) into 5,6-dihydroxindole or 5,6-dihydroxindole-2-carboxylic acid, which will be converted again to indole-5,6-quinone and indole-5,6-quinone carboxylic acid. Polimerization of these two will produce eumelanin. Most of melanin synthesis inhibitors inhibit melanogenesis by inhibiting tyrosinase activity.

UV radiation can induce skin aging because UV generates reactive oxygen species (ROS). ROS is reactive oxygen chemical molecule like peroxide, super peroxide, radical hydroxyl, and singlet oxygen. UV light and oxidative stress are the condition where ROS is found excessively in the body, and is believed linked to skin disorders (Thiele and Elsner, 2001).

According to the website published by Ministry of Environment and Forestry of Republic of Indonesia (Nurrani & Tabba, 2012), Pakoba (*Syzygium* sp) is an endemic plant of North Celebes. It is a big tree with height 10 – 20 m, wooden trunk, and a lot of branches and leaves. The wooden trunk is usually used for construction, and the fruit can be eaten although the taste is sour. The bark can be used as coloring agent for textile products. It is also reported that Pakoba leaf and bark are believed by the local people to be a cure for diabetes and cancer (Hidayah & Nurrani, 2017). In general some members of *Syzygium* family contain volatile oils that can be used as medicines. Nevertheless as far as the author's knowledge, Pakoba leaves have not yet been researched as anti-oxidant and tyrosinase inhibitor agent.

2. Materials and Methods

2.1. Materials

1,1-Diphenyl-2-picryl hydrazyl (DPPH) (Sigma Aldrich), methanol, aquadest, n-buthanol, chloroform, potassium dihydrogen phosphate (Merck), disodium hydrogen phosphate (Na_2HPO_4) (Merck), l-tyrosine (Sigma-Aldrich), mushroom tyrosinase (Sigma-Aldrich), hydroquinone (Sigma-Aldrich).

2.2. Collection and preparation of Pakoba leaves crude extract

Sample of leaves were collected from North Celebes in January 2016. Fresh samples were dried using oven at 60ºC. Samples of 200 g of dried Pakoba leaves were macerated in 2 liter of 80% methanol for 24 hours at room temperature. The maceration was done twice. After the methanol extract was filtered, it was evaporated using rotary evaporator. The extract were then frozen and kept at -20oC until further analysis.

2.3. Liquid-liquid extraction

Samples of 2.78 g crude extract was diluted by 75 ml aquadest. To the mixture was added 75 ml chloroform, mixed in separatory funnel. Then the chloroform layer was taken, continued by the water and butanol layer. In the end, there are three fractions: chloroform fraction, butanol fraction, and water fraction. These fractions were prepared for further analysis

2.4. Antioxidant activity test

The antioxidant activities of the extracts were determined using DPPH method (Proestos et al., 2013). First samples were dissolved in absolute methanol and then centrifuged to remove insoluble materials. In the Eppendorf tube, 1 ml of DPPH 0.17 mM was added to 0.8 ml sample, and then the mixture was vortexed until homogenous. The resulting samples were incubated in the room temperature in dark condition for 20 minutes. Color changes were observed and measured using UV spectrophotometer at λ 517 nm. DPPH and methanol were used as control. Vitamin C was used as positive control. Blanks were made using methanol. DPPH Scavenging Effect for the samples were calculated. This assay was done for several sample concentrations to get IC_{50} value. The concentrations used are 8, 16, 32, 64, and 128 ppm for crude extract, butanol fraction, and water fraction. For chloroform fraction the used concentrations are 16, 32, 64, 128, 256, and 1024 ppm. For each sample concentration (for all treatments), the experiments were repeated 3 times.

2.5. Anti - melanogenic activity test

This test was performed based on the method of Teixeira et al. (Teixeira et al., 2012) with some modifications. Pakoba crude extract was made into several concentrations using phosphate buffer 50 mM pH 6.8. After that, in the Eppendorf tube 140 µL phosphate buffer (50mM, pH 6.8), 120 µL extract, and 140 µL L-tyrosine (300 µg/ml) were mixed and then incubated for 5 minutes at 37ºC. After that, 40 µL of tyrosinase enzyme was added and incubated again for 30 minutes at 37ºC. Phosphate buffer as extract replacement was used as negative control. Hydroquinone was used as positive control. For blanks, phosphate buffer was used as tyrosinase enzyme replacement. Color changes were observed and measured using UV spectrophotometer at λ 475 nm. This assay was done for several sample concentrations to get IC_{50} value. The concentrations used are 100, 200, 250, 300, 350, 400, 600, 800, 1000 ppm for crude extract. For each sample concentration, the experiments were repeated 3 times.

3. Result and Discussion

The results of the antioxidant activity experiments are shown in Table 1. In that table, it can be seen that crude methanol extract has the best (lowest) IC_{50}, although it is still far above the control positive (vitamin C). Vitamin C is of course a very potent antioxidant. To get a better comparison, a published classification of antioxidant potency of an extract is used. Antioxidant activity of an extract can be classified as very strong if $IC_{50} < 50$ ppm, strong if IC_{50} 50 – 100 ppm, medium if IC_{50} 100 – 150, weak if IC_{50} 150 – 200, and very weak if $IC_{50} > 200$ ppm (Agustini et al., 2015). With this classification, the crude methanol extract can still be classified into very strong antioxidant.

Table 1: Antioxidant result

Extract	IC_{50} Value (ppm)
Crude methanol extract	22.66 ± 1.02
Chloroform fraction	511.56 ± 1.59
Butanol fraction	53.63 ± 1.45
Water fraction	53.30 ± 1.43
Vitamin C	8.55 ± 0.02

From the antioxidant IC_{50} of further separations of the crude methanol extract (chloroform fraction, butanol fraction, and water fraction) in Table 1, that show the IC_{50} are lower for polar solvent fractions, it can be concluded that the antioxidant active compound could be slightly polar to polar in nature. The higher value of the IC_{50} in the butanol and water fraction might be caused by the decomposition of the active compound. Antioxidant compounds are known to be easily degraded, for example by heating (Xu et al., 2017). The process of separating the crude methanol extract into further fractions did indeed involve some heating. Beside high temperature, antioxidants can also be degraded by exposure to light, oxygen, and drying, as well as the use of inappropriate solvents (Suryaningrum et al., 2006).

Since the drying process of the Pakoba leaf samples were done using oven, the antioxidant active compound might actually be more potent than what is reported in Table 1. Further research using freeze-drying method might be able to verify this hypothesis.

Unfortunately the promising antioxidant activity is not shared by the tyrosinase inhibition activity which is shown in Table 2. It can be seen there that the crude methanol extract has large IC_{50} for tyrosinase inhibition, indicating the activity is weak. It is about 100 times weaker than the positive control hydroquinone. Since the crude extract has only shown weak activity, further separations will not produce better results.

Table 2: Tyrosinase inhibition

Extract	IC_{50} Value (ppm)
Crude methanol extract	316.56 ± 11.04
Hydroquinone	3.65 ± 0.15

Although crude methanol extract is much weaker than hydroquinone, it is still comparably more potent in tyrosinase inhibition activity compared to some other plant extracts. In a research done for several Indian herbs (Vaibhav & Lakshaman, 2012), it was found that the methanol extracts of *Aloe vera*, *Azadiracta indica*, *Lawsonia inermis*, *Mangifera indica*, *Nyctanthus arbortistis*, *Ocimum santum*, and *Trigonella foenum graceum* all had tyrosinase inhibition activity less than 50% for extract concentration of 1000 ppm. For Pakoba leaves, the inhibition activity was 89.20% for 1000 ppm extract concentration. In another research conducted for several local plants in Amazonian forest, the most potent leaf extract was found out to be *Piranhea trifoliata* with IC_{50} of 318.37 ppm (Marcini et al., 2009). Pakoba leaf potency is not much lower than this.

Further research with different processing treatment might be beneficial since the active compound for tyrosinase inhibition might be easily degraded. This is important since the potent whitening agent hydroquinone has a lot of side effects (Singh et al., 2016).

The bioactivity of plant Pakoba extract has not been researched before as far as the knowledge of the authors. Other members of *Syzygium* genus which have been researched are Malay apple or *Syzygium malaccense* (Nunes et al., 2016), Jamun or *Syzygium cumini* (Mohamed et al., 2013) and clove or *Syzygium aromaticum* (Bakour et al., 2018). Nunes et al. (2016) presented the antioxidant activity of fruit extract of *Syzygium malaccense* in percentage of DPPH scavenging effect, expressed in unit of μmol TEAC (Trolox equivalent antioxidant activity)/ g dry weight. It was shown that the methanol extract of the fruit peel extract has higher antioxidant activity than the edible portion extract, with μmol TEAC / g dry weight 47.22 ± 0.26 and 25.92 ± 0.28 respectively. For *Syzygium aromaticum*, the essential oil is found to be a potent antioxidant with total antioxidant activity (TAC) of 3235.50 ± 237.40 mg equivalent of ascorbic acid (or vitamin C) / 100 mg essential oil (Bakour et al., 2018). It is interesting that the methanol extract of *Syzygium cumini* leaves have a higher antioxidant activity than its methyl chloride extract and essential oil. The DPPH scavenging effect of methanol extract of *Syzygium cumini* leaves is 70.45% for extract concentration of 50 μg/ml, compared to the DPPH scavenging effect of the methylene chloride extract and essential oil, which are only 64.55% and 55.87% respectively (Mohamed et al., 2013). Although the antioxidant activity cannot be directly compared to Pakoba extract due to different unit of result presentation, it can be seen that all samples of *Syzygium* genus show good antioxidant activities, and methanol extract seems to contain the most potent antioxidant agent. This comparison strengthens the argument that methanol extract of Pakoba has indeed the potential to become the source of good antioxidant.

4. Conclusion

Pakoba leaf methanol extract has been found to have very strong antioxidant activity. The active compound for this activity is deduced to be polar, although further research is needed to isolate and elucidate the compound.

As whitening agent, it is found out that Pakoba leaf might not be economically potential. Although Pakoba leaf does show tyrosinase inhibitory activity which is more potent than some other plant extracts, the potency is still much weaker than the synthetic whitening agent hydroquinone.

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