EFFECT OF EXTRACTION METHOD ON ANTIOXIDANT ACTIVITY OF PALM PALM LEAVES (*Elaeis guineensis* Jacq.)

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Submitted: September 25, 2023 Revised: December 29, 2023 Accepted: January 17, 2024

ABSTRACT

Oil palm (*Elaeis guineensis* Jacq.) is a plant that is native to Africa and South America. This plant can grow well in tropical areas, such as Indonesia. Indonesia is currently one of the largest producers of palm oil worldwide. As the area of palm oil plantations increases, the potential for waste from this plant continues to increase. The potential for palm oil leaf waste production in Indonesia is 658 kg dry matter/hectare/year. Palm oil leaf waste has been widely processed into organic fertilizers and animal feeds. This plant has the potential to be developed into a source of traditional medicinal ingredients. Based on the abundant sources of palm oil leaves and their pharmacological potential, palm oil leaves can be used as a source of raw materials for traditional medicine. This study aimed to compare the antioxidant activities of oil palm leaf extracts obtained by maceration and reflux to determine which extraction method produces the best antioxidant activity. Extraction was carried out using 70% ethanol solvent by maceration and reflux. The results of the phytochemical screening showed that oil palm leaves contain flavonoids, polyphenols, saponins, quinones, steroid-triterpenoids, and monoterpene-sesquiterpenes. Antioxidant activity using a UV-Visible spectrophotometer with DPPH free radicals showed that the extract obtained by maceration and reflux methods had IC_{50} values of 46.61 ± 1.76 µg/mL and 55.21 ± 2.91 µg/mL respectively. Therefore, extraction by maceration produces a better antioxidant activity.

Keywords: Palm leaves; Traditional medicine; Extraction; Active compound stability; Antioxidant

INTRODUCTION

The Palm Oil (*Elaeis guineensis* Jacq.) is a plant belonging to the *Arecaceae* family. This plant originates from Africa and South America and can grow well in tropical climates such as Indonesia. This plant is classified into three species: *Elaeis guineensis, Elaeis oleifera,* and *Elaeis odora*. *Elaeis guineensis* comes from the African continent, whereas *Elaeis oleifera* and *Elaeis odora* come from the South American continent. The *Elaeis oleifera* species grows slower in plant height, and the productivity of fresh fruit bunches is better.

Meanwhile, *Elaeis guineensis* species can produce higher amounts of oil. Most palm oil plantations in Indonesia use *Elaeis guineensis* species. Several varieties often found in Indonesia are divided based on the thickness of the shell and flesh, such as Dura, Pisifera, Tenera, and Macro Carya. The production of palm oil leaf waste has economic value, and one way to increase its value is to process it from something less valuable to something more valuable (Fauzi *et al*., 2012; Dirgantoro & Adawiyah, 2019; Harapan *et al*., 2019).

Oil palm plants (*Elaeis guineensis* Jacq.) have various contents. Palm oil contains beta-carotene, tocopherol, tocotrienol, and palmitic acid (Lubis and Widanarko, 2011). Palm oil leaves contain alkaloids, flavonoids, saponins, tannins, steroids, terpenoids, and

glycosides (Zumaro *et al*., 2021). Other studies have reported that palm leaves of the same species contain alkaloids, flavonoids, saponins, tannins, and steroid triterpenoids (Saputri, 2014). Palm oil leaves (*Elaeis oleifera* Kunth.) contain 6 flavonoid compounds, 3 tannin compounds, 7 steroid-triterpenoid compounds, and 2 glycoside compounds (Manurung, 2018). Most flavonoid compounds in palm leaves include catechin, apigenin, luteolin, and their derivatives (Tow *et al*., 2021). Agipenin, luteolin, and catechin derivatives, such as vitexin, isovitexin, orientin, isoorientin, (+)-catechin, and (−)-epicatechin, are the main bioactive metabolites in palm leaves (Fauziah & Izzah, 2019; Che Zain *et al*., 2020).

The leaves of the palm oil species *Elaeis guineensis* Jacq. Has antioxidant activity with an IC_{50} value of 133.58 ppm (Zumaro *et al*., 2021). In this study, the antioxidant activity was determined using the DPPH reduction method in a 96% ethanol extract of oil palm leaves, which was extracted using the maceration method. The methanol extract of palm leaves extracted using the ultrasonic method has antioxidant activity with an IC_{50} value of 17.73 ppm, and the IC_{50} values of the n-hexane, ethyl acetate, and water fractions are 53.12 ppm, 29.05 ppm, and 41.22 ppm respectively (Hui *et al*., 2017).

Leaves of the palm oil species *Elaeis oleifera* Kunth. It has antioxidant activity with an IC_{50} value of 6.75 ppm, and the IC_{50} values of the n-hexane, ethyl acetate, and water fractions are 26.02 ppm, 5.50 ppm, and 9.79 ppm respectively (Manurung, 2018). The antioxidant activity in this study was obtained using the DPPH reduction method in 80% ethanol extract, which was extracted using the maceration method.

RESEARCH METHODS

Equipment and Materials

The tools used in this study consisted of glass bottles, measuring cups, Erlenmeyer, spatula, aluminum foil, plastic wrap, ruler, measuring flask, test tube, test tube container, funnel, separating funnel, stirring rod, parchment paper, weighing bottles, porcelain cup, vials, slides, slide covers, filter paper, ash-free filter paper, pipettes, micropipettes, micropipette tips, scissors, volumetric flasks, drying cabinets, furnaces, hot plates, alcohol meters, cuvettes, blenders, stopwatch, rotary evaporator, water bath, analytical balance (Boeco, Germany), microscope (Olympus), oven (Strok), and UV-visible spectrophotometer (Shimadzu UV-1800).

Research Procedure

1. Raw Material Preparation

   Palm leaves were wet-sorted using running water and then drained. Subsequently, it was dried in a drying cabinet until dry. The dried leaves were powdered.
2. Characterization of Raw Material
   - Macroscopic Examination

     Observations were made on The smell, color, taste, and size of the oil palm leaf dry material powder were observed (Ministry of Health of the Republic of Indonesia, 2017).
   - Microscopic Examination

     The dry material was sprinkled on an object glass and dripped with chloral hydrate solution. The object glass was covered with a cover glass and observed under a microscope (Ministry of Health of the Republic of Indonesia, 2017).
3. Determination of Ash Content
   - Total Ash Content

     Dry material (2 gram) was placed in a crucible that had been ignited and torn. The crucible was heated on a hot plate until charcoal was obtained as the dry material. The crucible was then placed in a furnace at 500ºC every hour until a constant weight was obtained (Ministry of Health of the Republic of Indonesia, 2017).
   - Water Soluble Ash Content

     The ash obtained by determining the total ash content was boiled in 25 mL water for 5 minutes. The insoluble fraction was filtered using ash-free filter paper and

washed with hot water. The residue was heated in a crucible at 500 °C until constant weight was obtained (Ministry of Health of the Republic of Indonesia, 2017).

- Acid Insoluble Ash Content

  The ash obtained by determining the total ash content was boiled with 25 mL HCl for 5 minutes. The insoluble fraction was filtered using ash-free filter paper, washed with hot water, and ignited in a crucible 500ºC until a constant weight was obtained (Ministry of Health of the Republic of Indonesia, 2017).

4. Determination of Essence Content
   - Water Soluble Essence Content

     A total of 5 grams of dry material powder was placed into an Erlenmeyer flask, 100 mL of chloroform-saturated water was added, shaken for 6 hours, left for 18 hours and filtered. A total of 20 mL of the filtrate was evaporated in a cup that had been heated to 105 °C and stored. The remainder was heated to 105 °C until the weight remained constant (Ministry of Health of the Republic of Indonesia, 2017).
   - Ethanol Soluble Essence Level

     A total of 5 grams of dry material powder was put into an Erlenmeyer flask, 100 mL of ethanol, shaken for 6 hours, left for 18 hours, filtered and evaporated 20 mL of the filtrate until dry in a cup that had been heated to 105℃ and set aside. The remainder was heated at 105 °C until the weight remained constant (Ministry of Health of the Republic of Indonesia, 2017).
5. Determination of Water Content

   A total of 5 grams of dry material powder was added to 200 mL of toluene, which was saturated with water for a day before use, and then heated until separation occurred between the toluene and the water contained in the dry material, which was marked by a clear separation (Ministry of Health of the Republic of Indonesia, 2017).
6. Determination of Drying Losses

   Dry material powder (2 gram) was placed in a weighing bottle heated at 105 °C. It was then placed in an oven, the lid of the bottle was opened, heated at 105 °C every hour, cooled in a desiccator, weighed, and heated until a constant weight was obtained (Ministry of Health of the Republic of Indonesia, 2017).
7. Extracts Production
   - Maceration

     Oil palm leaf dry material powder (300 gram) was soaked in 2000 mL of 70% ethanol as a solvent, left for 3 days protected from sunlight, and stirred occasionally. After 24 hours, the filtrate was collected and filtered and the residue was macerated again. The extract was concentrated using a rotary evaporator and then thickened in a water bath.
   - Refluks

     A total of 150 grams of oil palm leaf dry material powder was placed in a round-bottom flask, and 1500 mL of 70% ethanol solvent was added and then heated to the boiling point of the solvent for 1 hour. This process was repeated 3 times and then filtered. The extract was concentrated using a rotary evaporator and then thickened in a water bath.
8. Determination of Extract Specific Gravity

   The weight of the empty pycnometer was then measured. Put the liquid extract into a pycnometer that has been set, then weigh it.
9. Quantitative Antioxidant Activity Testing
   - Preparation of DPPH Solution

     DPPH was dissolved in methanol to obtain a solution with a concentration of 50 ppm (Molyneux, 2004). All experiments were performed under dark conditions and protected from light (Pontoh *et al*., 2019).

- Determination of Maximum DPPH Wavelength

  The DPPH solution was added to methanol at a ratio of 1:1. Absorbance was measured at 400–800 nm, and the maximum DPPH wavelength was determined (Brand-Williams *et al*., 1995).
- Preparation of Quercetin Stock Solution

  Quercetin was dissolved in methanol to obtain a 100 ppm stock solution.
- Preparation of Quercetin Test Solution

  Quercetin test solutions with concentrations of 1, 2, 2.5, 3, 4, and 5 ppm were pipetted into a vial and added with a 1:1 DPPH solution. The sample was left in the dark for 30 minutes and the absorbance was determined at the maximum DPPH wavelength.
- Preparation of Sample Stock Solution

  Several palm oil leaf extract samples were dissolved in methanol to obtain a stock solution with a concentration of 1000 ppm.
- Preparation of Extract Test Solution (Maceration)

  Test solutions with concentrations of 15, 20, 30, 40, 50, and 60 ppm were pipetted into vials, and DPPH solution was added at a 1:1 ratio. The sample was left in the dark for 30 min and the absorbance was determined at the maximum DPPH wavelength.
- Preparation of Extract Test Solution (Reflux)

  Test solutions with concentrations of 20, 30, 40, 50, 60, and 70 ppm were pipetted into vials, and DPPH solution was added at a 1:1 ratio. The sample was left in the dark for 30 minutes and the absorbance was determined at the maximum DPPH wavelength.
- Measurement of Damping Percentage

  The Absorbance of the blank and samples was calculated using formula (1) (Fitriana *et al*., 2015).

$$\text{Attenuation activity (\%)} = \frac{Ab-As}{Ab}\ x\ 100\ \% \quad \text{.....(1)}$$

  Note:
  Ab = Blank absorbance
  As = Sample absorbance
- Determination of IC_{50} Value

  The IC_{50} value is a number that shows the concentration (µg/ml)/effective volume (mL) of the test sample and quercetin, which provides 50% DPPH reduction; therefore, the value 50 is substituted for the y value. After substituting the value of 50 for the y value, we obtained the x value as the IC_{50} value (Katrin & Bendra, 2015).

$$y = ax + b \quad \text{.....(2)}$$

  Note:
  y = 50 (% DPPH damping)
  x = Concentration (µg/ml)/IC_{50} value
  a = Regression constant
  b = Regression coefficient

RESULTS AND DISCUSSION

Macroscopic examination was carried out on dry oil palm leaf material (*Elaeis guineensis* Jacq.) including shape, color, smell, and taste. The results of the macroscopic examination can be seen in Table I. The results of the microscopic examination of oil palm leaves are shown in Figure 1.

Table I. Results of Macroscopic Examination of Oil Palm Leaf Dry Material

Macroscopic characteristics	Details
Form	Fibrous powder
Color	Green
Smell	Specific
Taste	Astringent and Bitter

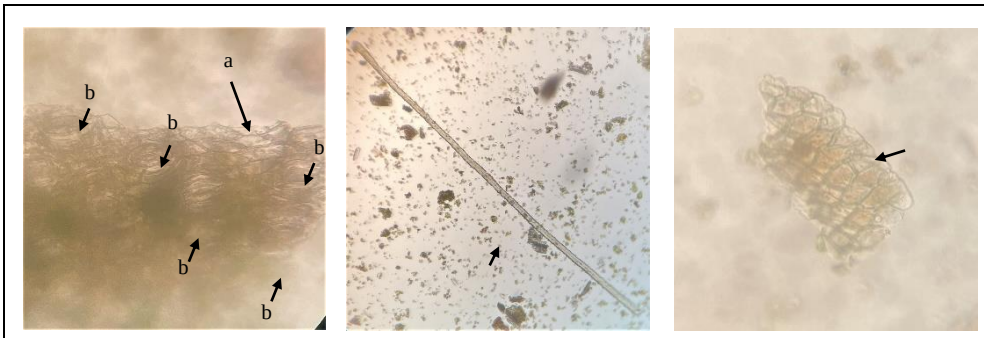

Figure 1. Results of Microscopic Examination of Oil Palm Leaves

Note:
a. Epidermis
b. Stomata
c. Sclerenchyma
d. Parenchyma with oil cell idioblasts

Oil palm leaf dry material was standardized to determine the quality of the materials used in the study. The dry material standardization includes the total ash content, water-soluble ash content, acid-insoluble ash content, water-soluble essence content, ethanol-soluble essence content, water content, and drying loss. The results are presented in Table II.

Table II. Results of Standardization of Oil Palm Leaf Dry Material

Parameter	Results
Total ash content (%w/w)	12,56 ± 0,084
Water soluble ash content (%w/w)	2,03 ± 0,272
Acid insoluble ash content (%w/w)	8,88 ± 0,290
Water soluble essence content (%w/w)	14,97 ± 0,85
Ethanol soluble essence content (%w/w)	10,26 ± 0,58
Water content (%v/w)	6,8 ± 0,20
Drying loss (%w/w)	4,72 ± 0,118

Determination of total ash content was carried out to provide an overview of physiological contamination originating from the plant itself in the form of inorganic elements such as magnesium, sodium, and calcium, as well as non-physiological pollution from outside the plant, such as air, soil, and water pollution. The higher the ash content, the lower the quality (Meriatna *et al*., 2017). The research showed that oil palm leaf dry material had a total ash content of 12.56 ± 0.084% w/w. The water-soluble ash content was determined to provide an overview of the presence of mineral compounds originating from the dry material, such as alkali metals and alkaline earth, which are water-soluble salts. The research showed that oil palm leaf dry material had a water-soluble ash content of 2.03 ± 0.272% w/w. The acid-insoluble ash content was determined to provide an overview of the mineral compounds originating outside the dry material, such as silver, mercury, lead, or

other impurities. The research showed that oil palm leaf dry material had an acid-insoluble ash content of 8.88 ± 0.290% w/w.

Determination of water-soluble essence levels aims to provide an overview of the number of secondary metabolite compounds in dry materials that are polar, so they can be attracted to water solvents, and secondary metabolite compounds that are semipolar-nonpolar, so they can be attracted to ethanol solvents. The research showed that oil palm leaf dry material had a water-soluble essence content of 14.97 ± 0.85% w/w. Meanwhile, the ethanol-soluble essence content was 10.26 ± 0.58%w/w. The results of this research show that the water-soluble essence content is greater than the ethanol-soluble essence content; therefore, it is suspected that the secondary metabolite compounds contained in oil palm leaf dry material have high polarity and are absorbed more in water.

Water content was determined to determine the amount of water contained in the dry material. The high water content in the dry material is a good growth medium for bacteria and fungi, causing damage to the compounds contained in the dry material. The water content was determined using toluene solvent, which was saturated with water. Saturation was carried out to avoid water adsorption by toluene, which yielded results smaller than the actual water content. The research showed that oil palm leaf dry material had a water content of 6.8 ± 0.20% v/w. This shows that the oil palm leaf dry material meets the water content requirements, that is, less than 10% v/w.

Drying shrinkage testing was performed to determine the amount of compounds lost during drying. The lower the drying shrinkage, the better (Safrina *et al*., 2019). The research showed that oil palm leaf dry material had a 4.72 ± 0.118% w/w drying loss value.

The specific gravity of the extract was determined using a pycnometer to determine mass per unit volume. Determination of the specific gravity is also related to the purity of the extract. Based on this test, oil palm leaf extract extracted using various extraction methods showed that the specific gravity of the macerated extract was 0.877 ± 0.0005 g/mL, and the specific gravity of the refluxed extract was 0.880 ± 0.0005 g/mL.

Phytochemical screening was performed to provide an overview of the classes of secondary metabolite compounds in the samples: dry material and oil palm leaf extract. The secondary metabolite compounds tested included alkaloids, flavonoids, polyphenols, tannins, saponins, quinones, steroid-triterpenoids, and monoterpene sesquiterpenes. The screening results of this study are presented in Table III.

The extraction process was performed using a filter solution. A 70% ethanol filter was used for this test. Ethanol 70% was chosen because this solvent is the only organic solvent with a low level of toxicity so it is safe and can be required for the development of raw materials for herbal medicines (Ginting *et al*., 2020; Hasanah & Novian, 2020; Aini *et al*., 2021; Aprianti *et al*., 2021). The extraction methods used in this study were maceration and reflux methods. The yield of the extract obtained using the maceration method was 14.93%. The yield of the extract obtained using the reflux method was 27.26%. The extract yield was calculated as the weight of the extract divided by the weight of the dry material powder used. The yield of the extract obtained using the reflux method was higher than that obtained using the maceration method. This occurs because, in maceration, osmosis of the solvent into the material occurs statically. After all, there was no assistance from other forces.

Table III. Results of Phytochemical Screening of Dry Material and Oil Palm Leaf Extract

Groups	Reactor	Screening Results		
		Dry material	Extract (Maceration)	Extract (Reflux)
Alkaloids	Mayer	-	-	-
	Dragendorff	-	-	-
Flavonoids	Mg powder, HCl 2N, Amyl alcohol	+	+	+
Polyphenols	$FeCl_3$	+	+	+
Tannin	Gelatin 1%	-	-	-
	Steasny	-	-	-
Saponins	Dilute HCl	+	+	+
Quinones	KOH 10%	+	+	+
Steroids-triterpenoids	Liebermann-Buchard	+	+	+
Monoterpenes-sesquiterpenes	Vanilin-SO_4	+	+	+

Note:
(+) = The presence of secondary metabolite compounds was detected
(-) = No secondary metabolite compounds were detected

Meanwhile, in reflux, adding heat helps break down plant cell walls and increases the solubility of the active compounds in the material, thereby improving the extraction process. In addition, during reflux, the solvent used for the extraction process remains fresh to avoid solvent saturation, which can increase the ability of the solvent to attract compounds (Susanti *et al*., 2014).

Quantitative antioxidant activity testing was performed using a UV-visible light spectrophotometer using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging method. This method was chosen because it is simple, does not take long, and is sensitive enough; therefore, it only requires a few samples. The measurement results can be observed from the change in the color of the test solution from purple to yellow. This color change occurs because of an electron capture reaction between the antioxidant compound, reducing agent, and DPPH radical compound added to the sample. This color change is directly proportional to the amount of DPPH compound, which is reduced to a more stable or neutral compound, so that it does not provide absorbance at the maximum DPPH wavelength of 515–520 nm. The higher the antioxidant activity of a sample, the greater the neutralization of DPPH radicals (Lung & Destiani, 2017). This method is easily affected by several factors, including light. Therefore, the DPPH solution must always be fresh and protected from light (Aryanti *et al*., 2021). The IC_{50} values of the oil palm leaf extracts are shown in Table IV.

Table IV. IC_{50} Value of Quercetin, Extract from Maceration and Reflux

No	Sample	IC_{50} (µg/mL)
1	Quercetin	3,27±0,03
2	Extract (Maceration)	46,61±1,76
3	Extract (Reflux)	55,21±2,91

The quercetin antioxidant test showed an IC_{50} value of 3.27 ± 0.03 µg/mL. Quercetin was chosen as a comparison based on test samples containing secondary metabolites such as catechin, apigenin, and luteolin, which are included in the flavonoid group. Therefore, plant isolates belonging to the flavonoid group were selected for comparison to determine the potential antioxidant activity of the sample. Quercetin is generally used as a comparison solution with other compounds that have very strong antioxidant activity because quercetin

also shows the ability to prevent the oxidation process of low-density lipoprotein (LDL) by capturing free radicals and binding transition metals (Maulana *et al*., 2019). The antioxidant activity of quercetin is five times greater than vitamin C. Suppose that the IC_{50} value of the sample is close to the IC_{50} value of quercetin. In this case, it can be said that the antioxidant activity of the sample is comparable to that of quercetin; therefore, it can be used as a strong antioxidant substitute.

The results of antioxidant testing for oil palm leaf extract samples showed that the IC_{50} value of the macerated extract was 46.61 ± 1.76 µg/mL, and the IC_{50} value of the refluxed extract was 55.21 ± 2.91 µg/mL. Based on these results, both the extracts exhibited potential antioxidant activity. It can be said that the macerated extract has very strong antioxidant power because it has an IC_{50} value < 50 µg/mL. Meanwhile, the reflux extract had strong antioxidant power because it had an IC_{50} value of 50–100 µg/mL. The oil palm leaf extract obtained via the maceration method showed better antioxidant activity than the extract obtained via the reflux method. This is because the reflux method involves heating during the process, which can damage secondary metabolite compounds that are not resistant to heat, such as flavonoids. Flavonoids have a conjugated aromatic system, which is easily damaged at high temperatures. In addition, several groups of flavonoids form glycosidic bonds with sugars, which are easily broken at high temperatures (Sa' adah *et al*., 2017).

The IC_{50} value obtained was analyzed by statistical testing using SPSS version 26 software, the Independent Samples T Test method, with a confidence level of 95% (α = 0.05). The results showed that the extraction method affected antioxidant activity. This is indicated by two-sided significance value (2-sided) of 0.012 and 0.018, which indicate a significant difference between the antioxidant activity of the extract obtained using the maceration extraction method and the extract obtained using the reflux method.

In a previous study, a 96% ethanol extract from oil palm leaves obtained by the maceration method provided antioxidant activity with an IC_{50} value of 133.58 µg/mL (Zumaro *et al*., 2021). In another study, the ethanol extract obtained using the reflux method exhibited antioxidant activity with an IC_{50} value of 461 µg/mL (Ahmad *et al*., 2018). These results are similar to those of the current study, in which the antioxidant activity of the palm leaf extract obtained using the maceration method was better than that obtained using the reflux method. Based on the results of phytochemical screening, both extracts were proven to contain secondarDry, such as flavonoids, polyphenols, saponins, quinones, steroids-triterpenoids, and foundenes-sesquiterpenes. These compounds are t,hought to play a role in their antioxidant potential bsteroid-triterpenoidson of radical compounds by donating H and converting them into nonreactive compounds (Kurniati, 2013).

CONCLUSION

It was proven that dry material, 70% ethanol extract of macerated palm leaves, and refluxed extract were detected to contain secondary metabolite compounds such as flavonoids, polyphenols, saponins, quinones, steroids-triterpenoids, and monoterpenes-sesquiterpenes. Testing antioxidant activity using the DPPH free radical reduction method on oil palm leaf extract obtained using the maceration method is better than the reflux method because it has the highest IC_{50} value. The IC_{50} values of the macerated and refluxed extracts were 46.61 ± 1.76 µg/mL and 55.21 ± 2.91 µg/mL, respectively. So, the extracts obtained by the maceration and reflux methods showed significant differences.

ACKNOWLEDGMENT

Universitas Jenderal Achmad Yani internal research grant with grant number Skep/194/Unjani/VI/2023.

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