COMPARISON OF EXTRACTION METHODS ON ANTIOXIDANT ACTIVITY, PHENOLIC AND FLAVONOID CONTENT IN 96% ETHANOL EXTRACTS OF LANGSAT LEAVES (*Lansium domesticum* Corr.)

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Abstract

The leaves of *Lansium domesticum* Corr., commonly known as langsat, are recognized for their antioxidant properties attributed to the existence of phenolic and flavonoid components. Variations in extraction methods can affect the levels of these compounds, thereby influencing their antioxidant activity. The objective of this study is to compare the antioxidant activity, phenolic and flavonoid content of 96% ethanol extracts of langsat leaves obtained through four different extraction techniques. Langsat leaves powder was extracted using maceration, percolation, reflux, and soxhlet, and methods with 96% ethanol as the solvent, resulting in ethanol-based extracts. Antioxidant activity was analyzed using the ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) assay with trolox as a standard. The phenolic and flavonoid contents were measured spectrophotometrically using Folin-Ciocalteu and $AlCl_3$ reagents, with gallic acid and quercetin as standards, respectively. Data on antioxidant activity, phenolic and flavonoid levels from the four extraction methods were statistically analyzed to evaluate differences among the techniques. The percolation method yielded the highest antioxidant activity, with an IC_{50} value of 89.48 ± 0.17 ppm. Furthermore, this approach yielded the greatest amounts of phenolic and flavonoid compounds, with values of 138.69 ± 0.456 mg GAE/g extract and 5.38 ± 0.576 mg QE/g extract, respectively. Thus, percolation emerged as the most effective extraction method for maximizing antioxidant activity, phenolic and flavonoid content compared to maceration, reflux, and soxhlet methods.

Keywords: antioxidants, phenolic, flavonoid, langsat leaves, extraction method

INTRODUCTION

Antioxidants are compounds that protect cellular integrity by mitigating damage caused by free radicals, which can contribute to the development of various diseases, including cancer, cardiovascular disorders, and premature aging (Martemucci *et al.*, 2022). There are two primary categories of antioxidants, namely synthetic and natural. Synthetic antioxidants often induce adverse effects, underscoring the need for the discovery of natural antioxidants derived from the environment. One potential candidate for natural antioxidant development is the leaves of the langsat tree (*Lansium domesticum* Corr.), which could offer a safer alternative to synthetic counterparts.

The leaves of *Lansium domesticum* extracted through maceration using 96% ethanol solvent have been shown to contain secondary metabolites, including phenolics, flavonoids, saponins, triterpenoids, and steroids (Yunus *et al*., 2018). According to Sulistiawati (2023), the findings indicate that the 96% ethanol extract of *Lansium domesticum* leaves exhibits antioxidant activity with an IC_{50} value of 110.493 ppm, classified as moderate when compared to trolox (IC_{50} value of 18.735 ppm). Furthermore, the extract contains phenolic and flavonoid contents amounting to 186.2 mg GAE/g and 3.126 mg QE/g, respectively.

Differences in extraction methods can influence the antioxidant activity and the total phenolic and flavonoid content yielded. For instance, methanol extracts of *Pluchea indica L.* leaves obtained through percolation exhibit higher flavonoid and total phenolic content compared to maceration, Soxhlet, and reflux methods (Safitri, 2018). Similarly, the highest phenolic content in ethanol extracts of *Kaempferia galanga* rhizomes was achieved through percolation compared to other methods (Khotimah, 2020). A study by Puspitasari and Proyogo (2017) reported that the total phenolic content in ethanol extracts of *Muntingia calabura* leaves was 1.16 mg GAE/g using maceration and 2.53 mg GAE/g using Soxhlet extraction. It can be inferred that the extraction method significantly influence the total phenolic content in ethanol extracts of *Muntingia calabura* leaves.

Research investigating the antioxidant activity and the determination of phenolic and flavonoid content in 96% ethanol extracts of *Lansium domesticum* leaves using various extraction methods has not been previously conducted. Consequently, this study was undertaken to evaluate and investigate the differences in efficacy among various extraction techniques in influencing the antioxidant activity, phenolic and flavonoid content in the ethanol extract.

METHODOLOGY

Materials and Tools

The materials utilized in this study included *Lansium domesticum* Corr. leaves, technical-grade 96% ethanol, 5% $FeCl_3$, lead acetate, Folin-Ciocalteu reagent (Merck), Na_2CO_3 (Merck), gallic acid (Merck), analytical-grade ethanol (Merck), quercetin (Merck), $AlCl_3$ (Merck), potassium acetate (Merck), ABTS (Merck), potassium persulfate (Merck), and Trolox (Phytotechnology Laboratories).

The equipment employed for the preparation of the 96% ethanol extract of *Lansium domesticum* Corr. leaves included a drying cabinet (Memmert), a blender (Maspion), a sieve with a mesh size of 40, a magnetic stirrer (Ohaus), and an electronic balance (Ohaus). Extraction processes utilized a maceration apparatus, a vacuum pump system (Rocker 600), and a rotary evaporator (Heidolph). For antioxidant activity assays and the calculation of tphenolic and flavonoid content, laboratory glassware sets (Iwaki Pyrex), micropipettes (Socorex), and UV-Vis spectrophotometers (Shimadzu UV-1800) were used.

Plant determination

The analysis was conducted at the Ecology and Biosystems Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Diponegoro, Semarang.

Langsat leaves material collection

The collection of plant material for this study was conducted with meticulous attention to detail and adherence to scientific protocols. The Langsat leaves were procured from an orchard located in Ngosari Village, Tlogowungu District of Pati Regency, Central Java, Indonesia.

Preparation of langsat leaves powder

The langsat leaves were initially sorted to remove impurities and subsequently washed under running water to eliminate any residual contaminants. Following the washing process, the leaves were drained until excess water was removed and weighed. The langsat leaves were then sliced into smaller sections and dried in a drying cabinet set at a temperature of 50°C. Once dried, the leaves were weighed again, ground using a blender, and sieved through a 40-mesh sieve. The moisture content of the powdered langsat leaves was measured using a moisture balance device. According to the requirements for simplicia, the moisture content should not exceed 10% (Ministry of Health of Indonesia, 1986).

Preparation of 96% ethanol extract of langsat leaves

a. Maceration Method

One hundred grams of powdered langsat leaves (Lansium domesticum) were placed in a dark container, followed by the addition of 750 mL of 96% ethanol as a solvent. The mixture was stirred until a homogeneous blend of powder and solvent was achieved. Subsequently, the container was sealed tightly, and its surface was covered with brown paper to protect it from direct sunlight. The maceration process lasted for three days, with stirring conducted for 15 minutes three times daily. The mixture was filtered after three days to acquire the first macerate (Macerate 1). The residue from this filtration underwent a second maceration (remaceration) using 250 mL of 96% ethanol for two days, after which it was filtered again to yield the second macerate (Macerate 2). Both macerates were then allowed to sediment overnight before being separated from their residues. The next step is to concentrate two macerate mixtures utilizing a rotary evaporator at a temperature of 50°C to produce a thick extract of 96% ethanol from the langsat leaves (Ministry of Health of Indonesia, 1986).

b. Percolation Method

One hundred grams of powdered *Lansium domesticum* leaves were placed into a 500 mL beaker glass, followed by the addition of 200 mL of 96% ethanol to moisten the plant material. The moistened material was then transferred into a percolator lined with filter paper and filled with solvent up to the upper limit. The solvent was collected from the percolator every three hours, and the percolation process was continued until the solvent exiting the percolator appeared clear. The collected percolate was stored in an Erlenmeyer flask and concentrated utilizing a rotary evaporator at 50°C to produce a thick extract of 96% ethanol from the langsat leaves (Ministry of Health of Indonesia, 1986).

c. Soxhlet Method

One hundred grams of powdered *Lansium domesticum* were enclosed in filter paper and tied firmly at both ends with thread. This package was then placed into the Soxhlet apparatus. Subsequently, 600 mL of 96% ethanol solvent was poured into a round-bottom flask connected to the Soxhlet apparatus. The extraction process was carried out at a temperature of 65ºC until the ethanol droplets exiting the apparatus became colorless. Following this, the resulting liquid extract was concentrated utilizing a rotary evaporator at a temperature of 50ºC to produce a thick extract of 96% ethanol from the langsat leaves (Ministry of Health of Indonesia, 1986).

d. Reflux Method

One hundred grams of powdered *Lansium domesticum* were placed into a round-bottom flask and mixed with 500 mL of 96% ethanol solution. The reflux process was conducted three times, each lasting for two hours. Subsequently, the resulting liquid extract was filtered and concentrated utilizing a rotary evaporator at 50°C to produce a thick extract of 96% ethanol from the langsat leaves (Ministry of Health of Indonesia, 1986).

The yield of the 96% ethanol extract from langsat leaves was determined using the following formula:

$$\text{Yield} = \frac{\text{Weight of extracted extracti (gram)}}{\text{Weight of powder used for the extraction process (gram)}} \text{ x } 100\% \quad (1)$$

Antioxidant activity test

a. Preparation of ABTS stock solution

The preparation of the ABTS solution involved dissolving 101.5 mg of ABTS powder in sufficient p.a. ethanol, followed by stirring until completely dissolved. This solution was then transferred to a 25 mL volumetric flask and diluted with p.a. ethanol to the mark, resulting in an ABTS solution with a concentration of 7.4 mM. Similarly, 166.2 mg of potassium persulfate was dissolved in an appropriate amount of p.a. ethanol and stirred until fully dissolved. This solution was subsequently placed in a 250 mL volumetric flask and brought to volume with p.a. ethanol, yielding a potassium persulfate solution with a concentration of 2.46 mM. Both solutions (25 mL of ABTS solution and 25 mL of potassium persulfate solution) were combined in a 50 mL volumetric flask, which was then covered with aluminum foil to prevent exposure to sunlight. The mixture was incubated for 12 to 16 hours to ensure complete reaction (Sharma *et al*., 2017).

b. Preparation of trolox stock solution

A 10 mg sample of Trolox powder was dissolved in an appropriate volume of analytical grade ethanol and stirred until completely dissolved. The prepared solution was subsequently transferred into a 10 mL volumetric flask and diluted to the calibration mark using analytical grade ethanol, yielding a trolox stock solution with a concentration of 1000 ppm (Anwar *et al*., 2022).

c. Preparation of a series of trolox concentrations

Trolox stock solution (1000 ppm) was precisely measured using a micropipette in aliquots of 25, 50, 75, 100, and 125 µL. Each aliquot was carefully transferred into a 5 mL volumetric flask and subsequently diluted to the calibration mark using analytical grade ethanol. This procedure generated a series of trolox solutions with concentrations of 5, 10, 15, 20, and 25 ppm (Anwar *et al*., 2022).

d. Preparation of samples stock solution

One hundred milligrams of 96% ethanol extract derived from *Lansium domesticum* leaves was dissolved in an appropriate volume of ethanol p.a. and stirred using a magnetic stirrer set at 300 rpm until complete dissolution was achieved. The resulting solution was subsequently filtered. After

filtration, the solution was quantitatively transferred into a 100 mL volumetric flask and subsequently diluted to the calibration mark using ethanol p.a., resulting in a stock solution with a concentration of 1000 ppm (Sami *et al*., 2020).

e. Preparation of a series of sample concentrations

The stock solution of the sample was measured using a micropipette in volumes of 150, 300, 450, 600, and 750 µL. After being moved into a 5 mL volumetric flask, each aliquot was diluted to the calibration mark using analytical-grade ethanol. This preparation yielded a series of sample solutions with concentrations of 30, 60, 90, 120, and 150 ppm (Sami *et al*., 2020).

f. Maximum wavelength measurement

The Trolox solution, prepared at a concentration of 15 ppm, was measured to a volume of 1 mL and subsequently transferred into a 5 mL volumetric flask. Following this, 1 mL of ABTS solution was introduced into the flask, and the total volume was adjusted to the calibration mark using ethanol p.a. The resulting mixture was then placed into a cuvette for absorbance analysis employing a UV-Vis spectrophotometer. Ethanol p.a. was used as the blank, and the absorbance was recorded within the wavelength range of 500–800 nm. The maximum wavelength was determined based on the highest absorbance value, corresponding to the peak absorption.(Anwar *et al*., 2022).

g. Operating time measurement

A 1 mL aliquot of a 15 ppm Trolox solution was transferred into a 5 mL volumetric flask. Subsequently, 1 mL of ABTS solution was added, and the mixture was diluted to the mark with ethanol p.a. The prepared solution was then placed in a cuvette for absorbance measurement using a UV-Vis spectrophotometer, with ethanol p.a. serving as the blank. Absorbance readings were recorded at the maximum wavelength over a period of 60 minutes, with measurements taken at 5-minute intervals (Anwar *et al*., 2022).

h. ABTS solution absorbance measurement

A volume of 1 mL of the ABTS solution was introduced into a 5 mL volumetric flask. Ethanol p.a. was then added to the flask until the calibration mark was reached. This procedure was replicated three times, and the solution was left to stand for the designated operating time. The solution was subsequently transferred into a cuvette, and its absorbance was determined using a UV-Vis spectrophotometer at the wavelength corresponding to its maximum absorbance (Sami *et al.*, 2020).

i. Antioxidant activity test of Trolox

A solution of Trolox at concentrations of 5, 10, 15, 20, and 25 ppm was prepared, with each concentration measured at 1 mL and transferred into a 5 mL volumetric flask. Subsequently, 1 mL of the ABTS stock solution was added, followed by the addition of ethanol p.a. until the solution reached the calibration mark. The mixture was then allowed to stand in a location shielded from direct sunlight for the duration of the operating time. Following this incubation period, the solution was placed into a cuvette for absorbance measurement using a UV-Vis spectrophotometer at the maximum wavelength. The absorbance values obtained were used to calculate the percentage of inhibition (Anwar *et al*., 2022).

j. Antioxidant activity test of samples

The antioxidant activity test of the samples was identical to the Trolox antioxidant activity test. The difference lies in the concentration of the sample solution used, which is 30, 60, 90, 120, and 150 ppm.

Calculation of total phenolic content (Puspitasari dan Prayogo, 2017)

a. Preparation of 7% Na_2CO_3 solution

A total of 7 grams of sodium carbonate was fully dissolved in distilled water. The resulting solution was then transferred into a 100 mL volumetric flask, and distilled water was added until the solution volume reached the calibration mark on the flask.

b. Preparation of gallic acid stock solution

Gallic acid was dissolved in ethanol (p.a.) until completely dissolved, using 10 mg of gallic acid. The solution was then transferred to a 10 mL volumetric flask and diluted to the calibration mark with ethanol (p.a.). The resulting stock solution of gallic acid had a concentration of 1000 ppm.

c. Preparation of a series of gallic acid concentrations

A range of gallic acid solutions with concentrations of 50, 100, 150, 200, 250, and 300 ppm was prepared by accurately pipetting volumes of 250 µL, 500 µL, 750 µL, 1000 µL, 1250 µL, and

1500 µL from the stock solution. Each aliquot was then transferred into a 5 mL volumetric flask and brought to volume with absolute ethanol of analytical grade.

d. Maximum wavelength measurement

A 200 µL aliquot of gallic acid solution (150 ppm) was pipetted into a 5 mL volumetric flask. Subsequently, 400 µL of Folin-Ciocalteu reagent was added, and the mixture was incubated for 8 minutes at ambient temperature. Following this, 4 mL of a 7% sodium carbonate (Na_2CO_3) solution was introduced, and the solution was diluted to the mark with analytical-grade ethanol. The resulting mixture was transferred to a quartz cuvette, and absorbance measurements were conducted using a UV-Vis spectrophotometer across a wavelength range of 500–800 nm.

e. Operating time measurement

A measured aliquot of 200 µL of gallic acid solution, prepared at a concentration of 150 ppm, was transferred into a 5 mL volumetric flask. Subsequently, 400 µL of Folin-Ciocalteu reagent was added to the flask, and the mixture was allowed to react for 8 minutes. After this incubation period, 4 mL of a 7% sodium carbonate (Na_2CO_3) solution was introduced, and the flask was filled to the calibration mark with ethanol of analytical grade. The resulting solution was then placed into a cuvette for spectrophotometric analysis. Absorbance measurements were conducted using a UV-visible spectrophotometer over a time interval ranging from 0 to 120 minutes at the predetermined maximum wavelength1. This temporal analysis was performed to determine the period at which the absorption reached stability, thus ensuring optimal measurement conditions for the assay.

f. Preparation of samples stock solution

The 96% ethanol extract of langsat leaves as much as 1000 mg was added with enough ethanol p.a solvent with the help of a magnetic stirrer to dissolve then filtered with filter paper. A 100 mL volumetric flask was filled with the solution, then ethanol was added gradually until the limit was reached.

g. Preparation of gallic acid standard curve

A solution of gallic acid at concentrations of 50, 100, 200, 250, and 300 ppm was prepared by transferring 200 µL of each concentration into a 5 mL volumetric flask. Subsequently, 400 µL of Folin-Ciocalteu reagent was added to the flask, and the mixture was allowed to stand for 8 minutes. Following this, 4 mL of a 7% sodium carbonate (Na_2CO_3) solution was added, and the volume was adjusted to the calibration mark using ethanol (p.a.). The resulting solution was transferred into a cuvette for absorbance measurement using a UV-Vis spectrophotometer at the maximum wavelength. To guarantee precision and dependability, this process was carried out three times.

h. Calculation of total phenolic content

A 200 µL sample solution was transferred into a 5 mL volumetric flask. Subsequently, 400 µL of Folin-Ciocalteau reagent was added, and the mixture was allowed to stand for 8 minutes. Following this, 4 mL of 7% Na_2CO_3 was added, and the solution volume was adjusted with pure ethanol until it reached the calibration mark. After that, the resulting mixture was introduced into a cuvette, and a UV-Vis spectrophotometer set to its maximum wavelength was used to measure its absorbance. To guarantee precision and dependability, this process was carried out three times.

Calculation of total flavonoid content (Puspitasari *et al*., 2019)

a. Preparation of 10% $AlCl_3$ solution

Ethanol was used to dissolve 500 mg of aluminum chloride ($AlCl_3$) until it was fully dissolved. The solution was then poured into a 5 mL volumetric flask, and ethanol was added gradually until the volumetric flask's limit line was reached.

b. Preparation 1 M CH_3COOK solution

After dissolving 500 mg of potassium acetate with ethanol p.a., the mixture was transferred to a 5 mL volumetric flask, and more ethanol p.a. was added until the limit was reached.

c. Preparation of quercetin stock solution

Ten milligrams of quercetin were added, and enough ethanol p.a. solvent was used to dissolve it. To create a quercetin stock solution with a 400 ppm concentration, the solution

was then transferred into a 25 mL volumetric flask, and ethanol was added gradually until it reached the limit line shown on the flask.

d. Preparation of a series of quercetin concentrations

Using a micropipette, aliquots of the quercetin stock solution were precisely measured in volumes of 25, 50, 75, 100, 125, and 150 µL. Each volume was then put into a measuring flask with a capacity of 5 mL, and ethanol p.a. was added until it reached the limit line. In this way, quercetin solutions with concentrations of 2, 4, 6, 8, 10, and 12 ppm were obtained.

e. Maximum wavelength measurement

A 5 mL volumetric flask was filled with 1000 µL of quercetin mother liquor at a concentration of 6 ppm. Following the addition of 200 µL of a 10% aluminum chloride ($AlCl_3$) solution and 200 µL of 1M potassium acetate, ethanol was added gradually until the volume reached the limit. After that, the solution was put into a cuvette, and a UV-Vis spectrophotometer operating in the 400–500 nm wavelength range was used to measure the absorbance.

f. Operating time measurement

A 5 mL volumetric flask was filled with 1000 µL of quercetin solution at a concentration of 6 ppm. 200 µL of 1M potassium acetate and 200 µL of 10% $AlCl_3$ were then added. Ethanol was then gradually poured into the volumetric flask until the desired limit was attained. In order to determine a steady absorption time, the solution was then placed in a cuvette, and the absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength for 0–60 minutes.

g. Preparation of samples stock solution

The preparation of the sample stock solution is the same as the preparation of the total phenolic content determination test sample stock solution.

i. Preparation of quercetin standard curve

A 5 mL volumetric flask was filled with quercetin solutions in levels up to 1000 µL, containing concentrations of 2, 4, 6, 8, 10, and 12 ppm. After that, 200 µL of a 10% aluminum chloride solution and 200 µL of 1 M potassium acetate, respectively, were added, and then ethanol was added gradually until the limit was reached. The solution was then placed in a cuvette, and the absorption was measured at the maximum wavelength using a UV-Vis spectrophotometer. Three iterations of this process were conducted to guarantee precision and dependability.

j. Calculation of total flavonoid content

The stock solution (1000 µL) was transferred into a 5 mL volumetric flask. Subsequently, 200 µL of 10% aluminum chloride ($AlCl_3$) and 200 µL of 1 M potassium acetate were added. The mixture was diluted to the mark with ethanol (p.a.). The resulting solution was then placed in a cuvette for absorbance measurement using a UV-Vis spectrophotometer at the maximum wavelength. This procedure was repeated three times to ensure the accuracy and reliability of the results.

Data analysis

a. Antioxidant activity

The result of the data obtained is the absorbance value of each concentration series used to calculate the percent inhibition with the formula:

$$\text{Percent inhibition} = \frac{\text{ABTS absorbance} - \text{sample absorbance}}{\text{ABTS absorbance}} \text{ x } 100\% \quad (2)$$

The % inhibition value of each concentration series was used for linear regression analysis of concentration series versus % inhibition to determine the IC_{50} value by the formula:

$y = bx + c$

$y = 50$

$x = IC_{50}$ value (ppm)

b. Calculation of phenolic and flavonoid content

The absorbance value of the sample is the information derived from the measurement of the total phenolic and flavonoid content. Additionally, the absorbance values of the samples were plotted into the standard curve equation of quercetin and gallic acid as a comparison in order to determine the total phenolic and flavonoid levels. The following formula is used to determine the phenolic or flavonoid content:

$$\text{Phenolic or flavonoid content} = \frac{C \times fp \times V}{g} \quad (3)$$

Here, C is the sample concentration (mg/mL or µg/mL) from a standard curve, fp is the dilution factor (if the sample was diluted), V is the sample volume (mL) used in the test, and g is the sample weight (g).

Statistical analysis

In order to assess the normality of the data, the Shapiro-Wilk or Kolmogorov-Smirnov test with a 95% confidence level was used to analyze the antioxidant activity data of IC50 values as well as the total phenolic and flavonoid content of the samples acquired by various extraction procedures. To further verify the data's uniformity, a variance homogeneity test was run. The analysis proceeded with the one-way ANOVA test and the Bonferroni test if the data turned out to be normal and homogeneous. However, the Kruskal-Wallis test would be employed to evaluate significance if the data did not match the criteria of homogeneity and normality. The differences between each extraction technique were found using the Mann-Whitney test for additional investigation.

RESULT AND DISCUSSION

Determination of langsat plants

The first step in research involves identifying the plant species to ensure accuracy during sampling. This identification process is conducted on all parts of the langsat plant. The results confirm that the analyzed samples are indeed langsat plants, specifically *Lansium domesticum Corr*. The identification details include characteristics such as scattered pinnate compound leaves and classification within the Meliaceae family, genus *Lansium*, and species *Lansium domesticum Corr.* as described by Steenis in 1992.

Preparation of ethanol extract of langsat leaves

The resulting langsat leaf powder was 735 grams, with a moisture content of 8.4%. The measurement of the water content of the langsat leaf powder has met the quality standard of <10% (Indonesian Ministry of Health, 1986). The extraction process was carried out with 4 extraction methods, namely soklet, reflux, percolation, and maceration of 50 grams of langsat leaf powder, each with 96% ethanol solvent. The calculation of yield is used to evaluate the efficiency of the extraction process (Kusuma and Aprileili, 2022). The yield of 96% ethanol extract of langsat leaves can be seen in Table I.

Percolation and maceration are cold methods while soklet and reflux are hot methods. The yield of the percolation method is greater than the maceration method. This happens because in the percolation method the solvent will be replaced continuously (always new) so that the solvent does not experience saturation until the search process runs perfectly while in the maceration method, the simplisia is immersed in the solvent so that the possibility of a saturated solution can occur before the search process is complete (Tutik *et al*., 2022). The yield of the reflux method is greater than the soklet method. This happens because the extraction process with the soklet method requires a longer duration for the interaction between the solvent and the langsat leaf powder. In contrast, the reflux method requires a shorter time because the interaction between solvent and langsat leaf powder occurs at the mixing stage (Mutiara and Wildan, 2020).

Table 1. Yield of 96% ethanol extract of Langsat Leaf

Extraction Method	Powder Weight (gram)	Extract Weight (gram)	Yield (%)
Percolation	50	10,3	20,6
Maceration	50	5,4	10,8
Soxhlet	50	7,7	15,4
Reflux	50	9,5	19,0

Antioxidant activity assay

In order to determine the wavelength at which the ABTS solution exhibits the highest absorbance, the maximum wavelength in the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) technique is determined. The right wavelength will give a more accurate and sensitive measurement of antioxidant activity. The establishment of the operating duration aims to identify the optimal time at which the antioxidant solution gives a stable absorbance, ensuring that the antioxidant activity data obtained are precise and reliable.. The result of determining the maximum wavelength between ABTS and trolox in this study is 748.70 nm (Figure 1) and operating time at 30 minutes (Table 2).

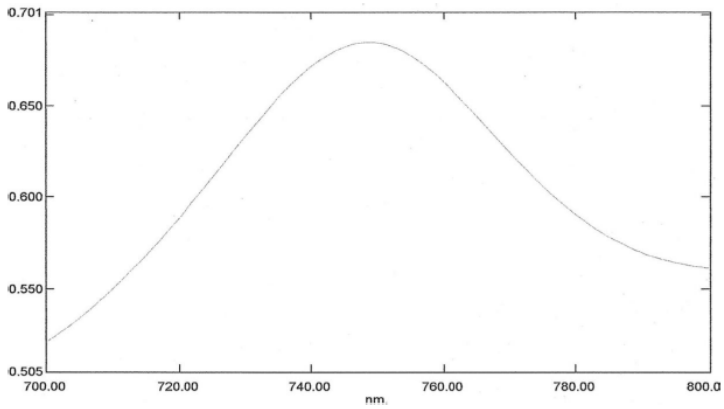

Figure 1. Maximum wavelength of ABTS-Trolox

Table 2. Operating Time of ABTS-Trolox

Waktu	Absorbansi
5	0,707
10	0.693
15	0,680
20	0,676
25	0,665
30	0,665
35	0,665
40	0,662
45	0,658
50	0,650
55	0,647
60	0,639

Antioxidant activity testing with the ABTS method was carried out with a comparison (positive control) of trolox. Trolox is a standardized positive control, thus providing a clear reference to compare the antioxidant activity of the tested samples. The IC_{50} value of trolox in this study was 18.583 ± 0.1 ppm (very strong category). This result shows only a slight variation compared to the research carried out by Anwar et al. (2022) which amounted to 19.29 ppm which is also included in

the very strong category. The results of antioxidant activity testing of 96% ethanol extract of langsat leaves obtained by 4 extraction methods can be seen in Table 3.

Table 3. Antioxidant Activity of 96% Ethanol Extract of Langsat Leaves

Extraction Methods	IC_{50} Value (ppm)
Percolation	89,48 ± 0,21 *
Maceration	107,53 ± 0,18 *
Soxhlet	92,52 ± 0,44 *
Reflux	114,28 ± 0,48 *

* = Three times replication

Based on Table 3, the order of antioxidant activity of 96% ethanol extract of langsat leaves based on extraction method from highest to lowest is percolation, reflux, soklet, and maceration. Statistical test results show that there are significant differences in maceration, percolation, reflux, and soklet methods. The percolation method gave the highest antioxidant activity of 89.48 ± 0.2 ppm (strong category) compared to other methods.

Calculation of phenolic content

The calculation of total phenolic content uses gallic acid comparator because gallic acid is a phenolic compound that is stable, easily obtained in a pure state, and has a simple structure and consistent properties, thus allowing the creation of an accurate calibration curve to calculate total phenolic content. The commonly used method is the Folin-Ciocalteu method, in which phenolic compounds in the sample will react with the Folin-Ciocalteu reagent in an alkaline atmosphere produced by the addition of sodium carbonate (Na_2CO_3). This reaction produces a blue complex whose color intensity is proportional to the phenolic concentration in the sample. The results of determining the maximum wavelength of gallic acid in this study are 744.80 nm and an operating time at 120th minute. Table 4 displays the findings of determining the total phenolic content of the 96% ethanol extract of langsat leaves that was produced using four different extraction techniques.

Table 4. Phenolic Content of 96% Ethanol Extract of Langsat Leaves

Extraction Method	Phenolic Content (mgGAE/gram extract)
Percolation	138,690 ± 0,51 *
Maceration	112,106 ± 0,88 *
Soxhlet	127,242 ± 1,25 *
Reflux	94,513 ± 1,12 *

* = Three times replication

Based on Table 4, the order of phenolic content of 96% ethanol extract of langsat leaves based on the extraction method from highest to lowest is percolation, soklet, maceration, and reflux. Statistical test results show that there are significant differences in maceration, percolation, reflux and soklet methods. The percolation method gives the highest total phenolic content of 138.690 ± 0.51 mgGAE/gram extract compared to other methods.

Calculation of flavonoid content

The calculation of flavonoid content using quercetin as a comparator is done because quercetin is one of the flavonoids of the flavonol group that has a stable chemical structure and can serve as a standard in the analysis. Quercetin has a keto group at atom C-4 and a hydroxyl group at atom C-3 or C-5, which makes it easy to react with certain reagents, such as aluminum chloride ($AlCl_3$), to form a yellow complex that can be measured using a UV-Vis spectrophotometer. This method utilizes the Lambert-Beer law, which states that the absorbance of a solution is proportional to the concentration of the solute. By making calibration curves of various concentrations of quercetin, the determination of flavonoid levels in samples can be done with high accuracy. The result of determining the maximum wavelength of quercetin in this study is 429.90 nm and operating time at

30 minutes. The outcome of the analysis for the total flavonoid content of 96% ethanol extract of langsat leaves obtained by 4 extraction methods can be seen in Table 5.

Table 5. Flavonoid Content of 96% Ethanol Extract of Langsat Leaves

Extraction Method	Flavonoid Content (mgQE/gram extract)
Percolation	5,379 ± 0,51 *
Maceration	4,245 ± 0,12 *
Soxhlet	4,659 ± 0,75 *
Reflux	3,747 ± 0,09 *

* = Three times replication

Based on Table 5, the order of flavonoid content of 96% ethanol extract of langsat leaves based on the extraction method from highest to lowest is percolation, soklet, maceration, and reflux. Statistical test results show that there are significant differences in maceration, percolation, reflux and soklet methods. The percolation method gives the highest total phenolic content of 5.379 ± 0.51 mgQE/gram extract compared to other methods.

The percolation method produced the highest antioxidant activity, total phenolic content, and total flavonoids of 89.48 ± 0.2 ppm (strong category); 138.690 ± 0.51 mgGAE/gram, and 5.379 ± 0.51 mgQE/gram extracts compared to maceration, soklet, and reflux methods. This happens because the extraction process in the percolation method is more efficient in removing bioactive compounds from plant materials than other methods (Hasanah *et al*., 2020).

The percolation method has a number of advantages that make it superior in producing higher antioxidant activity, total phenolic content, and total flavonoids compared to other extraction methods. One of the main advantages of percolation is its ability to perform continuous extraction by regularly replacing the solvent, thus preventing solvent saturation and increasing the transfer efficiency of active compounds from the sample into the solvent. This process allows thermolabile phytochemical compounds, such as flavonoids, to be extracted optimally without experiencing degradation due to excessive heat (Arrofiqi *et al*., 2024). In addition, percolation also allows for better precipitation of active compounds as the constant flow of solvent creates a significant concentration difference between the sample particles. Although this method requires a longer extraction time and a larger amount of solvent, the end result is an extract with high antioxidant content, making it very valuable in nutritional and pharmacological applications (Candra *et al*., 2021).

CONCLUSION

Different extraction methods will produce antioxidant activity, total phenolic content, and total flavonoids in 96% ethanol extract of langsat leaves. The percolation method produced the highest antioxidant activity, total phenolic content, and total flavonoids of 89.48 ± 0.2 ppm (strong category); 138.690 ± 0.51 mgGAE/gram, and 5.379 ± 0.51 mgQE/gram extract compared to maceration, soklet, and reflux methods.

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