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Antioxidant Activity of Porang (*Amorphophallus muelleri*) Leaves Ethanol Extract and Its Application as Cream Preparation

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Abstract

Antioxidants are compounds that can overcome the effects of free radicals. One of the plants that has antioxidant activity is the Porang plant (Amorphophallus muelleri). This study aims to determine the antioxidant activity of the Porang leaves' ethanol extract and fraction using the DPPH method. Porang leaves were extracted using maceration with 96% ethanol and separated by fractionation using n-hexane and ethyl acetate. The ethanol extract and the three fractions were tested for their activity as antioxidants using the DPPH method with ascorbic acid as positive control. Phytochemical screening and Total Flavonoid Content (TFC) assay were also conducted. Phytochemical screening showed the presence of flavonoids, tannins/polyphenols, and terpenoids. Quantitative test for Total Flavonoid Content (TFC) showed that the greatest flavonoid content was found in the ethyl acetate fraction, which was 101.93 ± 2.75 mgQE/g. The results showed that the strongest antioxidant activity was possessed by ethanol extract (IC_{50} 66.39 ± 0.44 µg/mL), followed by the ethyl acetate fraction (IC_{50} 72.19 ± 4.37 µg/mL), ethyl acetate insoluble fraction (IC_{50} 135.48 ± 2.54 µg/mL), and the weakest was n-hexane fraction (IC_{50} 198.31 ± 16.75 µg/mL). Hence, cream was prepared using ethanol extract which possesses the strongest antioxidant activity. Formula 4 (7.5% extract) showed the strongest antioxidant activity (IC_{50} 1637.12 µg/mL), followed by formula 3 (5% extract) with IC_{50} of 2590.377 µg/mL, formula 2 (2.5% extract) with IC_{50} of 3898.694 µg/mL, and formula 1 (cream base) with IC_{50} of 110727.4 µg/mL. All cream formulations which contained porang leaves extract (F2 – F4) had higher antioxidant activity than formula 1 (base), although their antioxidant activity was classified as very weak.

Keywords: Antioxidant, DPPH, Porang Leaves, Cream.

1 Introduction

Free radicals are unstable chemical compounds that contain unpaired electrons or electrons that are reactive [1]. Free radicals can penetrate the body and harm essential macromolecules in the cells, such as carbohydrates, proteins, fats, and DNA by taking their electrons. This process can lead to protein and DNA damage, lipid peroxidation, and ultimately cell death, contributing to the development of various conditions, including cancer, cataracts, premature aging, and other degenerative diseases [2]. On the skin, free radicals can cause premature aging, which is an aging process that occurs faster than it should, causing wrinkles or fine lines to appear on the skin or other parts of the body. To overcome this, it is necessary to apply antioxidant preparation to reduce or counteract the effects of free radicals on the skin [3]. Antioxidants are molecules that can inhibit the initiation or propagation process, that is when free radical molecules react with other neutral molecules to produce new free radical molecules [4].

One of the plants that has been proven to have antioxidant activity is the porang plant (*Amorphophallus muelleri*). Based on previous research by Anisah & Muhtadi (2021), the ethanol

extract of the leaves and stems of the Porang plant was shown to have antioxidant activity as measured using the DPPH method with IC_{50} values for leaves and stem extracts of 97.054 µg/mL and 260.202 µg/mL, respectively. In that study, it was explained that the leaves and stems of Porang contain alkaloids, polyphenols and steroids [5].

To date, studies on porang (Amorphophallus muelleri) leaf extracts have mainly focused on antioxidant activity, with limited exploration in topical applications. This study presents a novel approach by integrating fractionation and phytochemical profiling to elucidate metabolite distribution—rather than selecting the most active fraction—with the formulation of the crude extract into a cream and the evaluation of its concentration-dependent antioxidant activity after formulation, providing insight into its functional performance as an antioxidant cosmetic product.

2 Method

2.1 Sample Preparation

Porang leaves samples were obtained from Tunjungan District, Blora Regency, Central Java. The leaves are taken from old leaves, but before they turn yellow, the leaves are picked manually. Porang Leaves were determined with the aim of knowing the identity and correctness of the plants to be studied and tested. This determination process was carried out at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang. The leaves were sorted, then washed thoroughly with running water. The sample was chopped to speed up drying. The drying process was carried out by drying in direct sunlight with the sample covered with a black cloth. Then dry sorting was carried out on samples that had been dried and ground into powder, then weighed as the dry weight of simplicia [6].

2.2 Sample Extraction

Maceration method was used to extract porang leaves. The simplicia powder was extracted with 96% ethanol in a maceration jar for a total 3x24 hours at room temperature with a ratio of 1:10, with frequent stirring. Every 1x24 hours the maceration process is repeated by adding a new solvent (remaceration). The filtrate was filtered and then evaporated using a rotary evaporator to eliminate solvent. The yield of thick extract was calculated and kept in refrigerator (4ºC).

2.3 Extract Fractionation

The fractionation of the extract was performed using a solid–liquid partitioning method. Briefly, 1 g of solvent-free ethanolic extract (EE) was sequentially partitioned with 10 mL n-hexane (3×) and ethyl acetate (3×), each followed by vortexing for 5 minutes and centrifugation at 3000 rpm for 5 minutes. The soluble phases were collected and combined, while the remaining residue was designated as the ethyl acetate-insoluble fraction. All fractions, namely the n-hexane fraction (NH), ethyl acetate fraction (EA), and ethyl acetate-insoluble fraction (IS), were dried in a fume hood at room temperature. The yield of each fraction was calculated and expressed as percentage (%) relative to the initial weight of the simplicia powder [7].

2.4 Qualitative and Quantitative Analysis of Secondary Metabolites

2.4.1 Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) method was employed as qualitative analysis to determine the content of secondary metabolites in the extract and fraction of Porang leaves, as well as to identify compounds that have antioxidant activity. TLC was conducted using silica gel GF 254 as stationary phase and chloroform: ethyl acetate (9:1) as mobile phase. After elution, the TLC plate was then dried and observed under visible light, UV 254 nm, and UV 366 nm. Rf of each spots was calculated. Next, the plate were sprayed with 0.08 mM DPPH solution to identify the active compounds that have the potential to scavenge free radicals indicated by yellowish spots.

Furthermore, phytochemical screening was also conducted by spraying the plate using various spray reagents to identify the class of compounds existed in extract and fractions. The spray reagents used were Dragendorff solution to identify alkaloids, 5% $AlCl_3$ solution to identify flavonoids, 5% $FeCl_3$ solution to identify tannins/poliphenol, and Liebermann-Burchard solution to identify terpenoids [8].

2.4.2 Total Flavonoid Contents (TFC)

The determination of TFC was conducted to compare the flavonoid content in the extract and fractions of porang leaves.

a. Preparation of Quercetin Standard Curves

1 mg of quercetin standard was dissolved in 10 mL of methanol p.a to obtain a 100 ppm quercetin standard standard solution concentration. Several series of concentrations were made by dilution method, namely 10 ppm; 12.5 ppm; 15 ppm; 17.5 ppm; and 20 ppm. 100 μL of each solution was pipetted and added with 100 μL of 2% $AlCl_3$ solution in a microplate. Then the solution was incubated for one hour at room temperature. Next, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 435 nm.

b. Determination of Total Flavonoid Content (TFC) of Porang Leaves Extract and Fraction

Ethanolic extract and all fractions were weighed (1 mg), and then dissolved in 10 mL of methanol to obtain a solution concentration of 100 ppm. 100 μL of each sample solution was pipetted and then 100 μL of 2% $AlCl_3$ solution was added to the microplate. The sample solution was incubated for one hour at room temperature. Then the absorbance was measured with a UV-Vis spectrophotometer at a wavelength of 435 nm. Each sample was replicated three times and then the average absorbance was calculated [9].

The TFC content of porang leaves extract samples and fractions was calculated by distributing the absorbance value of each sample obtained previously into the linear regression equation of the calibration curve between the quercetin concentration and the absorbance value. Then the concentration values of all samples are redistributed into the TFC calculation formula (Eq. 1) [10]:

$$TFC = \frac{V\ (mL)\ x\ C\ \left(\frac{mg}{mL}\right)\ x\ Fp}{sample\ weight\ (g)} \quad \text{(Equation 1)}$$

Note:
TFC = *Total Flavonoid Content* (mgQE/g)
V = Volume of sample solution (mL)
C = Concentration of the compound in the sample solution (mg/mL)
Fp = Dilution Factor

2.5 Antioxidant Activity Test of Fractions and Creams by UV-Vis Spectrophotometry

All fractions and extract of porang leaves extract was subjected to antioxidant activity assay to determine the sample which possessed the strongest antioxidant. The sample with the strongest antioxidant activity then formulated into cream preparation. Next, the cream formulations were subjected again for antioxidant activity measurement. The procedure of the assay explained in this section.

2.5.1 Preparation of 0.254 mM DPPH Solution

10 mg of DPPH powder was dissolved with methanol p.a in a 100 mL volumetric flask until dissolved, then added methanol p.a up to the mark.

2.5.2 DPPH Maximum Wavelength Measurement

A total of 1 mL of 0.254 mM DPPH solution was taken and put in a test tube added with 3 mL of methanol p.a and vortexed until homogeneous (blank solution). The solution was incubated for 30 minutes in a dark room to prevent the formation of other radicals besides free radicals from DPPH. Next, 3 mL of the solution was poured into a cuvette and the wavelength between 400-600 nm was measured using a UV-Vis spectrophotometer. The wavelength is the maximum wavelength range of the DPPH according to the theory by observing the absorbance or maximum absorption produced by the UV-Vis spectrophotometer.

2.5.3 Preparation of Porang Leaves Extract and Fraction Test Solution

The stock solution (mother liquor) was prepared at a concentration of 1000 µg/mL by dissolving 10 mg of extract, ethyl acetate fraction, n-hexane fraction, and ethyl acetate-insoluble fraction in methanol p.a in a 10 mL volumetric flask and diluting to volume. Serial dilutions were then prepared to obtain concentrations of 0 (blank), 25, 50, 75, 100, 125, 150, 175, 200, and 225 µg/mL. For each concentration, 1 mL of 0.254 mM DPPH solution was added and diluted to 10 mL with methanol p.a, resulting in a final DPPH concentration of 0.0254 mM. The mixtures were incubated in the dark for 30 minutes prior to analysis.

2.5.4 Preparation of Ascorbic Acid Solution

Ascorbic acid (vitamin C) was used as a positive control. A stock solution (100 µg/mL) was prepared by dissolving 2.5 mg of ascorbic acid in methanol p.a and diluting to 25 mL in a volumetric flask. Serial dilutions were then prepared to obtain concentrations of 5, 7.5, 15, 20, and 25 µg/mL. For each concentration, 1 mL of 0.254 mM DPPH solution was added and the volume was adjusted to 10 mL with methanol p.a. The mixtures were homogenized and incubated in the dark for 30 minutes prior to analysis. [11].

2.5.5 Preparation of Cream Preparation Test Solution

Cream sample test solutions were prepared at concentrations of 10,000 and 100,000 µg/mL by dissolving 100 mg and 1000 mg of cream, respectively, in ethanol p.a and diluting to 10 mL with methanol p.a in a volumetric flask. Serial dilutions were then prepared to obtain concentrations of 0 (blank), 750, 1000, 1250, 1500, 1750, 2500, 5000, 7500, 10000, 12500, 15000, 17500, 20000, and 22500 µg/mL. For each concentration, 1 mL of 0.254 mM DPPH solution was added, and the volume was adjusted to 10 mL with methanol p.a. The solutions were homogenized and incubated in the dark for 30 minutes prior to analysis.

2.5.6 Absorption Measurement with a UV-Vis Spectrophotometer

Each serial solution of extract, fractions, cream, and ascorbic acid (positive control) was reacted with 0.254 mM DPPH solution and diluted with methanol p.a, followed by incubation in the dark for 30 minutes. After incubation, 100 µL of each reaction mixture was transferred into a 96-well microplate. The absorbance was measured at the predetermined wavelength using a UV–Vis microplate reader. All measurements were performed in triplicate, and absorbance values were corrected using the corresponding sample blank [11].

2.5.7 Determination of Percent of DPPH Radical Inhibition and IC_{50} Value

Percent of inhibition describes the ability of sample to inhibit DPPH free radicals. It was calculated using Eq. 2.

$$\%\ DPPH\ radical\ inhibition = \frac{Abs.control\ -Abs.of\ sample}{Abs.of\ control}\ x\ 100\%$$

(Equation 2)

From the percentage of inhibition obtained at each concentration, the IC_{50} values of extracts, fractions, vitamin C, and cream preparations were determined using linear regression analysis (y = a + bx), where *y* represents 50% inhibition and *x* corresponds to the IC_{50} value. The regression analysis was performed only within the linear inhibition range, as indicated by the coefficient of determination (R^2), which were 0.9754 (EE), 0.9788 (NH), 0.9673 (EA), 0.9981 (IS), 0.9537 (vitamin C), and 0.9958–0.9985 (cream formulations FI–FIV). The IC_{50} values were expressed as mean ± standard deviation from triplicate measurements [12].

2.6 Preparation of Antioxidant Cream

The antioxidant cream was prepared using the sample which has the strongest antioxidant activity. Based on the antioxidant activity assay of extracts and fractions, it was found that the ethanol extract had the highest antioxidant activity (IC_{50} 66.39 ± 0.44 µg/mL). The cream formulation in this study refers to the preformulation that has been performed previously by Mailana *et al* (2016) [13] (**Table 1**).

Stearic acid, cetyl alcohol, and propyl paraben were melted in a porcelain cup over a water bath at 70°C as the oil phase. The mixture was stirred until dissolved and homogeneous. Next, the aquadest was heated to a temperature of 70°C on a waterbath as the aqueous phase and methyl paraben was added little by little until it dissolved. Then added glycerin, and TEA stirred until homogeneous. The previously prepared oil phase is added to the water phase in the hot mortar. The mixture is stirred to a temperature of 25°C until a creamy mass is formed. The ethanol extract as active ingredient was added into the cream mass and stirred until homogeneous. Each cream formula made was 10 grams and was placed in a pot container. Next, a physical evaluation of the cream preparation was carried out and the antoxidant activity assay was performed to compare antioxidant activity of all cream fomula [13].

Table 1. Formulation of Antioxidant Cream

Ingredients	Concentrations (%)				Function
	F1	F2	F3	F4	
Extract of porang leaves	0	2.5	5	7.5	Active substance
Stearic acid	11	11	11	11	Emulsifying agent
Cetyl alcohol	4	4	4	4	Stabilizing agent
TEA	2	2	2	2	Stabilizing agent
Glycerin	18	18	18	18	Humectant
Methyl paraben	0.15	0.15	0.15	0.15	Preservative
Prophyl paraben	0.02	0.02	0.02	0.02	Preservative
Aquadest	Ad 100	Ad 100	Ad 100	Ad 100	Solvent

2.7 Evaluation of Cream Preparations

The evaluation of the cream preparations included organoleptic properties, homogeneity, pH, adhesion, spreadability, and emulsion type, each performed in triplicate; however, the absence of stability testing represents a limitation of this study [14].

2.7.1 Organoleptic Test

This test was carried out by visually observing the cream preparations that have been produced, including shape, color, and smell.

2.7.2 Homogeneity Test

The homogeneity test was conducted by taking 0.5 gram of the cream preparation, then the cream was smeared thinly on a dry and clean glass object. The slide was closed using another slide. Next, it was observed whether the cream preparations made were homogeneous or not homogeneous. Cream was considered homogeneous if it forms an even texture and there are no lumps.

2.7.3 pH test

pH was tested using a digital pH tester. The pH was calibrated using buffer solution and then after washing with distilled water, the electrode was dipped into cream preparations. The value shown in the tester was the pH value.

2.7.4 Adhesion Test

The adhesion test was carried out by taking 100 mg of cream and placing it between two glass objects whose area had previously been measured. Then on top of the object glass is added a load weighing 1 kg and allowed to stand for 5 minutes. Next, the glass object is mounted on the test kit, by removing the 21 gram load and recording the time until the two objects are released.

2.7.5 Spreadability Test

As much as 0.5 gram of cream was placed in a petri dish. Then the petri dish is closed and a load weighing multiples of 50 to 250 grams is added on top of it, then allowed to stand for 1 minute and the results of the diameter of the distribution are measured.

2.7.6 Emulsion Type Test

This emulsion type test was carried out using methylene blue solution and water. If the cream was the O/W type, the aqueous phase will be stained by the methylene blue solution because methylene blue dissolves in water. The second method was by dissolving the cream in water, if the cream dissolves in water then the cream is of the O/W type.

2.8 Data Analysis

The data analyzed included descriptive and quantitative data. Descriptive data is data obtained from observations on cream evaluation, namely organoleptic tests, homogeneity, pH value, and cream emulsion type. Meanwhile, quantitative data include TFC, antioxidant activity (IC_{50}) of the extract, fractions, and cream preparation, as well as cream evaluation such as adherence test and the spreadability test.

3 Results and Discussions

3.1 Extraction of Porang Leaves

The results of Porang leaves determination indicate that the sample was *Amorphophallus muelleri* Blume from the Araceae family. From 4 kg of wet porang leaves yielded 750 grams of porang leaves powder, hence the yield percentage was 18.75%. The porang leaves powder was extracted using maceration using 96% ethanol. Maceration enabled the contact between the sample and the solvent and avoided damage to the components contained in the sample which are not heat-resistant [15]. Furthermore, 96% ethanol is a universal solvent so it can make it easier to withdraw the compounds contained in the sample. The maceration was followed by evaporation using a rotary evaporator at 55°C, yielding 101.34 grams of extract (yield percentage 13.51%).

3.2 Fractionation of Porang Leaves

The fractionation method used in this research is solid-liquid partitioning. N-hexane was used as non-polar and ethyl acetate as semi-polar solvent. Fractionation using different polarity solvents aimed to attract non-polar to polar compounds contained in porang leaves extracted, according to their polarity. It is known that *n*-hexane could attract non-polar compounds, for example chlorophyll, steroids/triterpenoids, and a small portion of carotenoids. Whereas ethyl acetate could attract lycopene and *β*-carotene. Meanwhile, the filtrate that is insoluble in ethyl acetate is called the insoluble fraction (IS) which attracts compounds with polar properties, for example, flavonoids and xanthin [16,17]. The fractionation process yielded NH, EA, and IS as much as 19.49%; 1.15%; and 44.46%, respectively. The highest yield was found in the insoluble fraction, so it can be concluded that the most dominant component from the extract was polar compounds.

3.3 Thin Layer Chromatography (TLC)

TLC was carried out as qualitative analysis, followed by DPPH reagent spray to identify whether the sample contained antioxidant compounds. Positive result indicated by yellow-colored spots [18]. Furthermore, various spray reagents were used for phytochemical screening of extract and fractions. Eluent of chloroform: ethyl acetate (9:1) was selected after optimization using various solvents and ratio.

Table 2. TLC Results of Porang Leaves Extract and Fraction

Sample	Spots after observation (Rf)		
	Visible light	UV 254	UV 366
EE	0.01 (green) 0.04 (green/blue) 0.38 (yellow)* 0.69 (blue) 0.75 (blue) 0.94 (blue) 1.00 (black)	0.01 (dark) 1.00 (dark)	0.01 (pink) 0.04 (pink) 0.38 (yellow) 0.69 (pink) 0.75 (pink) 0.94 (pink) 1.00 (dark orange)
NH	0.01 (light brown) 0.05 (blue) 1.00 (black)	0.01 (dark) 0.05 (dark) 1.00 (dark)	0.01 (pink) 0.05 (pink) 0.38 (yellow) 0.66 (pink) 0.69 (pink) 0.91 (pink) 1.00 (pink)
EA	0.01 (brown) 0.04 (brown) 0.35 (yellow)*	0.01 (dark) 0.04 (dark) 0.35 (dark)	0.01 (brown) 0.04 (pink) 0.35 (yellow) 0.66 (pink) 0.91 (pink) 1.00 (pink)
IS	0.01 (yellowish brown)	0.01 (dark)	0.01 (brown) 0.03 (pink)

Note: * Spots that showed yellow color when sprayed with DPPH reagent

Based on TLC analysis, it can be concluded that there were differences in the chromatogram profiles between porang leaves extract (EE) and fractions (NH, EA, and IS), indicating different phytochemical components contained in extracts and fractions (**Fig. 1**, **Table 2**). Relatively nonpolar compounds (spots with Rf ~0.9) existed in EE, NH, and EA. Spots at Rf ~0.3-0.6 were dominantly observed in the EA fraction, indicating that they are relatively semi-polar. Whereas for IS, the spot cannot be eluted by the eluent, indicating the presence of polar compound.

After spraying with DPPH, spots on Rf 0.38 in EE and Rf 0.31 in EA showed yellow color, indicating the antioxidant activity dominantly possessed by EE and EA.

3.4 Phytochemical Screening

The phytochemical screening was performed using TLC with spray reagents to identify the phytochemical component in extract and fractions. It is known that alkaloids, flavonoids, tannins, and saponins can act as antioxidants, donating their hydrogen atoms to free radicals (4). From the result in **Table 3**, it can be concluded that EE and EA contained flavonoid, tannin, and terpenoid. The spots in Rf 0.38 (EE) and Rf 0.35 (EA) that showed positive results after spraying DPPH were identified as flavonoids. Based on prior research by Suryani *et al* (2023), ethanolic extract of porang leaves contained flavonoid, tannin, and terpenoid [19]. Whereas research by Erikania & Rosalina (2022) stated that ethanolic extract of porang leaves also contained alkaloid [20]. It is possible that the extract contained alkaloid but the alkaloid spots in TLC were overlapped. Further research on TLC mobile phase optimization is needed to confirm this result.

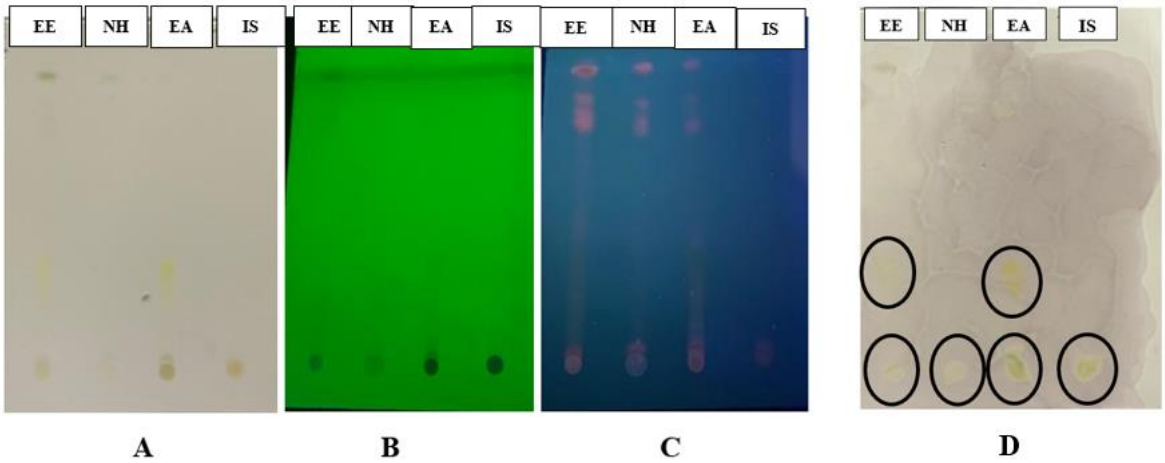

Figure 1. TLC Profile of Ethanol Extract and All Fractions Before Sprayed with DPPH (A) under visible light, (B) UV 254 nm, (C) UV 366 nm, and (D) after sprayed with DPPH under visible light
Note: EE : Ethanol Extract; NH : n-Hexane Fraction; EA : Ethyl Acetate Fraction; IS : Insoluble Fraction

Table 3. Phytochemical Screening of Extract and Fractions

Sample	Spots after reagent spray (Rf)			
	Alkaloid (Dragendorff) (+) reddish	Flavonoid (5% $AlCl_3$) (+) yellowish green	Tannin (5% $FeCl_3$) (+) dark blue/black	Terpenoid (Liebermann-Burchard) (+) blue
EE	-	(+) 0.01 0.38	(+) 0.01 1.00	(+) 0.04 0.69 0.75 0.94
NH	-	(+) 0.01	-	(+) 0.05 0.66 0.69 0.91
EA	-	(+) 0.01 0.35	(+) 0.01	(+) 0.38
IS	-	(+) 0.01	(+) 0.03	-

3.5 TFC and Antioxidant Activity

The TFC analysis on porang leaves extract (EE) and fraction (NH, EA, and IS) was performed spectrophotometrically based on the complex formation between $AlCl_3$ and the C-4 keto and C-3/C-5 hydroxyl groups of flavones and flavonols [21]. Quercetin, a flavonol, was used as standard for TFC analysis, hence, the result shown as mgQE (Quercetin Equivalent)/g sample.

The antioxidant activity assay of the porang leaves ethanol extract (EE) and fractions (NH, EA, IS) used the principle of UV-Vis spectrophotometry with the DPPH method. If a sample contained antioxidant compounds, the compound would donate its hydrogen atoms so that it binds to the DPPH reagent that showed color change from purple to pale yellow and a decrease in the absorbance of the sample. The maximum wavelength was 516 nm, which showed concordance with the research by Molyneux (2004) that stated the maximum wavelength for DDPH is 516-520 nm [22].

Table 4. The Antioxidant Activity of Porang Leaves Extract and Fractions

Samples	TFC (mg QE/g)	IC_{50} (μg/mL)	Antioxidant Category*
EE	76.79 ± 3.57[a]	66.39 ± 0.44[a]	Strong
NH	71.27 ± 1.15[b]	198.31 ± 16.75[b]	Weak
EA	101.93 ± 2.75[c]	72.19 ± 4.37[c]	Strong
IS	86.98 ± 3.29[d]	135.48 ± 2.54[d]	Moderate
Ascorbic acid	-	8.89 ± 0.70[e]	Very strong

Different letters within a column indicate significant differences ($p<0.05$). All analyses were the mean of three replicate measurements ± standard deviation. *antioxidant activity category by Molyneux (2004) (22)

The coefficient of determination (R^2) analysis showed that the ethyl acetate fraction exhibited the strongest correlation between total phenolic content and antioxidant activity (R^2 = 0.9693), indicating that phenolic compounds were the major contributors to the free radical scavenging activity in this fraction. In contrast, the ethanolic extract (EE) demonstrated the highest antioxidant activity despite having a weak correlation (R^2 = 0.1029), suggesting that its activity may result from the synergistic interaction of diverse bioactive compounds ranging from nonpolar to polar constituents present in the crude extract (**Table 4**). This result was in concordance with TLC chromatogram and phytochemical screening. Compounds contained in EE could act sinergistically to provide antioxidant effects. Antioxidant activity among extract and fractions from the strongest to the weakest were as follows: EE > EA > IS > NH. Whereas the TFC result from the greatest were EA > IS > EE > NH. These results indicate that the antioxidant activity was not solely attributed to flavonoids but may also involve other phenolic and non-phenolic compounds. Structurally, flavonoids and tannins possess hydroxyl groups that can donate hydrogen atoms or electrons to stabilize free radicals, while terpenoids may contribute through radical scavenging and membrane-protective mechanisms. Therefore, the combination of multiple bioactive compounds with different antioxidant mechanisms may explain the stronger antioxidant activity observed in EE [23]. Although EE and EA were categorized as having strong antioxidant activity, EE was selected as the active ingredient for cream formulation because it exhibited the strongest antioxidant activity overall. Although EE and EA were in the same antioxidant category (strong), EE was further chosen as active ingredients in antioxidant cream formulation because it exhibits stronger antioxidant activity.

3.6 Cream Preparation and Evaluation

In this study, the preparation of antioxidant cream preparations was conducted using EE as the active ingredient of the cream. There were 4 cream formula with different EE concentrations, namely F1 as a base; F2 with 2.5% EE; F3 with 5% EE; and F4 with 7.5% EE (**Fig. 2**). Base formula (F1) was made to compare the antioxidant activity of cream base with cream formula added with active extract ingredients (F2-F4).

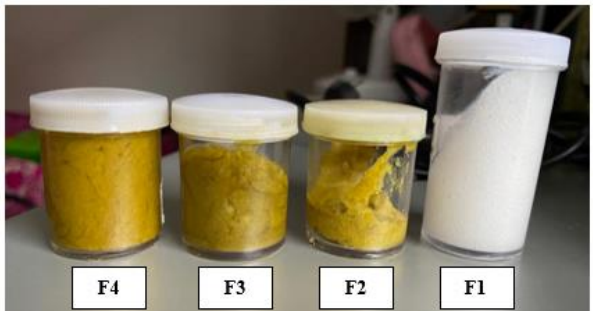

Figure 2. Cream Preparation from Porang Leaves Extract

Table 5. Evaluation of Antioxidant Cream Preparation

Cream formula	Organoleptic	Homogeneity	pH	Adhesion (seconds)	Spreadability range (cm)	
					50g	250g
F1	White color, no smell, semisolid	Homogeneous	6.47 ± 0.15[a]	4.38 ± 0.62[a]	5.08 ± 0.08[a]	6.75 ± 0.05[a]
F2	Yellow color, faint extract smell, semisolid	Homogeneous	6.25 ± 0.13[a]	4.85 ± 0.52[a]	4.25 ± 0.05[b]	6.00 ± 0.50[b]
F3	Dark yellow color, faint extract smell, semisolid	Homogeneous	6.23 ± 0.10[a]	5.81 ± 0.54[b]	4.08 ± 0.08[b]	5.75 ± 0.05[b]
F4	Dark yellow color, faint extract smell, semisolid	Homogeneous	6.20 ± 0.13[a]	8.09 ± 0.49[c]	4.17 ± 0.13[b]	5.42 ± 0.10[c]

Different letters within a column indicate significant differences ($p<0.05$). All analyses were the mean of three replicate measurements ± standard deviation.

Evaluation results showed that all cream formulas meet the requirement for cream preparation (Table 5). The requirements for a good pH value in the cream preparation test are the same as the skin pH (4.5-6.5) [14]. Creams that have a pH of less than 4.5 can cause skin irritation, whereas creams that have a pH of more than 6.5 can cause scaly skin. All formulas have met the requirements for good adhesion (> 4 seconds). The greater the concentration of the ethanol extract added, the longer the adhesion time, and the smaller the spreadability. The spreadability results at the maximum applied load (250g) met the acceptable range for topical preparations (5–7 cm) [24]. All cream formulas have oil-in-water (O/W) emulsion type. The advantage of the O/W type is that it can provide a hydration effect so that it can increase the penetration of active substances due to the higher water content [25].

Table 6. Antioxidant Activity of Porang Leaves Ethanol Extract Cream

Formula	Concentrations (ppm)	Average Absorbance	Inhibition (%)	IC_{50} Value (µg/mL)	Linear Regression Equations
F1 (Base)	22500	0.568	5.330	110727.400	y = 0.0005x – 5.3637 r^2 = 0.9863
	20000	0.577	3.830		
	17500	0.585	2.400		
	15000	0.587	1.832		
	12500	0.597	0.499		
F2 (2.5% EE)	2250	0.554	29.880	3898.694	y = 0.0121x + 2.8258 r^2 = 0.9958
	1750	0.529	23.861		
	1500	0.509	21.389		
	1250	0.473	18.302		
	1000	0.454	14.440		
F3 (5% EE)	2250	0.367	43.320	2590.377	y = 0.0191x + 0.5238 r^2 = 0.9978
	2000	0.393	39.305		
	1750	0.426	34.208		
	1500	0.462	28.640		
	1000	0.519	19.845		
F4 (7.5% EE)	2250	0.213	67.104	1637.120	y = 0.0287x + 3.5058 r^2 = 0.9985
	2000	0.257	60.307		
	1750	0.299	53.830		
	1500	0.347	46.409		
	1250	0.398	38.532		

3.7 Antioxidant Activity of Cream Preparation from Porang Leaves Extract

Antioxidant activity of cream preparation was measured using DPPH method. The antioxidant activity of the cream increased along with the increase in the concentration of the extract added to each formula (Table 6). The formula with the addition of extracts (F2 – F4) showed much higher antioxidant activity than the base (F1). This shows that the porang leaves extract has a significant effect on increasing antioxidant activity in cream preparations. However, at the highest concentration of extract (F4), the antioxidant activity was still categorized as very weak.

The reduced antioxidant activity after formulation may be attributed to several factors, including dilution of the extract within the cream base, limited release of active compounds from the semisolid matrix, poor solubility of antioxidant constituents in the assay medium, interference from excipients, incomplete extraction of active compounds prior to DPPH analysis, and turbidity effects during UV–Vis absorbance measurement [26-29]. The best cream formula based on its activity as an antioxidant is F4. However, it is necessary to optimize the formulation by increasing the concentration of the extract and optimizing the composition of the cream additives to produce more optimal antioxidant activity.

4 Conclusion

The ethanol extract and fractions of porang (*Amorphophallus muelleri*) leaves possess antioxidant activity. The highest antioxidant activity was found in the ethanol extract (IC_{50} 66.39 ± 0.44 µg/mL) and ethyl acetate fraction (IC_{50} 72.19 ± 4.37 µg/mL), which were classified as strong antioxidants. Both ethanol extract and ethyl acetate fraction contained with flavonoid, tannin, and terpenoid according to the phytochemical screening using TLC method.

The cream prepared from ethanolic extract from porang leaves (F1-F4) met the requirements of a good cream preparation, including the organoleptic test, homogeneity test, pH test, adhesion and spreadability test. All the cream was O/W type.

The ethanolic extract of porang leaves formulated into cream preparations exhibited weak antioxidant activity (IC_{50} = 1637.12–3898.69 µg/mL), which was substantially lower than that of the crude extract. Nevertheless, formulations containing porang leaf extract (F2–F4) showed markedly higher antioxidant activity compared with the cream base (F1; IC_{50} = 110727.4 µg/mL), indicating that the extract contributed antioxidant compounds to the formulation. Further optimization of the cream formulation is still needed to enhance its antioxidant activity.

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